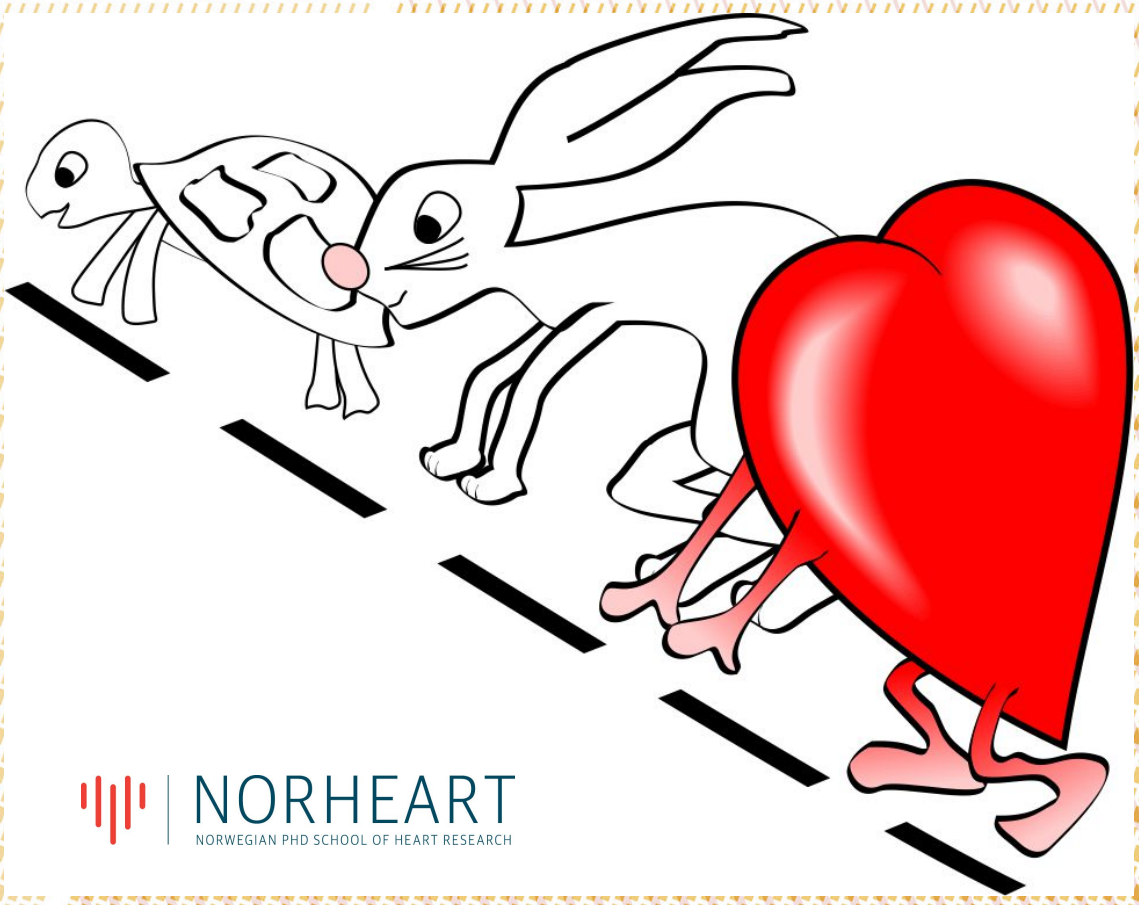
Faculty of Health Sciences
Department of Medical Biology

Novel inotropic strategies for treating acute heart failure: A large animal study on cardiac function and energetics

Jens Petter Bakkehaug

A dissertation for the degree of Philosophiae Doctor – May 2016



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Contents

1. Acknowledgements

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2. List of papers

1. Bakkehaug JP, Næsheim T, Engstad ET, Kildal AB, Myrmel T, How OJ. Reversing dobutamine induced tachycardia using ivabradine increases stroke volume with neutral effect on cardiac energetics in left ventricular postischemia dysfunction. *Acta Physiol (oxf)* 2016; In press. E-pub:2016 May 4. doi: 10.1111/apha.12704.

2. Bakkehaug JP, Kildal AB, Engstad ET, Boardman N, Næsheim T, Rønning L, Aasum E, Larsen TS, Myrmel T, How OJ. Myosin activator omecamtiv mecarbil increases myocardial oxygen consumption and impairs cardiac efficiency mediated by resting myosin ATPase activity. *Circ Heart Fail*. 2015;8:766–775.

2.1 Letter to the editor for paper 2

Teerlink JR, Malik FI, Kass DA. Letter by Teerlink et al regarding article,"Myosin activator omecamtiv mecarbil increases myocardial oxygen consumption and impairs cardiac efficiency mediated by resting myosin ATPase activity". *Circ Heart Fail* 2015;8:1141.

2.2 Response to the letter regarding paper 2

Bakkehaug JP, Kildal AB, Engstad ET, Boardman N, Næsheim T, Rønning L, Aasum E, Larsen TS, Myrmel T, How OJ. Response to letter regarding article, "Myosin activator omecamtiv mecarbil increases myocardial oxygen consumption and impairs cardiac efficiency mediated by resting myosin ATPase activity". *Circ Heart Fail* 2015;8:1142.

3. Rønning L, Bakkehaug JP, Rødland L, Kildal AB, Myrmel T, How OJ. Dobutamine plus ivabradine, but not omecamtiv alone, partially restores diastolic dysfunction by increasing lusitropy and filling time in the ischemic pig heart. Manuscript.

