Aus der Medizinischen Klinik m. S. Kardiologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Right Ventricular Cardiomyocyte Ca^{2+} Homeostasis and Myofilament Function in Heart Failure with Preserved Ejection Fraction and Right Ventricular Dysfunction

Rechtsventrikuläre, kardiomyozytäre Ca^{2+} Homöostase und Myofilamentfunktion bei Herzinsuffizienz mit erhaltener Ejektionsfraktion und rechtsventrikulärer Dysfunktion

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von

Niklas Hegemann

aus München

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Abbreviations

Ca^{2+}	calcium
CCD	charge-coupled device
cMyBP-C	cardiac myosin binding protein C
cTnI	cardiac troponin I
E/e’	echocardiographic marker of diastolic dysfunction
ECG	electrocardiogram
EF	ejection fraction
Fig.	figure
Fr	french (catheter size)
FS	fractional shortening
ESC	European Society of Cardiology
HF	heart failure
HFpEF	heart failure with preserved ejection fraction
HFmrEF	heart failure with mildly reduced ejection fraction
HFrEF	heart failure with reduced ejection fraction
LV	left ventricle
LVEDP	left ventricular end-diastolic pressure
MS	metabolic syndrom
NaCl	sodium chloride
RV	right ventricle
RVD	right ventricular dysfunction
SGLT-2	sodium glucose co-transporter 2
SR	sarcoplasmic reticulum
SERCA	sarcoplasmic reticulum Ca^{2+} ATPase
TAPSE	tricuspid annular plane systolic excursion
T2DM	type-2 diabetes mellitus
W	week
WGA	wheat germ agglutinin

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Abstract (English)

Background: Approximately 50% of patients with signs and symptoms of heart failure (HF) are suffering from diastolic dysfunction, termed heart failure with preserved ejection fraction (HFpEF). Right ventricular (RV) dysfunction (RVD) is seen frequently (30%) in these patients and associated with poor clinical outcomes and increased mortality. Currently, there are no specific therapeutic options available addressing RVD in HF. On this account, we studied a well described small animal model of HFpEF, the ZSF-1 obese rat, to investigate a potential RV involvement and underlying pathomechanisms.

Results: Echocardiographic and hemodynamic examinations confirmed the previously described HFpEF phenotype of the ZSF-1 obese rat with preserved left ventricular (LV) ejection fraction (LVEF), LV hypertrophy (increased LV mass) and diastolic dysfunction as evident by increased E/e' ratio and LV end-diastolic filling pressures. Assessment of the RV revealed distinct changes in structure, such as an increased ventricular diameter and thickening of the free wall, and function, i.e. decreased RVEF. Analysis of Ca^{2+} handling and contractility in isolated RV cardiomyocytes showed distinct changes. Ca^{2+} transient amplitude was drastically reduced while diastolic $[Ca^{2+}]_i$ remained normal. In addition, the frequency-dependent increase of systolic $[Ca^{2+}]_i$ was lost. However, RV cardiomyocyte cell shortening amplitude was preserved. RV myofilament Ca^{2+} sensitivity was increased, which was associated with a hyperphosphorylation of S-282 of the cardiac myosin binding protein C (cMyBP-C) – a known regulator of myofilament Ca^{2+} sensitivity.

Conclusion: RVD is present in this established rat metabolic HFpEF model *in-vivo*, associated with profound changes in RV cardiomyocyte Ca^{2+} homeostasis and increased myofilament Ca^{2+} sensitivity, likely due to hyperphosphorylation of the sarcomeric protein cMyBP-C.

Abstract (German)

Hintergrund: Etwa 50% aller Patienten mit klinischen Zeichen einer Herzinsuffizienz leiden an der sogenannten diastolischen Herzinsuffizienz bzw. Herzinsuffizienz mit erhaltener Ejektionsfraktion (HFpEF). Im Rahmen dieser Erkrankung leiden etwa 30% der Patienten ebenfalls unter einer rechtsventrikulären Dysfunktion (RVD), die mit erhöhter Mortalität in dieser Patientengruppe assoziiert ist. Aktuell sind keine spezifischen Therapeutika für die RVD bei Herzinsuffizienz verfügbar. Aus diesem Grund haben wir ein gängiges Kleintiermodell für HFpEF, die ZSF-1 obese Ratte, auf eine potentielle RVD untersucht, um im Weiteren die zu Grunde liegenden Pathomechanismen zu evaluieren.

Ergebnisse: Echokardiographische und hämodynamische Untersuchungen bestätigten den zuvor beschriebenen HFpEF Phänotyp in der ZSF-1 obese Ratte. Es zeigten sich eine erhaltene linksventrikuläre Ejektionsfraktion (LVEF), LV Hypertrophie im Sinne einer gesteigerten LV Masse, sowie eine diastolische Dysfunktion, manifestiert als erhöhte E/e' Ratio und gesteigerte LV end-diastolische Druckverhältnisse. Die Analyse des RV fand relevante, strukturelle Veränderungen im Sinne einer vergrößerten rechten Herzkammer, sowie einer verdickten freien Wand des RV, als auch funktional eine erniedrigte RVEF vor. In isolierten RV Kardiomyozyten konnten wir signifikante Änderungen in der Ca^{2+} Homöostase beobachten. Hierzu zählen eine stark verringerte Ca^{2+} Transienten-Amplitude bei erhaltener diastolischer $[Ca^{2+}]_i$. Die frequenzabhängige Zunahme der systolischen $[Ca^{2+}]_i$-Amplitude ging verloren, wobei die Kontraktilität der Kardiomyozyten erhalten blieb, woraus sich eine gesteigerte Myofilament-Ca^{2+}-Sensitivität in den RV Kardiomyozyten der ZSF-1 obese Tiere ableitete. Dies war assoziiert mit einer Hyperphosphorylierung des kardialen Myosin-bindenden Proteins C (cMyBP-C), einem zuvor beschriebenen Regulator der Myofilament-Ca^{2+}-Sensitiv.

Schlussfolgerung: Im etablierten ZSF-1 Ratten HFpEF Model besteht eine RVD, welche mit fundamentalen Änderungen in der kardiomyozytären Ca^{2+}-Homöostase und gesteigerte Myofilament-Ca^{2+}-Sensitivität assoziiert ist und vermutlich durch eine Hypersphosphorylierung des cMyBP-C vermittelt wird.

1. Introduction

1.1 Heart failure

Initially, heart failure (HF) has been simply regarded as the clinical manifestation of systolic dysfunction of the heart: Lewis, 1933: ”*a condition in which the heart fails to discharge its contents adequately*”[1]. However, HF, as opposed to numerous other conditions, cannot be defined by a single diagnostic marker, but is a much more complex clinical syndrome with various characteristics. The American College of Cardiology / American Heart Association, European Society of Cardiology (ESC) and Japanese Circulation Society all agree on the classification as clinical syndrome featuring signs and symptoms such as (exertional) dyspnea, edema formation and fatigue as well as some form of functional or structural cardiac abnormality[2-4]. While this consensus definition covers the clinical manifestations it still neglects the great array of underlying pathomechanisms and is rather imprecise as a standalone diagnostic term.

1.2 Epidemiology

Current estimations see approximately 60 million people worldwide to be living with some form of HF[5] with numerous studies reporting HF prevalence in western countries to be in the lower single digit percentages between 1% and 4% across the whole population, while among people older than 65 years incidence drastically increases to over 10%[6]. Whereas on the one hand the ageing of western societies contributes to a continuously increasing incidence, and the prevalence of HF increases with age, recent reports indicate that HF among the young is on the rise as well, documented by a Swedish study which highlighted 18-44 year olds to be diagnosed 43-50% more frequently in the early 2000s compared to the late 1980s[7]. It is currently speculated that this could be the consequence of increased prevalence of obesity and the often ensuing onset of diabetes mellitus type 2 (T2DM), which are both known risk factors for the onset of HF[5]. These alarmingly increasing numbers facilitate that the current state of research on available treatment options does not sufficiently meet the medical needs across the HF spectrum.

1.3 Heart failure classification

The "*2021 European Society of Cardiology Guidelines for the diagnosis and treatment of acute and chronic heart failure (...)*"[2] give a comprehensive outline of different heart failure entities and subclasses (Fig. 1).

Heart Failure with reduced Ejection Fraction (HFrEF)	**Heart Failure with mildly reduced Ejection Fraction** (HFmrEF)	**Heart Failure with preserved Ejection Fraction** (HFpEF)
Signs ± Symptoms[a]	Signs ± Symptoms[a]	Signs ± Symptoms[a]
LVEF ≤40%	LVEF 41–49%[b]	LVEF ≥50%
-	-	Cardiac structural and/or functional abnormalities consistent with the presence of LV diastolic dysfunction/raised LV filling pressures, including raised natriuretic peptides[c]

Figure 1: Definition of HF subclasses according to 2021 European Society of Cardiology Guidelines[2]. a) Signs may not be present in the early stages of HF (especially in HFpEF) and in optimally treated patients. b) For the diagnosis of HFmrEF, the presence of other evidence of structural heart disease (e.g. increased left atrial size, LV hypertrophy or echocardiographic measures of impaired LV filling) makes the diagnosis more likely. c) For the diagnosis of HFpEF, the greater the number of abnormalities present, the higher the likelihood of HFpEF.