3. Abbreviations

ACS	Acute coronary syndrome
AHF	Acute heart failure
BDM	2,3-Butanedione monoxide
CBF	Coronary blood flow
CE	Cardiac efficiency
CHF	Chronic heart failure
CME	Coronary microembolization
CO	Cardiac output
CS	Cardiogenic shock
Cx	Circumflex coronary artery
DFT	Diastolic filling time
dP/dt_{max}	Peak positive derivative of LV pressure
dP/dt_{min}	Peak negative derivative of LV pressure
ECC	Excitation-contraction coupling
ECMO	Extracorporeal membrane oxygenation
EDV	End-diastolic volume
EDP	End-diastolic pressure
EDPVR	End-diastolic pressure volume relationship
EF	Ejection fraction
E_{es}	End-systolic elastance, slope of the ESPVR
ESV	End-systolic volume
ESPVR	End-systolic pressure volume relationship
ESP	End-systolic pressure
FFA	Free fatty acids

HF	Heart failure
HR	Heart rate
LAP	Left atrial pressure
LAD	Left anterior descending coronary artery
LV	Left ventricle
L V-A_{INTG}	Area under the curve of the negative ventriculo-atrial pressure gradient during diastole
L V-A_{MAX}	Maximal pressure difference between left atrium and left ventricle during diastole
MAP	Mean arterial pressure
MPAP	Mean pulmonary arterial pressure
MBF	Myocardial blood flow
MVO_2	Myocardial oxygen consumption
OM	Omecamtiv mecarbil
PA	Pulmonary artery
PCI	Percutaneous coronary intervention
PVA	Pressure-volume area
PRSW	Preload-recruitable stroke work
RCT	Randomized controlled trial
SET	Systolic ejection time
SV	Stroke volume
SW	Stroke work
Tau	Time constant of isovolumetric relaxation
V_0	X–intercept of ESPVR

4. Introduction

Preface- clinical relevance
A woman (60 years old) with no prior history of heart disease arrives at the hospital with an acute ST-segment myocardial infarction. Prehospital thrombolysis is unable to reverse ST-segment elevations on the electrocardiogram. Six hours have passed since the first appearance of chest pain. She is confused, presents with mottled skin, and is immediately admitted to percutaneous coronary intervention (PCI) for revascularization of a coronary main stem occlusion. Post-revascularization, she is hemodynamically unstable, with accompanying signs of tissue hypoperfusion. In the ICU, a Swan-Ganz catheter is placed and measures a cardiac index of 1.8 L/min/m^2 and a left ventricular (LV) filling pressure of 22 mmHg. Ultrasound of the heart shows hypokinesisa/akinesisa in the lateral and anterior wall of the LV. She is intubated and put on a respirator because of increasing confusion and respiratory distress. To increase cardiac output (CO), dobutamine infusion is increased stepwise in addition to employment of noradrenaline to achieve a mean arterial pressure (MAP) above 60 mmHg. However, CO is still inadequate, with no resolution of her metabolic acidosis. Additionally, a progressive sinus tachycardia of 130 bpm has become a major concern, as the short diastolic time severely impairs ventricular filling and coronary perfusion. How should this patient be treated?

4.1 Acute heart failure

4.1.1 Epidemiology of HF

During the past 50 years, the advances in the prevention, diagnosis, and management of cardiovascular disease (CVD) have been tremendous. Age-adjusted CVD-related deaths have declined by approximately two-thirds in industrialized nations[1]. There has also been a dramatic reduction in mortality rates associated with acute coronary syndrome (ACS)[2], valvular and congenital heart disease, uncontrolled hypertension, and many arrhythmias.
Heart failure (HF) stands out as an exception to this positive trend. HF is the leading cause of hospitalization in patients>65 years old[3]. In Europe, chronic HF has a prevalence of 1-2% (10%>60 years). Although the age-adjusted death rate has declined [4,5] and the mean age of death

from HF has risen during the last decades, the 5-year mortality is still approximately 50%, comparable to that of colorectal cancer [6]. Temporal trends suggest a stable or perhaps decreasing incidence of HF over the last decades[7].

Patients with acute HF (AHF), as opposed to patients with chronic heart failure (CHF), are unstable and need urgent interventions and/or treatment. According to the European Society of Cardiology (ESC) guidelines, AHF can be defined as follows[8]: "The rapid onset of symptoms and signs secondary to abnormal cardiac function. The cardiac dysfunction can be related to systolic or diastolic dysfunction, to abnormalities in cardiac rhythm, or to preload and afterload mismatch. It is often life-threatening and requires urgent treatment" [8].

AHF can be divided into 6 distinct clinical syndromes:

- Acute decompensated HF
- Hypertensive AHF
- Pulmonary oedema
- Cardiogenic shock (CS)
- High output failure
- Right HF

It is evident that patients present with a large clinical spectrum of symptoms, signs and associated prognoses. ACS is the dominant cause of both acute decompensated CHF and de novo AHF[9]. Other common aetiologies include valvular pathology, arrhythmias, viral myopathies, endocrine myopathies, and others. Patients who develop AHF have either acute decompensated chronic HF [10](2/3) or de novo AHF[11](1/3). Overall, the 1-year mortality after hospitalization for AHF ranges from 25 to 35%[12]. Patients with de novo AHF have significantly better survival than those with decompensated CHF. The in-hospital mortality ranges from 2 to 40%[10] depending on the clinical subtype of HF. Patients suffering from CS have the worst prognosis. Hermansen et al.[13] showed that CS was present in 23% of patients with AHF hospitalized at UNN Tromsø, with a related mortality of 46%. Importantly, 2-year survival for hospital survivors is the same for CS and less severe AHF, thus motivating efforts to improve AHF prognosis by optimizing initial supportive treatment.

The presenting symptoms of AHF include breathlessness, fatigue, and tiredness and are accompanied by the following signs: tachycardia, tachypnoea, pulmonary rales, pleural effusion, hepatomegaly, and peripheral oedema. Oliguria, hypotension and hyperlactataemia occur when the condition evolves into CS.

4.1.2 Pathophysiology of ACS-induced HF

In 1935, Tennant and Wiggers published a study showing that coronary artery occlusion leads to systolic left ventricular (LV) dysfunction [14]. Oxygen was identified as the link between myocardial blood flow (MBF) and systolic function. Later, Ross Jr [15] performed several studies further exploring the close correlation between changes in systolic wall thickening and decreases in sub-endocardial blood flow, termed perfusion-contraction matching. The major determinants of myocardial oxygen demand are heart rate (HR), wall stress, and LV contractility[16]. Wall stress is directly related to systolic blood pressure and LV diameter and inversely related to wall thickness. At rest, the oxygen extraction of the heart is 75% compared to total body extraction of approximately 30%. Because of its limited ability to further increase oxygen extraction, the myocardium depends on changes in MBF to increase oxygen supply. In exercising healthy individuals, the MBF can increase 5-fold[17]. Imbalance in the oxygen supply and demand of the myocardium may lead to ischaemia.

Reduced LV contractility is a hallmark of LV ischaemia/postischaemia[18] and is an important predictor of mortality after myocardial ischaemia[19]. Contractility describes the intrinsic force and velocity of myocardial contraction (inotropic state) independent of HR and loading conditions. In the acute phase, with ongoing ischaemia and acidosis, Ca^{2+} transients and Ca^{2+} sensitivity of the myofilaments are reduced[20]. The resultant reduction in contractility is evident as decreases in preload-recruitable stroke work (PRSW),with a concomitant increase in the end-systolic volume (ESV) and a decrease in the LV ejection fraction (EF)[18].

Diastolic function is dependent on Ca^{2+} reuptake at the sarcomere, which is reduced in ischaemia. This diastolic dysfunction is displayed as incomplete or delayed early relaxation (Tau, dP/dt_{min}) or acute stiffening of the ventricle, causing late diastolic constraint[21].

The potential for regeneration of cardiac function is primarily dependent on timely reperfusion[22]. Furthermore, a reduction in LV function of unknown magnitude is not directly accounted for by myocardial necrosis and is not relieved by reperfusion. This condition, initially termed “myocardial stunning”[23], is reversible within days and is caused by metabolic abnormalities, oxidative stress, Ca^{2+} overload and oedema[24]. Therefore, treatment of acute ischaemic HF depends on timely reperfusion of obstructed coronary vessels in combination with haemodynamic support to secure optimal recovery of the post-ischaemic LV dysfunction.

The acute reduction of LV function initiates acute adaptive and compensating mechanisms (Figure 1) mediated by intravascular baroreceptors. Among the most important compensating

mechanisms is activation of the sympathetic system (α– and β-adrenergic receptors) and renin-angiotensin-aldosterone system (RAAS), resulting in fluid retention, vasoconstriction, increased contractility and tachycardia.

Continuous neurohormonal activation, oxidative stress, inflammation and haemodynamic abnormalities follow the acute phase. Over weeks and months, this myriad of mechanisms together lead to remodelling of the heart, with myocardial damage and fibrosis[25]. In addition, renal function is compromised to varying degrees. There is dilatation of the LV with increases in ESV, EDV and left atrial (LA) diastolic pressure. Stroke volume (SV) can be sustained at the expense of increased filling pressures and wall tension. When this situation is stable, the patient has compensated chronic HF. Over time, the remodelling further reduces LV compliance, with a resulting increase in LV filling pressure[26]. Reduced renal function contributes to fluid and salt retention. At one point, the condition rapidly deteriorates, with signs of acute decompensated HF or pulmonary oedema; at worst, CS occurs.