Largely based on echocardiographic read-outs of left ventricular systolic function (left ventricular ejection fraction, LVEF), they provide a classification not only used for clinical diagnoses but also for pre-clinical small animal models of cardiovascular disease and heart failure. In the following, these subclasses will be summarized with a focus on HFpEF, as it is within the central scope of the current study.

1.3.1 Heart failure with reduced ejection fraction (HFrEF)

HFrEF describes the state of systolic failure of the heart and primarily the LV, most commonly caused by ischemic heart disease, hypertension, valvular disease or idiopathic dilated cardiomyopathy[8]. This is routinely assessed clinically as well as in pre-clinical studies by echocardiographic examination, evaluating the LVEF or percentage of blood volume that is ejected from the LV. The threshold for the diagnosis of systolic insufficiency of HFrEF is set to be ≤40%[2-4]. As cardiac output decreases, the sympathetic nervous system as well as the renin-angiotensin-aldosterone system are activated to maintain the cardiovascular homeostasis[9] (Fig. 2). These compensatory mechanisms, however, cause deleterious consequences in the long-term. Advancing systolic HF is commonly accompanied by LV structural abnormalities[10]. The left heart starts to dilate and adhere to a rather spherical in contrast to its regular elliptical shape. This global remodeling can

be also tracked on a cellular level as cardiomyocyte hypertrophy and interstitial accumulation of collagen[11, 12].

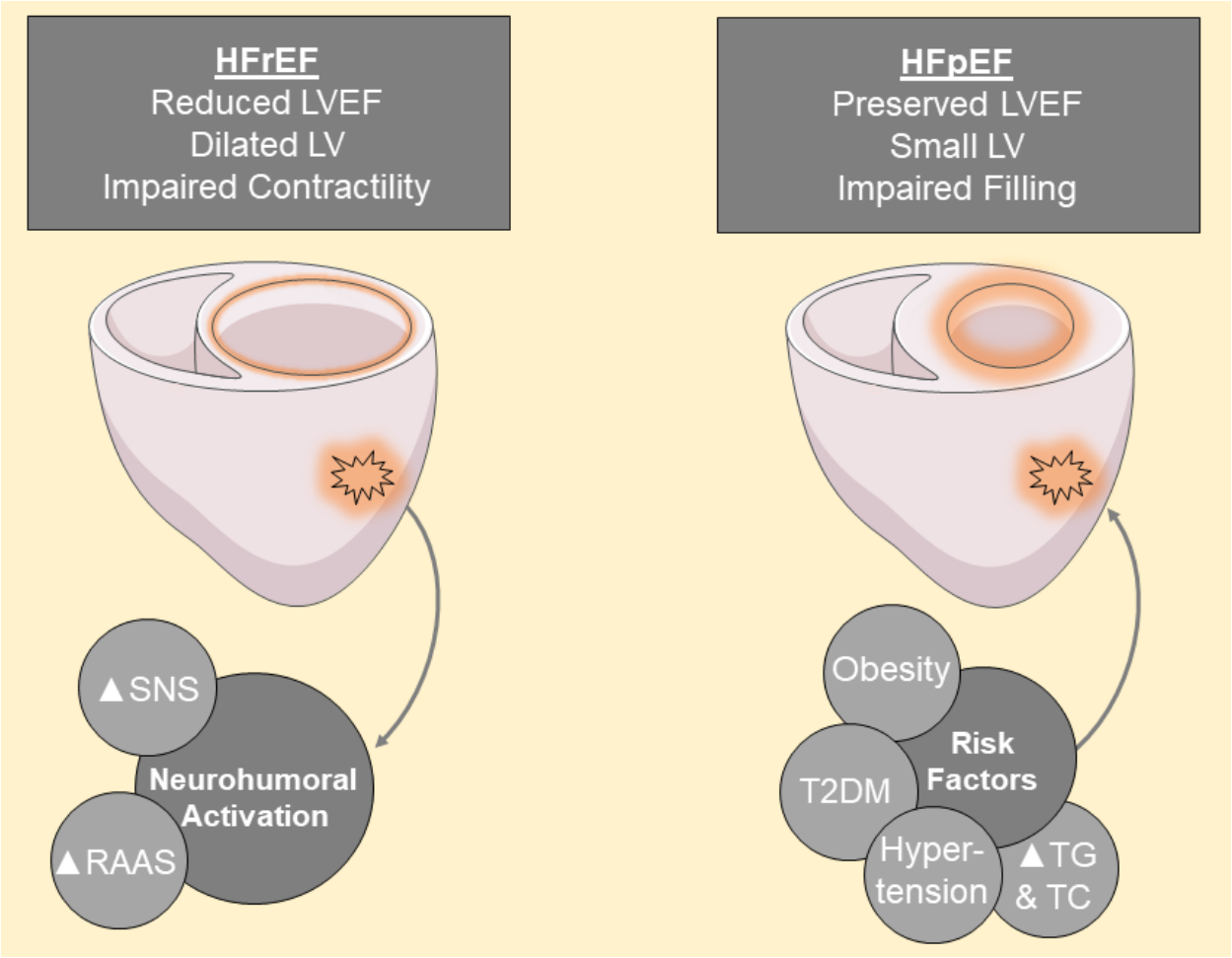

Figure 2: Heart Failure with reduced Ejection Fraction (HFrEF) vs. Heart Failure with preserved Ejection Fraction (HFpEF). HFrEF, characterized by reduced left ventricular ejection fraction (LVEF), dilation of the LV and overall impaired contractility, causes neurohumoral stimulation by activating the sympathetic nervous system (SNS) als well as the renin-angiotensin-alodsteron system (RAAS). HFpEF, defined by a preserved LVEF, a smaller LV due to concentric hypetrophy and impaired filling, is most commonly caused by external risk factors like obesity, type-2 diabetes mellitus (T2DM), hypertension and elevated triglycerides (TG) and total cholesterol (TC). This figure uses images from Servier Medical Art by Servier licensed under a CC 3.0 unported license.

1.3.2 Heart failure with mildly reduced ejection fraction (HFmrEF)

HFmrEF concerns patients with a LVEF between 41% and 49% and presents an intermediate form of HF[2] which is seen in approximately 13-24% of all HF patients. In some cases it seems to be a transitional state of patients initially diagnosed with heart failure with preserved ejection fraction (HFpEF) and now further deterioration of diastolic function and onset of systolic dysfunction[13], which might be caused by the onset of coronary artery disease[14]. On the other hand, HFrEF patients with improved systolic function under adequate therapeutic intervention could also fall into this category[15]. The

current consensus is that clinical presentation and outcome in HFmrEF tends to be closer related to HFrEF characteristics[16].

1.3.3 Heart failure with preserved ejection fraction (HFpEF)

HFpEF describes a patient group with preserved ejection fraction, defined as 50% or greater while showing prominent signs and symptoms of HF[2]. These patients suffer from diastolic dysfunction, refering to impaired active relaxation of the LV resulting in inadequate filling during ventricular diastole which in turn causes lower stroke volume and cardiac output. Impaired filling is closely related to increased myocardial stiffness, LV hypertrophy and ultimately reduced LV compliance, becoming also apparent as increased LV filling pressures. As a consequence, the left atrium (LA) is also commonly enlarged caused by backward congestion from the LV which increases the likelihood for the onset of atrial fibrillation. A frequently used echocardiographic parameter to evaluate diastolic dysfunction and disease severeness is the ratio of early peak mitral inflow velocity *E* to tissue oscillation at the mitral valve annulus ***e'***, a parameter that strongly correlates with invasively measured elevated LV end-diastolic filling pressures[17].

Today, HFpEF is reported to account for roughly 50% of HF patients with an increasing tendency[18] and has currently no approved targeted treatment to improve prognosis. In agreement, the recently published 2021 ESC guidelines on the management of HF state that "*To date, no treatment has been shown to convincingly reduce mortality and morbidity in patients with HFpEF(...)*"[2]. Large clinical trials evaluating the use of Angiotensin-II receptor 1 blockers - either alone (CHARM-Preserved and I-PRESERVED) or in combination with neprilysin inhibitors (PARAGON-HF) - or spironolactone (TOPCAT) have failed to meet their respective primary endpoints[2]. SGLT-2 inhibitors seem to be the first drug to relevantly improve mortality and hospitalizations as outlined in the recently published EMPEROR-Preserved study[19]. However, as the latter also included patients that meet the criteria for the diagnosis of HFmrEF (LVEF <50%), these results need to be interpreted with care.