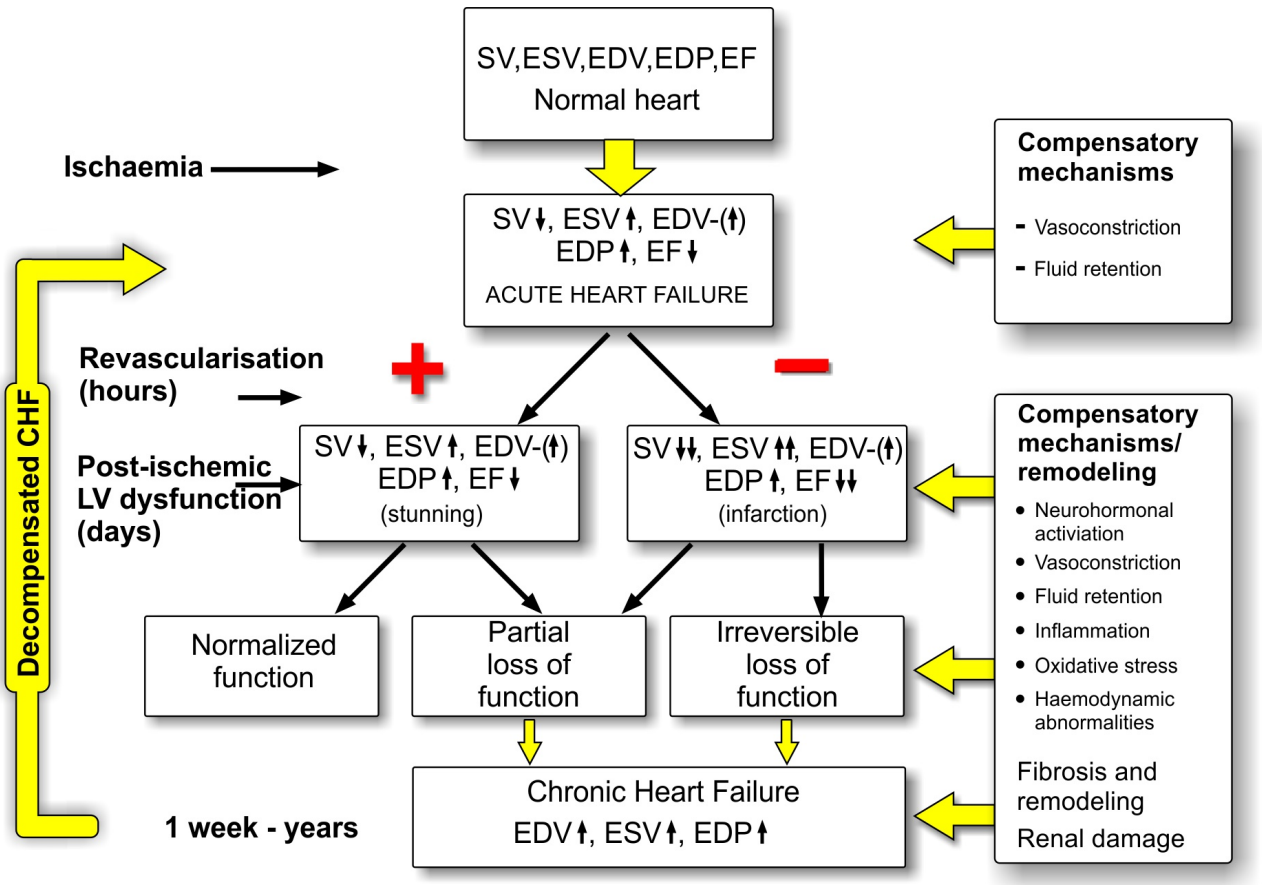

Figure 1. Development of acute and chronic ischaemic heart failure. Schematic illustration of the development of ischaemic acute and chronic heart failure. SV, stroke volume; ESV, end-systolic volume; EDV, end-diastolic volume; EDP, end-diastolic pressure; EF, ejection fraction; CHF, chronic heart failure.

4.1.3 Treatment of AHF

In the initial phase, correction of the precipitating cause is the first priority. Timely reperfusion is the main predictor of mortality after acute coronary occlusion[27]. Another important treatment goal is hypertension and arrhythmia management.

After immediate management, the treatment principle is reduction of the LV pressure-volume (PV) load. Unloading improves haemodynamics and symptoms. First-line treatment is the use of fluid removal (loop diuretics) and/or vasodilatation (nitrogen monoxide-donors) to decrease pre- and afterload, with concomitant reductions in wall tension, LV filling pressures and oxygen consumption. To counteract severely depressed LV function, administration of volume and/or inotropic agents may be indicated. Patients with AHF who respond haemodynamically to unloading with reduced ESV and EDV have a reduced risk of mortality[28].

After these patients are stabilized, they are considered to have CHF and are treated according to current guidelines with neurohormonal inhibition (β-blockers and ACE-antagonists)[29]. This therapy is sufficient in patients with moderate AHF and in the majority of patients with decompensated CHF. However, neuroendocrine inhibition is potentially harmful in unstable patients[30].

In low-output states with signs of organ hypoperfusion (such as CS), inotropes should be used[29]. Reports have indicated the use of inotropes in 10-39% of all admissions due to AHF[11,31]. The first-line inotropic drug is dobutamine[32,29]. Intracellular effects of catecholamines are discussed in paper 3. In brief, at myocytes, dobutamine acts via β-adrenergic receptors to greatly enhance the transient amplitude of cytosolic Ca^{2+} (inotropy) and to speed up sarcoplasmic reticulum (SR) Ca^{2+} reuptake (lusitropy)[20] (Figure 2). The Ca^{2+} fluxes are haemodynamically mirrored in the whole heart by indexes such as dP/dt_{min} and dP/dt_{max}. In principal, dobutamine reverses some of the altered Ca^{2+} fluxes and counteracts the reduced myofilament Ca^{2+} sensitivity caused by ischaemia[20]. Side effects of dobutamine include tachycardia, hypermetabolism, apoptosis[33], and a debated dose-dependent oxygen waste caused by increased Ca^{2+} handling[34]. These side effects have motivated researchers to develop alternative inotropes that do not alter intracellular Ca^{2+} levels, such as Ca^{2+} sensitizers and myosin activators.

A novel synthetic cardiac inotrope with a unique mechanism of action, omecamtiv mecarbil (OM), is presently in phase 2 of clinical research. This inotrope is classified as a myosin activator and was discovered through high-throughput screening with a cardiac myosin ATPase bioassay [35]. OM increases the LV EF by enhancing the systolic ejection time (SET) and sarcomere

shortening. Analogously, this enhancement has been described as "more hands pulling on the rope" [36]. The intracellular effects of OM are discussed in paper 3. Briefly, this compound accelerates the transition from weakly to strongly bound actin-myosin, which is the rate-limiting step in actin-myosin crossbridge formation (Figure 2). At cardiomyocytes, this enhancement is displayed as atypical inotropic action with a negative treppe effect (force-frequency relationship) and offset in the relationship between intracellular calcium amplitude and sarcomere shortening [37]. This compound has been identified as a potential strengthener of cardiac muscle and is presently being tested in a phase 2 study of chronic HF[38]. By acting directly on myosin ATPase, Ca^{2+} handling is not affected. In contrast, increasing myosin ATPase activity and reducing time for diastole may come at a cost.

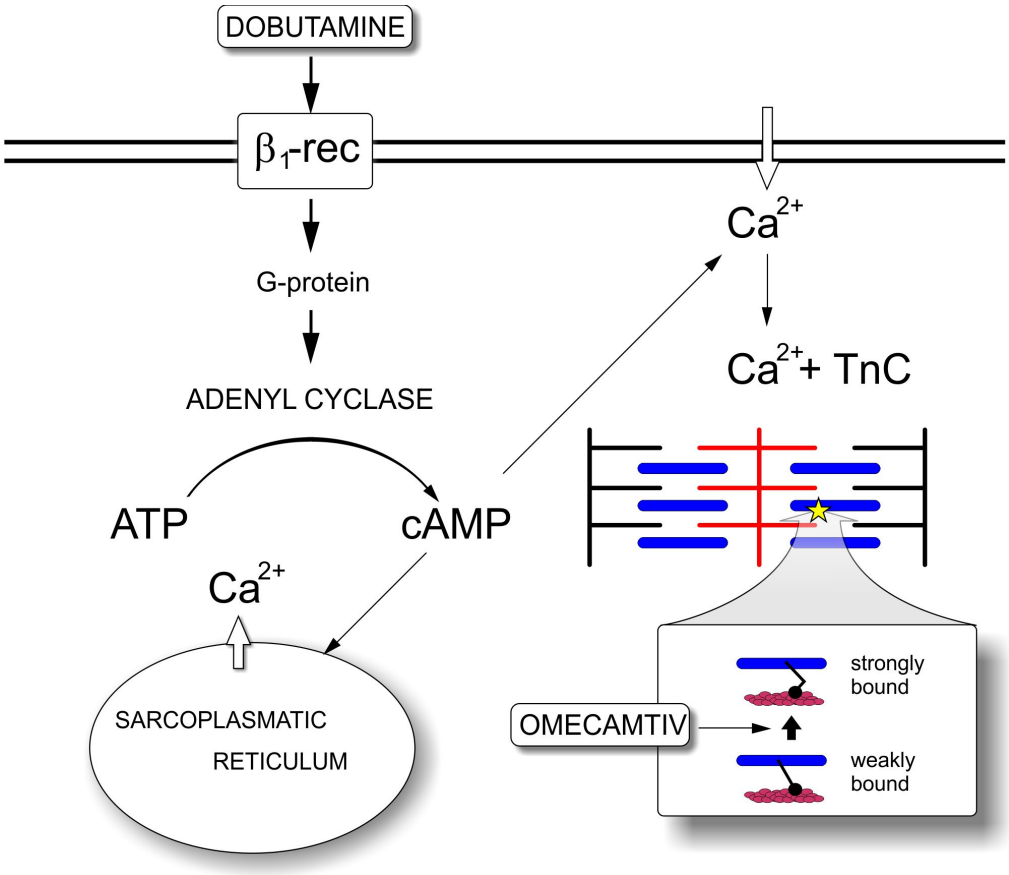

Figure 2. Simplified schematic illustration of intracellular actions of β-adrenergic agonists and omecamtiv mecarbil (OM) in cardiac myocytes. β-Receptor stimulation, through a stimulatory G-protein unit, activates the adenyl cyclase system, which results in increased concentrations of cAMP. cAMP further activates membrane-bound Ca^{2+} channels and releases Ca^{2+} from the SR, which leads to Ca^{2+}-mediated positive inotropy by increasing the contractility of the actin-myosin-troponin system, and enhanced chronotropic response.
OM binds directly to myosin ATPase and accelerates the transition from weakly bound to strongly bound actin-myosin.

4.1.4 Controlling HR in AHF

A heart cycle consists of isovolumetric contraction, systolic ejection time (SET), isovolumetric relaxation, and diastolic filling time (DFT) (Figure 3). With decreasing RR intervals, DFTs are relatively shorter than SETs[39,40].

Tachycardia is a double-edged sword in the pathogenesis and treatment of AHF. Acute and chronic neuroendocrine compensation causes tachycardia that compensates for a loss of SV at the expense of increased myocardial oxygen consumption (MVO_2) and reduced DFT. To maintain SV in this setting, the shortened diastole implies enhanced filling rates. Normally, in physiological tachycardia (i.e., exercise), the ventricle compresses the myocardium in the preceding systole beyond the resting shape, generating restoring forces that are released in diastole. The ventricle recoils to its passive shape and sucks blood into the cavity [41]. This phenomenon, termed diastolic suction, is dependent on contractility. Consequently, acute ischaemia is believed to reduce suction. Therefore, tachycardia may induce decompensation due to diastolic suction-HR mismatch.

HR reduction alone may be beneficial in both chronic HF and AHF[42,43], as this may reduce myocardial oxygen demand and the risk of sub-endocardial ischaemia[44]. β-Antagonists have proven to be fundamental in the treatment of CHF[45]. Several mechanisms are responsible for their beneficial effect; however, the negative inotrope effect accompanying β-blockers limits their use to haemodynamically stable AHF patients with reduced EF[29,46].