Exact pathomechanisms in the development of HFpEF are not conclusively investigated. However, recent studies label a range of co-morbidities as the origin of systemic low-grade inflammation and microvascular dysfunction that fuel the onset of cardiac stiffness

and remodeling including interstitial fibrosis[20] (Fig. 2) in agreement with the clinical diagnosis of metabolic syndrome (MS). MS is defined as a combination of different risk factors, including (abdominal) obesity, high triglycerides, low- and high-density lipoprotein cholesterol, hypertension and elevated blood glucose / insulin resistance in accordance with the diagnosis of maturity-onset / type 2 diabetes mellitus[21, 22]. As a feature of many chronic disorders, MS results in general leukocyte infiltration and systemically increased levels of pro-inflammatory cytokines and oxidative stress. In addition, microvascular dysfunction and rarefaction are observed which result in increased peripheral resistance and hence increase blood pressure and afterload for the left heart as well as poor tissue perfusion, including the myocardium, impairing oxygen and nutrient supply. Compensatory mechanisms like myocardial hypertrophy inevitably ensue and change the structural composition of the LV, including cardiomyocyte hypertrophy and dysfunction as well as increased collagen deposition, fueling further stiffening and worsening diastolic dysfunction[23].

1.4 Role of the right ventricle in (left) Heart Failure

The right ventricle (RV) has been often regarded as the neglected ventricle, since the LV is the critical entity in pumping blood towards the aorta and producing an adequate cardiac output that meets the body's metabolic needs. In recent years, the RV has regained attention and its role as predictor of clinical outcome and death in a range of cardiac and pulmonary conditions has been highlighted[24]. However, even though HFrEF patients are commonly (48%)[25] affected by RVD it has been disregarded for quite some time and has only recently regained attention as the center of research efforts around HF management.

RV and LV are fixed within the pericardial sac, sharing the interventricular septum as well as a portion of myocardial fibers that enclose both ventricles. These anatomical circumstances already give way to the idea that dysfunction of the one will inevitably affect performance of the other. The commonly deprived RV function in HFrEF patients[26] is thought to arise as a consequence of numerous pathological processes:

- Congestion in the left heart causes an increase in pulmonary vascular pressure and ultimately an increased RV afterload, putting strain on the RV and fueling RV remodeling and dysfunction
- RV systolic function and output is directly dependent on LV systolic function through the shared septal myocardium and its contractility[27]
- Structural remodeling of the left heart can cause profound changes in cardiac geometry which results in disarray of septal myocardial fibers, further weakening RV function, and possibly also leading to inadequate tricuspid valve coaptation[27]
- Intrinsic RV dysfunction[28, 29]

While RVD is more often diagnosed in patients suffering from systolic HF, a concomitant diagnosis of RVD in HFpEF is also seen in approximately 30%[30] of patients. In this subgroup, RVD boasts as a major predictor of poor clinical outcome and mortality[31] which calls for a routine assessment of RV function in these patients to identify individuals at higher risk for disease progression and premature death.

Our 2020 review article on RVD in HFpEF[23] divides the mechanisms for the onset of RVD in HFpEF into intrinsic myocardial dysfunction and RV afterload-dependent[23] dysfunction. As the LV gets stiffer and filling is impaired, blood backs up into the left atrium and ultimately the pulmonary circulation. Chronification of this state causes pulmonary vascular remodeling in agreement with the diagnosis of pulmonary hypertension due to left heart disease (World Health Organization Type II), resulting in increased afterload for the RV with functional as well as structural consequences, such as RV enlargement and (myocyte) hypertrophy. An intrinsic component of RVD is commonly known for HFrEF and has also been mentioned for HFpEF, however, the exact underlying pathomechanisms are neither known nor satisfyingly addressed in the currently available literature[30, 32].

1.5 Cardiac Ca^{2+} handling and contractility

Central to general cardiac function is contractility, which is centrally dependent on proper Ca^{2+} trafficking and subsequent activation and adequate functioning of the contractile apparatus within myocytes, whose subunit, the sarcomere, is composed of thin and thick

myofilaments (Fig. 3). The latter consists of an F-actin double helix, two strands of tropomyosin with troponin complexes, containing troponins I, T and C[33]. While troponin C is the Ca^{2+} binding component of the complex, triggering the contractile process, troponin T has only a structural role, constituting the tropomyosin-binding unit whereas troponin I is referred to as the inhibitory subunit, as it prevents myosin from interacting with actin on the thin filament[34]. The thick filament is primarily made up of the protein myosin. Each myosin has two so called heads which contain an ATPase that is required for the process of cross-bridging between myosin and actin or the thick and thin filament, respectively. Among a ladder of myosin binding proteins, the cardiac myosin binding protein C (cMyBP-c) has been proposed to limit extension of myosin heads[35] and therefore putatively affect contractile range and function of the myofilaments.

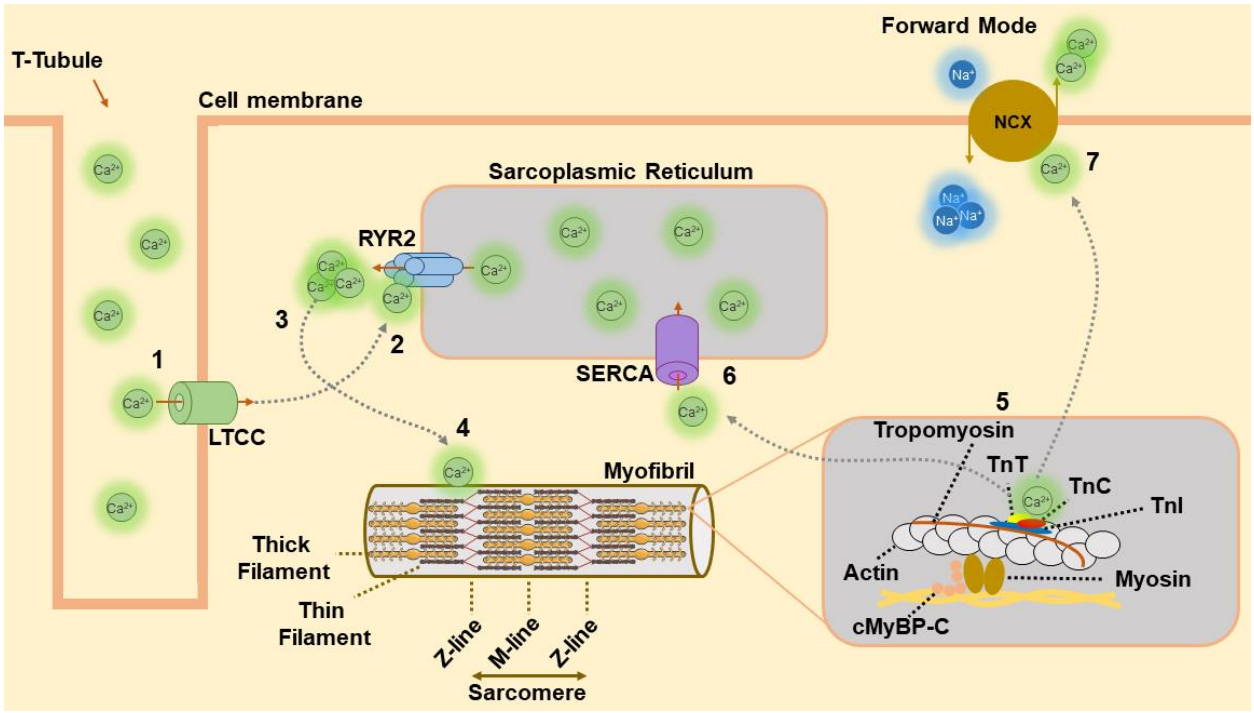

Figure 3: Cardiomyocyte Ca^{2+} trafficking. 1) Ca^{2+} enters the cell via voltage-gated L-type Ca^{2+} channels (LTCC) located in the t-tubuli system. 2) Ca^{2+} binds intracellularly to ryanodine receptors (RYR2) on the sarcoplasmic reticulum (SR) to release further Ca^{2+} (3) from the intracellular store, the SR. 4+5) Ca^{2+} then binds to troponin C (TnC) on the thin filament, triggering the contractile process. 6+7) Ensuing relaxation is caused by Ca^{2+} coming off of TnC and then being cycled back into the SR via the SR Ca^{2+} ATPase (SERCA) and out of the cell via the sodium- Ca^{2+} exchanger in forward mode. This Figure uses images from Servier Medical Art by Servier licensed under a CC 3.0 unported license.

Upon cardiomyocyte excitation, Ca^{2+} enters the cardiomyocyte through voltage-gated L-type Ca^{2+} channels causing the ryanodine-receptor mediated release of more Ca^{2+} from the intracellular Ca^{2+} store, the sarcoplasmic reticulum (SR), to raise the intracellular levels from 0.1 to 1.0mM[23, 36]. Ca^{2+} then binds to the troponin complex on the thin filament, specifically troponin C. This causes a conformational change and allows for cross-bridging of thin and thick filaments and therefore the actual contraction. Ensuing

relaxation is dependent on Ca^{2+} coming off of troponin C, which is then being pumped back into the SR by the SR Ca^{2+} ATPase (SERCA) or out of the cell by the sodium-calcium exchanger[37]. Numerous studies have already evaluated LV cardiomyocyte function, proving that dysregulation of cardiac Ca^{2+} handling are key mechanisms that are altered in HF[38-40]. However, there is a clear knowledge gap towards RV cellular function specifically in HFpEF which hinders us from gaining a deeper understanding of underlying pathomechanisms on the level of cardiomyocytes and therefore the etiology of RVD onset and deterioration in this condition.

1.6 Myofilament sensitivity

The myofilament sensitivity to Ca^{2+} has been widely used and described as a tool for assessing myocardial function. This concept simply describes the relationship between the amount of free Ca^{2+} in the cytoplasm to bind to troponin C and the actual force or magnitude of contraction generated[37]. In heart failure, depending on the etiology of disease, increased or decreased myofilament Ca^{2+} sensitivity has been described[41]. These effects are often attributable to either mutations of sarcomeric proteins or disease-associated differential phosphorylation of Ca^{2+} sensitivity regulators, like c-MyBPc or troponins[42].

1.7 Study aim and hypotheses

The central aim of the current study is to elucidate on the underlying pathologies and functional-structural adaptation of the RV in HFpEF, thereby aiding in the identification of potential pharmacotherapeutic targets. The main hypotheses of the study are as follows:

1.) *In-vivo* evidence of RVD

RVD is prevalent in the multimorbid ZSF-1 obese rat model of HFpEF as defined by *in-vivo* echocardiographic evidence for structural remodeling and functional decline of the RV.

2.) *In-vitro* evidence of RVD

Isolated RV cardiomyocytes from ZSF-1 obese rats show altered Ca^{2+} handling properties and contractility *in-vitro.*

3.) Involvement of myofilament Ca^{2+} sensitivity regulators

Alterations of sarcomeric proteins regulating cardiomyocyte contractility and myofilament function, such as cMyBP-C and troponins, are associated with the outcome of hypotheses one and two.

To investigate the hypotheses stated above, the present study by Hegemann, et al. (2021)[43] evaluates a frequently used small animal model of HFpEF, the ZSF-1 obese rat, on RVD *in-vivo* using echocardiography and invasive hemodynamics as well as *in-vitro* assessing Ca^{2+} levels and contractility in isolated RV cardiomyocytes using ratiometric fluorescence microscopy.

2. Experimental outline and methods

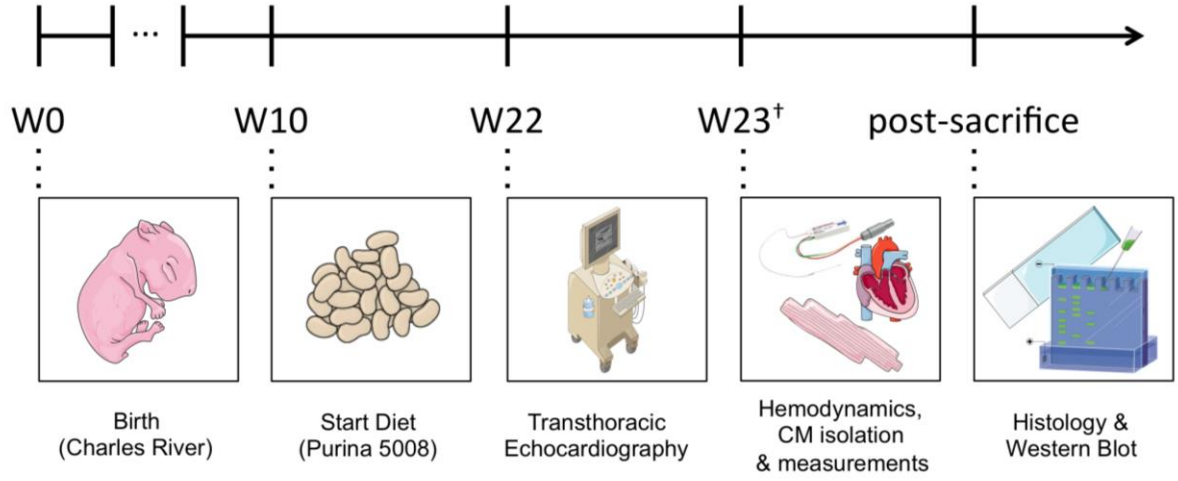

Figure 4: Experimental outline. Week 0 (W0) – birth of the animals at Charles River facilities. Week 10 (W10) – Animals arrive at our animal facility and start high-caloric diet. Week 22 (W22): Animals undergo transthoracic echocardiography. Week 23 (W23) – animals undergo invasive left-heart catheterization with subsequent animal sacrifice, cardiomyocyte isolation and Ca^{2+} and contractility measurements. Post-sacrifice – histological and western blot analysis of right ventricular tissue from sacrificed animals.This Figure uses images from Servier Medical Art by Servier licensed under a CC 3.0 unported license.

The following paragraphs summarize the experimental plan and the main methods for the current study. A more detailed description of the individual techniques can be found in the corresponding publication[43].

The experiments outlined here were performed with approval of the local authorities (Landesamt für Gesundheit und Soziales) under the the reference number G0317/17 and in agreement with the ARRIVE guidelines and guidelines for the Care and Use of Laboratory Animals (National Institute of Health, USA). ZSF-1 obese and Wistar Kyoto

rats, the latter serving as a control group, were obtained at 10 weeks of age (Charles River Laboratories, MA, USA) and fed a high-caloric diet (Purina 5008: 26.6% protein, 16.5% fat, 56.9% carbohydrates; LabDiet, MO, USA)[43]. The ZSF-1 obese rat is a hybrid between a female Zucker diabetic fatty and a male spontaneously hypertensive heart failure rat. It harbors two distinct leptin mutations that cause the observed hyperphagia in this model. It has previously been evaluated as a HFpEF model by our group as well as others[44-46] and not only agrees with the clinical diagnosis of HFpEF but also replicates common risk factors and comorbidities in terms of MS[47].

2.1 Transthoracic echocardiography

Animals were subject to transthoracic echocardiography at 22 weeks of age. A Fujifilm Visualsonics Vevo 2100 Imaging System was used for these experiments in conjunction with a MS250 (13-24 MHz) ultra-high frequency linear array transducer. The procedure was performed under inhalational anaesthesia (1.5 l/min O_2 supplemented with 1,5% isoflurane). The animals were fixed in a dorsal position on a temperature-regulated ECG table for surveillance of physiological parameters (heart rate, body temperature, respiratory rate). Image acquisition ensued according to common standards and data analysis was performed using the Vevolab software (Fujifilm Visulasonics)[43].

2.2 Invasive hemodynamics and animal sacrifice

At 23 weeks, invasive cardiac hemodynamic measurements were conducted as a terminal procedure. Induction of anaesthesia was performed as described for echocardiography. The animals were placed onto a heating pad to ensure maintenance of physiological temperatures. Intubation with an orotracheal tube ensued with ventilation by a specialized rodent ventilator (SAR-1000 Small Animal Ventilator, CWE, USA) as well as attachment to a surface ECG. After blunt preparation of the external jugular veins a polyethylene catheter was inserted into the left jugular vein for fluid application where needed (0.9% NaCl) whereas a second catheter was placed into the right jugular vein for saline calibration (10% NaCl) of the pressure-volume catheter. After preparation of the right carotid artery, a 2Fr pressure-volume conductance catheter (Millar SPR-838) was inserted and guided through the aorta and aortic valve into the LV under pressure control. After a stabilization period, readings of LV pressures were performed. After animal sacrifice, organ weights were documented for a subset of animals including ventricular

weights as well as kidney and liver. RV tissue was harvested by surgically separating the RV free wall from the heart. After weight documentation, tissue was frozen and stored at -80°C for molecular analyses[43].

2.3. Histology

For histological analyses, cardiac specimens were fixed in 4% paraformaldehyde and afterwards embedded in paraffin. 4-8 µm thick tissue sections were stained with either haematoxylin / eosin or picrosirius red staining according to common standards by Prof. Klopfleisch at the veterinary pathology of the Freie Universität Berlin. Slides were subsequently scanned for image analysis. Fluorescent staining was performed using wheat germ agglutinin (WGA) conjugated to the fluorescent dye Alexa-488. Slides were imaged using an Invitrogen EVOS M5000 imaging system. Analysis of RV cardiomyocyte cross-sectional area and fibrosis was performed using FIJI (ImageJ). Specimens for these analyses originate from a separate cohort at 21 weeks[43].