A pharmacological intervention that separates inotropic and chronotropic responses has not been available until the recent development of ivabradine. This funny channel (I_f) antagonist is approved for clinical use in patients with stable angina and chronic HF[29]. Ivabradine acts by inhibiting the I_f channels in the sinoatrial node, thereby decelerating the spontaneous depolarization of pacemaker cells[47] and leading to a lower HR. In acute myocardial ischaemia, attenuating tachycardia by a selective negative chronotrope (ivabradine) has beneficial effects beyond reduced total oxygen demand, including an increased coronary perfusion time, reduced risk of sub-endocardial ischaemia[48], and reduced risk of major ventricular arrhythmias[49]. Notably, a recent publication by Kleinbongard et al.[50] indicated that the non-HR-related cardioprotective effects of ivabradine are probably caused by reduced ROS production. In addition, using ivabradine, we are now able to attenuate the chronotropic effect of β-adrenergic drugs without affecting their inotropy[51,52]. Recently, two clinical studies demonstrated promising outcomes of combined dobutamine-ivabradine treatment of severe AHF[53,54].

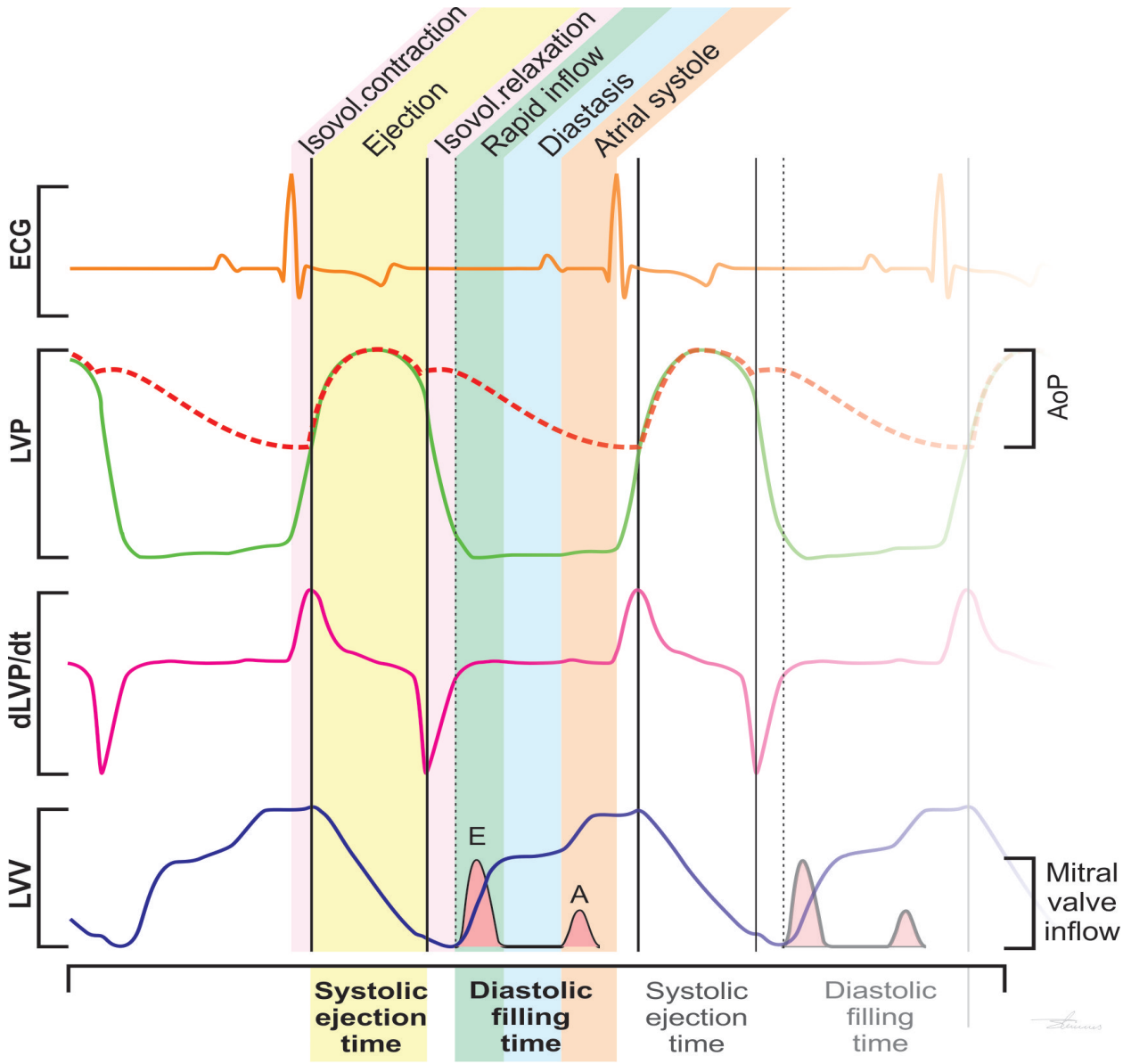

Figure 3. A modified Wiggers diagram (with permission, originally from[55]). dP/dt_{max} and dP/dt_{min} denote the peak positive and negative derivatives of LV pressure and are indices of contraction and early relaxation of the ventricle, respectively. The time constant of isovolumetric relaxation (Tau) is another index of early LV diastolic function. Tau is the estimated time between aortic valve closure and mitral valve opening as estimated by the method described by Weiss[56]. Systolic ejection time (SET) was defined in papers 1-3 as the time between dP/dt_{max} and dP/dt_{min} in pigs and between the minimum aortic pressure and the dicrotic notch in ex vivo mouse hearts. Diastolic filling time (DFT) was simplified defined as the cardiac cycle minus SET.