2.4 RV cardiomyocyte isolation, staining and Ca^{2+} analysis

After animal sacrifice, retrograde perfusion and enzymatic digestion of the heart via the aorta was performed using a Langendorff perfusion setup. The RV was severed from the digested heart and a cell suspension was prepared and filtered into a 50 ml falcon tube. A stepwise Ca^{2+} reintroduction (0.1-1.0 mM) was performed using Tyrode's solutions. RV cardiomyocytes were transferred onto laminin pre-coated glass slides and incubated with fura-2 AM for 12 minutes (both Thermo Fisher Scientific, Waltham, MA, USA) at a concentration of 1 µmol/l in Tyrode's solution with 1 mM Ca^{2+}. The cells were then washed and transferred to a temperature controlled imaging stage (set to 37°C) connected to the ratiometric fluorescence microscope setup (PMT400 photomultiplier; Axiovert 200 microscope, Zeiss, Jena, Germany; MyoPacer EP and Fluorescence System Interface, both IonOptix, Westwood, MA, USA). For contractility imaging, sarcomere shortening was imaged with a CCD camera (MyoCam-S, IonOptix, Westwood, MA, USA). As part of the imaging routine, cells were paced with 1, 2 and 4 Hz stimulation. Background- and autofluorescence-correction were performed. Data analysis was carried out using the *Transient Analysis Tool*, as well as *IonWizard* by IonOptix. Myofilament sensitivity analysis was performed by peak intracellular [Ca^{2+}] to systolic sarcomere length ratio.

Additionally, sarcomere shortening was plotted against intracellular [Ca^{2+}], generating a hysteresis loop[43]. A linear regression line was fitted to calculate the slope return from the peak sarcomere shortening[22, 23].

2.5 Western blot

A lysis buffer was utilized to homogenize the tissue samples at 4°C before being run on 4–12% Bis-Tris polyacrylamide gels. Afterwards they were transferred to nitrocellulose membranes, which were probed overnight at 4°C with anti-phospho Ser 23/24 troponin I (PhosphoSolutions, Aurora, CO, USA) and anti-troponin (Novus, Centennial, CO, USA) or anti-phosphoSer282 cMyBP-C (Enzo) and anti-cMyBP-C (Novus, Centennial, CO, USA). As second antibodies Anti-rabbit IgG linked with IRDye 800CW or anti-mouse IgG linked with 680RD (LI-COR, Lincoln, NE, USA) were used. For signal detection, an Odyssey CLx System was used in combination with the Image Studio software (LI-COR, Lincoln, NE, USA) to analyze band intensities[43].

2.6 Blood glucose

At 23 weeks, prior to the final experiments, blood glucose levels were evaluated via tail vein sampling using a conventional blood glucose analyzer (Bayer, Leverkusen, Germany)[43].

2.7 Statistics

Data are presented as mean ± standard error of the mean. Single data points indicate individual biological replicates. Mann-Whitney, t-test or two-way analysis of variance were performed for statistical analysis. Significance was assumed at $P<0.05$.

3. Results

The ZSF-1 obese animals presented with structural remodeling in terms of LV hypertrophy (increased LV mass), a preserved LVEF and reduced Cardiac Index (cardiac output normalized to calculated body surface area). Diastolic dysfunction was evident as increase in E/e' ratio. In direct agreement, invasive hemodynamics revealed increased LV filling pressures (left ventricular end-diastolic pressure) as well as systolic pressures. With respect to metabolic features, the ZSF-1 obese rats had strongly increased blood glucose levels and showed lipemic blood samples[43].

Analysis of the RV by echocardiography revealed increased diastolic and systolic dimensions, reduced RVEF as well as an increase of the RV free wall thickness. Gravimetric data supported the RV hypertrophic phenotype by an increased RV-to-tibia length ratio (Fig. 5A-C). Histological evaluation of RV cardiomyocyte cross sectional area additionally revealed RV hypertrophy on the level of single cardiomyocytes (Fig. 5D).

Ratiometric Ca^{2+} measurements showed a profound decrease in the Ca^{2+} transient amplitude (Fig. 5E) and a loss of the frequency-dependent increase of systolic $[Ca^{2+}]_i$ concentrations while diastolic $[Ca^{2+}]_i$ was not different compared to the control[43].

Contractility of RV cardiomyocytes, assessed as sarcomere shortening, as well as sarcomere length were not altered in ZSF-1 obese animals[43].

Analysis of myofilament sensitivity of RV cardiomyoctes from ZSF-1 obese animals by plotting a contraction-$[Ca^{2+}]_i$ hysteresis loop revealed a leftward shift and an increase in the slope of the returning portion of the loop (Fig. 5E). In agreement, peak $[Ca^{2+}]_i$ to systolic sarcomere length ratio was reduced[43].

Molecular analysis of RV tissue by western blot showed a significant increase in cMyBP-C phosphorylation at serine residue 282 (Fig. 5F). On the other hand, cTnI showed no significant difference for phosphorylation status of serine residue 23, 24, yet a strong trend was observed[43].

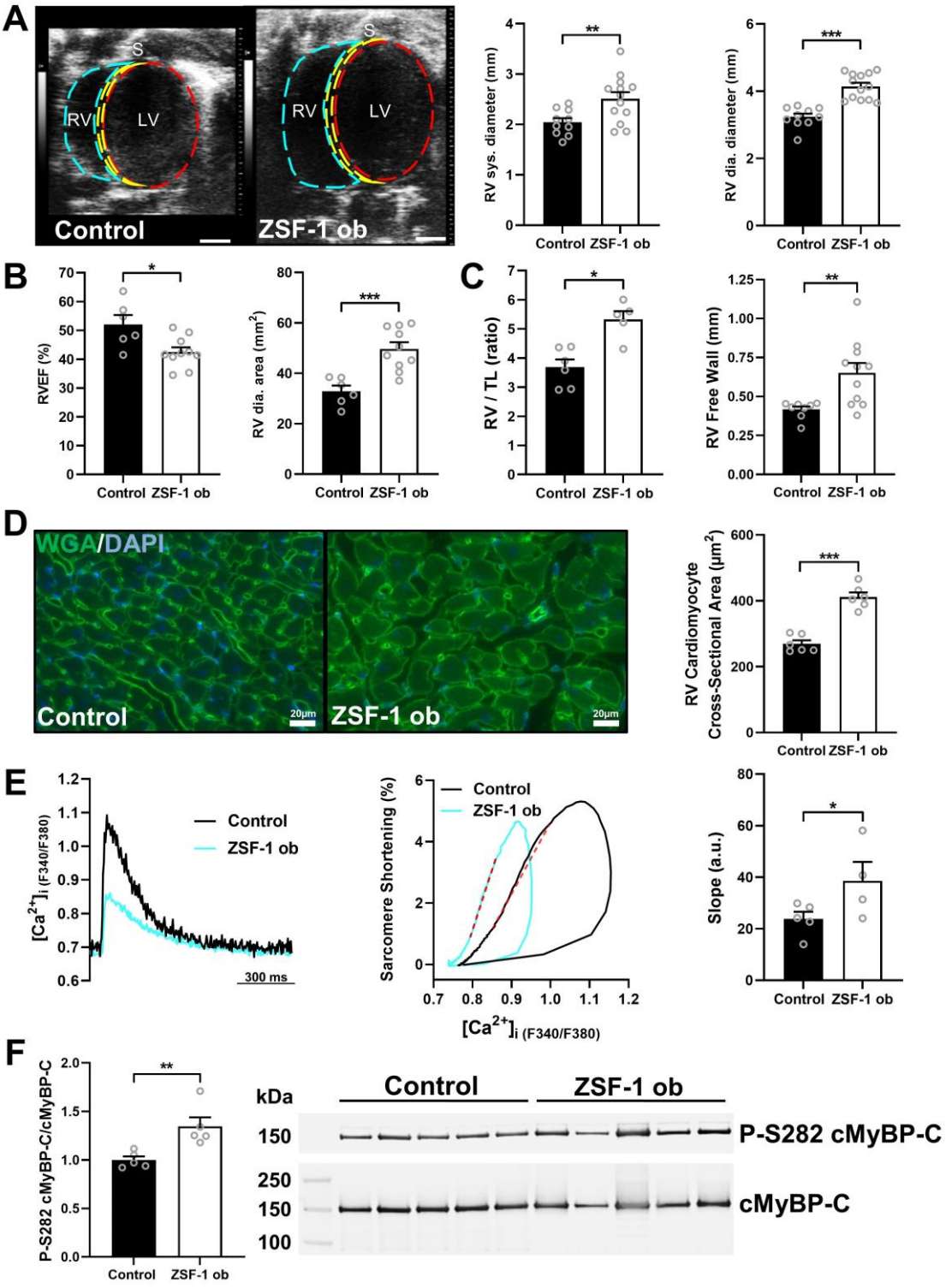

Figure 5: Key results from the publication by Hegemann et al. (2021). A) Representative echocardiographic parasternal long axis B-Mode images of controls (WKY) and ZSF-1 obese (ZSF-1 ob) rats with quantification of RV systolic (sys.) and diastolic (dia.) dimensions (n=10-13). B) Quantification of RV ejection fraction (RVEF) and diastolic area from B-Mode four-chamber view images (n=6-10). C) RV hypertrophic evaluation by RV to tibia length (TL) ratio from post-sacrifice analysis (n=5-6) and RV free wall thickness assessment from echocardiographic parasternal long-axis B-Mode images (n=8-11). D) Representative fluorescence images of wheat germ agglutinin (WGA)/DAPI stained RV tissue sections and quantification of RV cardiomyocyte cross-sectional area (n=6). E) Representative Ca^{2+} transients of isolated RV cardiomyoctes, averaged sarcomere shortening – $[Ca^{2+}]_i$ hysteresis loops with quantification of the slope of the return loop (n=4-5). F) Western blot analysis of cardiac myosin binding protein C (cMyBP-C) phosphorylation at serine residue 282 (P-S282) (n=5). For further details and extended data see the original publication, that this figures is based on[43].

4. Discussion

The current study provides a comprehensive evaluation of RV function *in-vivo* and *in-vitro* in a well-established rat model of HFpEF, the ZSF-1 obese rat[48-51]. Initial data could adequately replicate the previously reported HFpEF phenotype in our animals. In addition, we detected fundamental structural and functional changes of the RV accompanied by impaired RV cardiomyocyte Ca^{2+} handling and increased myofilament Ca^{2+} sensitivity, possibly regulated by cMyBP-C hyperphosphorylation.

Our phenotypic analysis of the ZSF-1 obese rat is in direct agreement with previous data by us and others[44, 45, 49, 50]. We detected a preserved LVEF, signs of LV structural remodeling as evident by an increased LV mass, as well as diastolic dysfunction apparent as increased E/e' ratio and LVEDP[43]. While the model adequately replicates the clinical disease characteristics of HFpEF[2], it also features the most critical and common co-morbidities and risk factors of developing HFpEF referring to MS[21]. In our study, the animals present with obesity, increased blood glucose levels[43] and hypertension (data not shown). On this account, the ZSF-1 obese rat promises to be especially valuable for the research around HFpEF.

Since literature on HFpEF in the past often solely focused on the deterioration of LV function and its clinical management, the foundation for in-depth (pre)clinical evaluation of the evidently critical role of the RV in HFpEF, appropriate animal models with proven RV dysfunction, is missing. On this account, our RV phenotypic data on the ZSF-1 obese rat addresses an unmet need in the field of preclinical HF research. We approached the assessment of RV function in our animals using state of the art small animal echocardiography as described by others[52, 53], which is also the primary tool for clinical evaluation of the RV[54]. Our analysis revealed increased chamber dimensions and a reduced RVEF of 42% (Fig. 5A+B) in the ZSF-1 obese animals in direct agreement with clinical data that sees mild RV systolic dysfunction in HFpEF patients ranging around 45%[55]. However, other measures like RV fractional shortening (FS) or tricuspid annular plane systolic excursion (TAPSE) showed no significant alteration[43]. We argue, that RV FS measurements as proposed by the literature[52, 53] may be inadequate to detect subtle functional changes since measurements are taken towards the kinetically less active base of the RV. Secondly, TAPSE was not reduced in the ZSF-1 obese rats. However,

when normalized to RV size, a difference is detectable (data not shown). We claim, that this normalization should be considered because tissue excursion of the same extent is putatively linked to differential output in differentially sized ventricles, such as seen in childhood vs adulthood (small vs big heart)[56].

RV free wall thickness measured by echocardiography and increased RV weight as measured by post-sacrifice gravimetric assessment demonstrate the presence of RV hypertrophy in our model (Fig. 5C). In further agreement, histological assessment exhibited RV cardiomyocyte hypertrophy evident as increased cross-sectional area (Fig. 5D), while no significantly higher RV fibrosis was seen[43]. Our findings regarding the RV in our model seem plausible for a borderline functionally compensatory state of the RV with hypertrophic remodeling and discrete reduction in systolic function[57]. These results are in line with hypothesis one, which called for "*In-vivo evidence for RVD*". Boosting the validity of our data, the recent study by Oliveira et al.[58] also described RV hypertrophy and dysfunction in their ZSF-1 obese animal study.

At the time of publication, our study was the first to investigate RV function in HFpEF down to the level of isolated RV cardiomoycytes in a preclinical setting, while recently other studies have picked up and reported similar findings[58, 59]. Our RV cardiomyocyte Ca^{2+} handling and contractility data revealed profound dysregulation in the ZSF-1 obese rats. Ca^{2+} transient analysis showed a marked decline of peak $[Ca^{2+}]_i$ and amplitude (Fig. 5E), as well as a blunted frequency-dependent increase of systolic $[Ca^{2+}]_i$, whereas sarcomere length and contractility were not significantly altered[43]. This confirms the second hypothesis of *"in-vitro evidence for RVD".* While reduced $[Ca^{2+}]_i$ amplitudes are characteristic for impaired Ca^{2+} handling in HF[60], the preserved diastolic $[Ca^{2+}]_i$ points towards a unique RV specific phenomenon.

Analysis of myofilament Ca^{2+} sensitivity by peak $[Ca^{2+}]_i$ to systolic sarcomere length ratio provides evidence for an increased myofilament Ca^{2+} sensitivity[43] which has also been reported for some types of HF[41]. To further evaluate this finding, other studies commonly plot $[Ca^{2+}]_i$ to force/contractility, creating a hysteresis loop and assessing the slope of the relaxation phase as measure for myofilament calcium sensitivity[61]. This analysis revealed a leftward shift of the loop and an increased slope during the cardiomyocyte relaxation for the ZSF-1 obese animals (Fig. 5E), in line with previous reports of increased

myofilament Ca^{2+} sensitivity[61-63], further confirming our findings. This mechanism likely acts as a compensatory mechanism in response to reduced systolic $[Ca^{2+}]_i$ and thereby largely preserves the contractile function, as seen in our sarcomere shortening analysis[43].

To elucidate molecular mechanisms of myofilament Ca^{2+} sensitivity in our model, we investigated two of its well-known regulators – cTnI and cMyBP-C[64, 65] – by western blot. As post-translational modifications, i.e. phosphorylation, are crucial to their function, we investigated previously reported central phosphorylation sites of the two proteins. While phosphorylation status of S-23,24 cTnI in the LV of ZSF-1 obese rats[66] was not altered in the RV of our animals[43], S-282 cMyBP-C was significantly hyperphosphorylated (Fig. 5F). Past studies have outlined how this likely improves myofilament Ca^{2+} sensitivity by causing cMyBP-C to rather bind to actin than the myofilament subfragment two. The latter bond is thought to act as a form of tether, limiting the myosin head's range of motion therefore the loss in turn will increase contractility[64, 67]. This argues in favor of hypothesis three, *"Involvement of myofilament* Ca^{2+} *sensitivity regulators".*

We performed a comprehensive examination of the ZSF-1 obese rat model and especially the RV phenotype. Based on our findings, there are a number of experiments that could be performed perspectively to fill in on some related scientific questions that have evolved from our current data.

With regards to the performed Ca^{2+} measurements and the preserved diastolic $[Ca^{2+}]_i$ and reduced systolic $[Ca^{2+}]_i$ in RV cardiomyocytes, we currently have no hard evidence towards the underlying mechanisms. However, from previous studies concerning HFrEF, we know that SERCA expression and/or activity has been frequently found to be reduced, resulting in diminished SR Ca^{2+} load[68]. On this account, future experiments on isolated RV cardiomyocytes could include additional evaluation of the SR Ca^{2+} load to investigate if the intracellular Ca^{2+} pool might be reduced and responsible for our observations. This could be performed by application of caffeine which causes a concentration dependent release of Ca^{2+} from the SR[69] which can be quantified by the ratiometric fluorescence microscopy methodology used in our current experiments. Alternatively, SERCA expression analysis (by qPCR or western blot) and/or activity assays could be performed. Other potential causes for the reduced systolic $[Ca^{2+}]_i$ could be a reduced Ca^{2+} induced

Ca^{2+} release caused by alterations of the t-tubular structure and reduced density of L-type Ca^{2+} channels, as described for various forms of cardiac disease[70].

Making forward looking statements towards potential translation of the presented findings into a novel targeted therapeutic for HFpEF with RVD can only be speculative at the current stage. However, it has to be duly noted that current approaches towards systolic HF treatment already have picked up on altering the hearts sensitivity to Ca^{2+}. The prime example is the Ca^{2+} sensitizing agent levosimendan. It is capable of stabilizing the complex of Ca^{2+} bound to troponin C, thereby increasing contractility and reducing adenosine triphosphate and consequently oxygen consumption[71]. Levosimendan therefore increases affinity of Ca^{2+} to troponin C. These inotropic effects have been reported for both LV and RV[72, 73]. In contrast, the available literature leads us to believe, that the compensatory effects of increased myofilament Ca^{2+} sensitivity in RV cardiomyocytes of the ZSF-1 obese animals are rather mediated by altered efficacy of Ca^{2+} binding to the myofilaments via increased range of mobility of myosin heads through actions of hyperphosphorylated cMyBP-C.