4.2 Cardiac function

4.2.1 Systolic function

No ideal index of contractility exists. CO, SV and EF are often applied in clinical practice despite load and HR dependency. Nevertheless, invasive techniques provide tools that are not available in clinical practice. The PV loop (Figure 4) and the first derivative of the LV ejection pressure over time (dP/dt) are common ways to describe systolic and diastolic properties. Unfortunately, these indices are also limited by load and HR dependency [57]. Two indexes that incorporate different loads are the preload recruitable stroke work (PRSW) and the end-systolic elastance, E_{es} (slope of the end-systolic pressure volume relation, ESPVR). The SW/EDV relationship is termed PRSW, and increased slope (M_w) indicates increased contractility. PRSW is relatively independent of load and HR[58]; however, one shortcoming is that EDV is determined by both systolic and diastolic properties. Obtaining PV loops at different abrupt load interventions and extrapolating a line through the ESPVR provide a slope termed E_{es} and an *x*-intercept named V_o (se Figure 5). In theory, an increase in slope with an unchanged V_o indicates increased contractility, while an increase in V_o with an unchanged slope represents reduced contractility. However, in vivo studies have proven the ESPVR to be curvilinear[57] and dependent on HR and afterload[59].

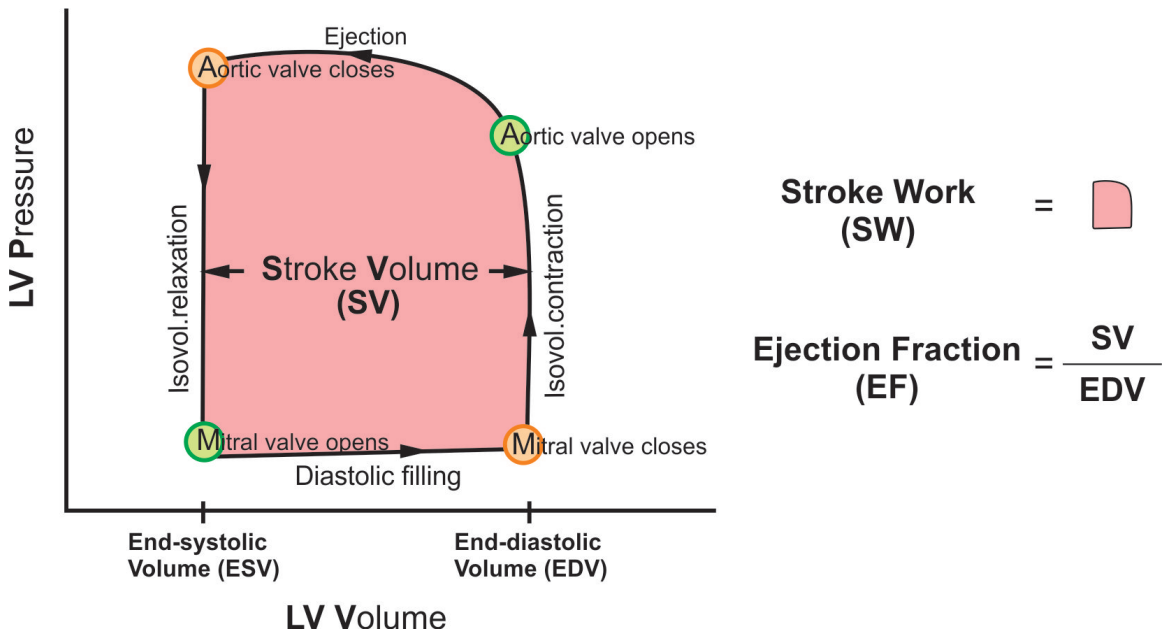

Figure 4. A typical pressure-volume (PV) loop showing the LV ejection phase indices SV, SW and EF derived from one heartbeat. To follow the events of one cardiac cycle, the loop shows the events of one cardiac cycle when followed counter-clockwise (with permission, originally from [55]).

4.2.2 Diastolic function

In myocardial ischaemia, microvascular dysfunction, cell necrosis and wall motion abnormalities impair active relaxation. Interstitial oedema and fibrocellular infiltration will further directly affect LV chamber stiffness and indirectly affect the diastolic filling of the heart.

The diastolic period can be subdivided into early diastole, active myocardial relaxation, and the passive process of late diastolic filling. dP/dt_{min}, which is the first derivative of isovolumetric relaxation[56], and Tau, which is the time constant of isovolumetric relaxation, describe early diastole. Tau is independent of preload. The strain rate of myocardial shortening also describes early diastolic function.

Late diastolic function can be described by the slope of the end-diastolic pressure volume relationship (EDPVR) (Figure 5). In addition, we can measure the driving force across the mitral valve in diastole. The maximum atrioventricular gradient ($L\ V\text{-}A_{Max}$) is the maximal pressure difference between the left atrium and the left ventricle during diastole, and the atrioventricular pressure gradient integral ($L\ V\text{-}A_{INTG}$) is the area under the curve of the negative pressure gradient during diastole. These two indices integrate early and late diastolic myocardial functions with loading conditions.

4.3 Cardiac efficiency

The heart maintains its pumping action by converting chemical energy in metabolic substrates into mechanical energy. As with any mechanical pump, only part of the energy invested is converted to external power. More than 50% of the energy is converted to heat, and the mechanical energy is split into energy used to develop ventricular pressure and energy used for external work (stroke work [SW]). The ratio of cardiac work to myocardial energy expenditure is termed cardiac efficiency (CE).