5. Conclusion

While established concepts of RVD in HF and especially HFpEF have been addressed and reviewed by us and others in the past[23, 74], we herewith provide the first insight into the consequences of RV remodeling and dysfunction on intrinsic RV cardiomyocyte function to gain further insight towards a deeper understanding of RVD in HFpEF. We identified profound changes in RV function and structure in a relevant small animal model of HFpEF *in-vivo* and *in-vitro*, with drastic changes in RV cardiomyocyte Ca^{2+} homeostasis and compensatory increased myofilament Ca^{2+} sensitivity, likely linked to increased phosphorylation of cMyBP-C (Fig. 3). Whereas HFpEF has gained a lot of attention in the scientific community over the past years, we believe to substantially aid to an area of research that has been at least partially neglected, the role of the RV in HFpEF.

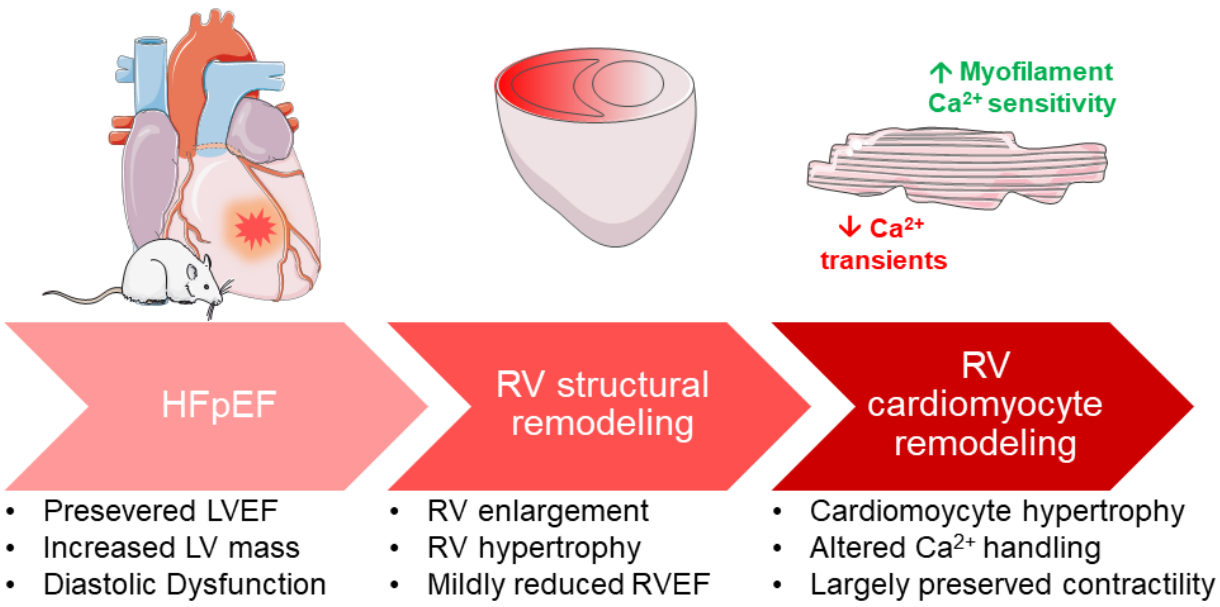

Figure 6: Graphical summary of the study results. This Figure uses images from Servier Medical Art by Servier licensed under a CC 3.0 unported license.

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I. Statuatory declaration

I, Niklas Hegemann, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic:

Right ventricular Cardiomyocyte Ca^{2+} Homeostasis and Myofilament Function in Heart Failure with preserved Ejection Fraction and Right ventricular Dysfunction

Rechtsventrikuläre, kardiomyozytäre Ca^{2+} Hämostase und Myofilamentfunktion bei Herzinsuffizienz mit erhaltener Ejektionsfraktion und rechtsventrikulärer Dysfunktion

independently and without the support of third parties, and that I used no other sources and aids than those stated. All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

_____	_____
Date	Signature

II. Declaration of contribution

Niklas Hegemann has contributed to the following publication:

Hegemann N*, Primessnig U*, Bode D, Wakula P, Beindorff N, Klopfleisch R, Michalick L, Grune J, Hohendanner F, Messroghli D, Pieske B, Kuebler WM, Heinzel FR. Right ventricular dysfunction in HFpEF is linked to altered cardiomyocyte Ca2+ homeostasis and myofilament sensitivity. ESC Heart Fail. 2021 May 17. doi: 10.1002/ehf2.13419. Epub ahead of print. PMID: 34002482. *shared first authors

(2019 Journal Impact Factor: 3.902)

Contribution in detail:

- Participated in project conception (and design of selected experiments)
- Performed echocardiographic examinations including data analysis
- Aided in conducting hemodynamic measurements
- Performed organ removal, gravimetry and blood sampling
- Helped with isolation of cardiomyocytes
- Conducted staining of isolated cardiomyocytes and subsequent ratiometric Ca^{2+} and contractility measurements
- Carried out analysis of cellular data
- WGA-staining of cardiomyocytes
- Analysis of histological specimens
- Preparation of the initial manuscript and it's revised version
- Statistical analysis and figure preparation (except for Fig. 5)

_____

Signature of doctoral candidate

III. Journal summary list „Cardiac and Cardiovascular Systems“ 2019

Journal Data Filtered By: **Selected JCR Year: 2019** Selected Editions: SCIE,SSCI Selected Categories: **"CARDIAC and CARDIOVASCULAR SYSTEMS"** Selected Category Scheme: WoS
Gesamtanzahl: 138 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	CIRCULATION	158,218	23.603	0.205020
2	EUROPEAN HEART JOURNAL	59,968	22.673	0.140620
3	JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY	101,927	20.589	0.190280
4	Nature Reviews Cardiology	7,100	20.260	0.021130
5	CIRCULATION RESEARCH	51,539	14.467	0.071470
6	JAMA Cardiology	4,740	12.794	0.030110
7	JACC-Cardiovascular Imaging	10,110	12.740	0.027550
8	BASIC RESEARCH IN CARDIOLOGY	4,704	11.981	0.006380
9	EUROPEAN JOURNAL OF HEART FAILURE	12,784	11.627	0.028700
10	JACC-Heart Failure	4,117	8.750	0.019180
11	JACC-Cardiovascular Interventions	11,371	8.432	0.037330
12	CARDIOVASCULAR RESEARCH	21,526	8.168	0.019950
13	JOURNAL OF HEART AND LUNG TRANSPLANTATION	12,465	7.865	0.028140
14	Cardiovascular Diabetology	6,179	7.332	0.011390
15	PROGRESS IN CARDIOVASCULAR DISEASES	4,193	6.763	0.008340
16	European Heart Journal-Cardiovascular Pharmacotherapy	521	6.696	0.001640
17	Circulation-Heart Failure	6,773	6.033	0.018490
18	European Journal of Preventive Cardiology	5,589	5.864	0.015370
19	HEART RHYTHM	12,246	5.731	0.028620
20	Circulation-Cardiovascular Imaging	5,574	5.691	0.016320

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
21	JOURNAL OF THE AMERICAN SOCIETY OF ECHOCARDIOGRAPHY	11,347	5.508	0.018230
22	Circulation-Cardiovascular Interventions	5,012	5.493	0.018140
23	JOURNAL OF CARDIOVASCULAR MAGNETIC RESONANCE	5,205	5.361	0.011120
24	Clinical Research in Cardiology	3,321	5.268	0.007280
25	HEART	18,108	5.213	0.030140
26	Circulation-Cardiovascular Quality and Outcomes	4,728	5.071	0.014350
27	CANADIAN JOURNAL OF CARDIOLOGY	6,980	5.000	0.017630
28	European Heart Journal-Cardiovascular Imaging	6,359	4.841	0.023110
29	TRENDS IN CARDIOVASCULAR MEDICINE	2,695	4.755	0.003920
30	REVISTA ESPANOLA DE CARDIOLOGIA	3,672	4.642	0.004610
31	Journal of the American Heart Association	17,149	4.605	0.070620
32	Circulation-Cardiovascular Genetics	3,090	4.534	0.008600
33	JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY	28,491	4.451	0.034300
34	Circulation-Arrhythmia and Electrophysiology	6,344	4.393	0.016630
35	AMERICAN HEART JOURNAL	19,814	4.153	0.026810
36	JOURNAL OF MOLECULAR AND CELLULAR CARDIOLOGY	14,031	4.133	0.017960
37	CARDIOVASCULAR DRUGS AND THERAPY	2,114	4.069	0.003340
38	Circulation-Genomic and Precision Medicine	375	4.063	0.002220
39	Hellenic Journal of Cardiology	987	4.047	0.001000
40	EUROPACE	9,973	4.045	0.024750

Selected JCR Year: 2019; Selected Categories: "CARDIAC and CARDIOVASCULAR SYSTEMS"

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
41	EuroIntervention	5,542	3.993	0.016590
42	ATHEROSCLEROSIS	24,587	3.919	0.036590
43	Frontiers in Cardiovascular Medicine	1,303	3.915	0.004020
44	ESC Heart Failure	1,276	3.902	0.004120
45	AMERICAN JOURNAL OF PHYSIOLOGY-HEART AND CIRCULATORY PHYSIOLOGY	26,114	3.864	0.020400
46	Global Heart	1,074	3.862	0.003180
47	European Heart Journal-Acute Cardiovascular Care	1,555	3.813	0.005430
48	NUTRITION METABOLISM AND CARDIOVASCULAR DISEASES	6,026	3.700	0.008820
49	ANNALS OF THORACIC SURGERY	35,221	3.639	0.040380
50	HEART FAILURE REVIEWS	2,697	3.538	0.005130
51	EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY	16,682	3.486	0.025820
52	JOURNAL OF CARDIAC FAILURE	4,983	3.435	0.008730
53	JOURNAL OF NUCLEAR CARDIOLOGY	3,600	3.366	0.004570
54	Journal of Cardiovascular Translational Research	1,656	3.312	0.003140
55	INTERNATIONAL JOURNAL OF CARDIOLOGY	31,193	3.229	0.068160
56	RESPIRATORY MEDICINE	11,934	3.095	0.013490
57	Annals of Cardiothoracic Surgery	1,828	3.058	0.005060
58	CURRENT PROBLEMS IN CARDIOLOGY	567	2.966	0.000740
59	Journal of Cardiovascular Computed Tomography	1,809	2.892	0.004850
60	American Journal of Cardiovascular Drugs	1,063	2.674	0.001580

ESC Heart Failure (Rank 44/138; 68.48th Percentile)

3

Selected JCR Year: 2019; Selected Categories: "CARDIAC and CARDIOVASCULAR SYSTEMS"

IV. Publication: Hegemann et al., (2021) *“Right-ventricular dysfunction in HFpEF is linked to altered cardiomyocyte Ca2+ homeostasis and myofilament sensitivity”*

Hegemann N, Primessnig U, Bode D, et al. Right-ventricular dysfunction in HFpEF is linked to altered cardiomyocyte Ca2+ homeostasis and myofilament sensitivity. ESC Heart Fail. 2021;8(4):3130-3144. doi:10.1002/ehf2.13419

https://doi.org/10.1002/ehf2.13419

V. Curriculum vitae

Due to reasons of data protection, my curriculum vitae is not published in the electronic version of my thesis.

VI. List of publications (1)

Ott C, Pappritz K, **Hegemann N**, John C, Jeuthe S, McAlpine CS, Iwamoto Y, Lauryn JH, Klages J, Klopfleisch R, Van Linthout S, Swirski F, Nahrendorf M, Kintscher U, Grune T, Kuebler WM, Grune J. Spontaneous Degenerative Aortic Valve Disease in New Zealand Obese Mice. J Am Heart Assoc. 2021 Dec 7;10(23):e023131. doi: 10.1161/JAHA.121.023131. Epub 2021 Nov 15. PMID: 34779224. (Impact Factor: 5.50)

Lerchbaumer MH, Lauryn JH, Bachmann U, Enghard P, Fischer T, Grune J, **Hegemann N**, Khadzhynov D, Kruse JM, Lehner LJ, Lindner T, Oezkan T, Zickler D, Kuebler WM, Hamm B, Eckardt KU, Muench F. Point-of-care lung ultrasound in COVID-19 patients: inter- and intra-observer agreement in a prospective observational study. Sci Rep. 2021 May 21;11(1):10678. doi: 10.1038/s41598-021-90153-2. PMID: 34021219; PMCID: PMC8139973. (Impact Factor: 4.37)

Hegemann N, Primessnig U, Bode D, Wakula P, Beindorff N, Klopfleisch R, Michalick L, Grune J, Hohendanner F, Messroghli D, Pieske B, Kuebler WM, Heinzel FR. Right-ventricular dysfunction in HFpEF is linked to altered cardiomyocyte Ca2+ homeostasis and myofilament sensitivity. ESC Heart Fail. 2021 Aug;8(4):3130-3144. doi: 10.1002/ehf2.13419. Epub 2021 May 17. PMID: 34002482; PMCID: PMC8318431. (Impact Factor: 3.90)

Bode D, Rolim NPL, Guthof T, **Hegemann N**, Wakula P, Primessnig U, Berre AMO, Adams V, Wisløff U, Pieske BM, Heinzel FR, Hohendanner F; OptimEx Study Group. Effects of different exercise modalities on cardiac dysfunction in heart failure with preserved ejection fraction. ESC Heart Fail. 2021 Mar 25. doi: 10.1002/ehf2.13308. Epub ahead of print. PMID: 33768692. (Impact Factor: 3.90)

Bode D, Semmler L, Wakula P, **Hegemann N**, Primessnig U, Beindorff N, Powell D, Dahmen R, Ruetten H, Oeing C, Alogna A, Messroghli D, Pieske BM, Heinzel FR, Hohendanner F. Dual SGLT-1 and SGLT-2 inhibition improves left atrial dysfunction in HFpEF. Cardiovasc Diabetol. 2021 Jan 7;20(1):7. doi: 10.1186/s12933-020-01208-z. PMID: 33413413; PMCID: PMC7792219. (Impact Factor: 9.95)

Heinzel FR, **Hegemann N**, Hohendanner F, Primessnig U, Grune J, Blaschke F, de Boer RA, Pieske B, Schiattarella GG, Kuebler WM. Left ventricular dysfunction in heart failure with preserved ejection fraction-molecular mechanisms and impact on right ventricular function. Cardiovasc Diagn Ther. 2020 Oct;10(5):1541-1560. doi: 10.21037/cdt-20-477. PMID: 33224773; PMCID: PMC7666919. (Impact Factor: 2.61)

Bode D, Wen Y, **Hegemann N**, Primessnig U, Parwani A, Boldt LH, M Pieske B, R Heinzel F, Hohendanner F. Oxidative Stress and Inflammatory Modulation of Ca^{2+} Handling in Metabolic HFpEF-Related Left Atrial Cardiomyopathy. Antioxidants (Basel). 2020 Sep 14;9(9):860. doi: 10.3390/antiox9090860. PMID: 32937823; PMCID: PMC7555173. (Impact Factor: 5.13)

VI. List of publications (2)

Pappritz K, Grune J, Klein O, **Hegemann N**, Dong F, El-Shafeey M, Lin J, Kuebler WM, Kintscher U, Tschöpe C, Van Linthout S. Speckle-tracking echocardiography combined with imaging mass spectrometry assesses region-dependent alterations. Sci Rep. 2020 Feb 27;10(1):3629. doi: 10.1038/s41598-020-60594-2. PMID: 32108156; PMCID: PMC7046677. (Impact Factor: 3.99)

Grune J, Beyhoff N, **Hegemann N**, Lauryn JH, Kuebler WM. From bedside to bench: lung ultrasound for the assessment of pulmonary edema in animal models. Cell Tissue Res. 2020 May;380(2):379-392. doi: 10.1007/s00441-020-03172-2. Epub 2020 Feb 3. PMID: 32009189; PMCID: PMC7210222. (Impact Factor 4.04)

Bode D, Lindner D, Schwarzl M, Westermann D, Deissler P, Primessnig U, **Hegemann N**, Blatter LA, van Linthout S, Tschöpe C, Schoenrath F, Soltani S, Stamm C, Duesterhoeft V, Rolim N, Wisløff U, Knosalla C, Falk V, Pieske BM, Heinzel FR, Hohendanner F. The role of fibroblast - Cardiomyocyte interaction for atrial dysfunction in HFpEF and hypertensive heart disease. J Mol Cell Cardiol. 2019 Jun;131:53-65. doi: 10.1016/j.yjmcc.2019.04.016. Epub 2019 Apr 18. PMID: 31005484; PMCID: PMC7065048. (Impact Factor: 5.05)

VII. Acknowledgements

First and foremost, I would like to express my deep gratitude to my supervisor, Prof. Dr. Frank Heinzel, who gave me the opportunity to pursue this exciting project as part of his research group. My thanks extend to Uwe, David, Paulina and Brian who were also centrally involved in the project – not to forget my fellow students Peter, Jan and Linden.

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Also a shout-out to my fellow hockey enthusiasts from Neukölln – there was literally no better way to clear my head than having practice with you after being in the lab all day (of course with the obligatory beer afterwards).

Finally, I would like to dedicate this thesis to my mother. You taught me what perseverance is and got me to where I am today.