Myocardial oxygen consumption (MVO_2) is used as indirect calorimetry to measure total myocardial energy expenditure because the majority of ATP production in the normoxic myocardium is derived from oxidative phosphorylation (>90%)[60]. In addition, this method assumes a constant ratio between the different metabolic substrates throughout the experiment. The healthy heart switches between lipids and carbohydrates as energy substrates, depending on

availability. The efficiency of ATP production, which is expressed as the ratio of ATP to O_2 consumed (P:O), differs depending on the mix of substrates oxidized: the P:O is ~15% higher for oxidation of glucose only vs fatty acids only[61].

From studies of cardiac energetics, we have learned that failing hearts have a limited energy reserve and reduced efficiency[62]. An increase in cardiac work comes at a higher cost of contraction, with increased susceptibility to arrhythmia and ischaemic injury. Decreased fatty acid oxidation and increased reliance on glucose oxidation and glycolysis also occur[63]. The increase in glucose oxidation is regarded as adaptive because fatty acids are energetically less efficient compared to glucose.

Several indices are used to express cardiac work. SW is the product of LV pressure (P_{max}–P_{min}) and SV obtained at different steady state preload reductions and was first described by Bing & Hammond in 1949[64]. Under normal conditions, the SW/MVO_2 ratio is ≈25% and is termed external efficiency. Analyses of SW do not rely on absolute volumes (ESV and EDV) but are highly load dependent.

In Suga's 1979 publication[65], he introduced the concept of pressure volume area (PVA) and found PVA to be a good predictor of MVO_2, independent of contractile state and loading conditions. The ratio of PVA to MVO_2 is termed "total mechanical efficiency". His intention was to create an index that could differentiate between energy used for activation (unloaded heart) and contractility of the heart. In brief, PVA consists of the area bounded by the PV loop (SW) and the triangular area limited by the line of the ESPVR and EDPVR, as obtained by a transient vena cava occlusion (Figure 5). PVA is plotted against MVO_2 using the same unit (J/beat). PVA is calculated by the formula:

PVA= SW[ESP×(ESV-V_0)/2][EDP×(ESV-V_0)/4][65] where SW is calculated from the PV data and ESP and ESV are end-systolic pressure and volume, respectively; V_0 is the interpolated *x*-intercept of the quadratic-fitted ESPVR during steady state recordings; and EDP is end-diastolic pressure.

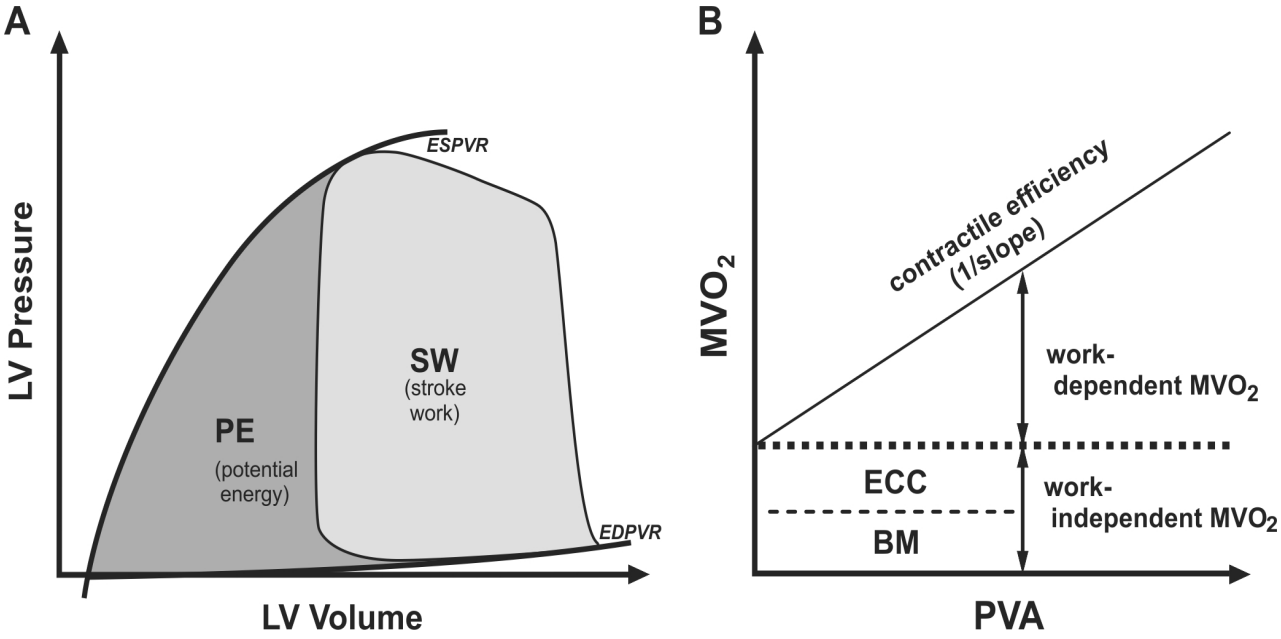

Figure 5. Pressure-volume area (PVA) concept. A: The PVA consists of the area of the PV loop (SW) and the triangular area (PE), which is limited by the ESPVR, EDPVR and the descending limb of the PV loop. B: The relationship between MVO_2 and PVA, which shows work-dependent and work-independent MVO_2. The inverse slope of the PVA-MVO_2 relationship is the contractile efficiency of the heart.

The *y*-intercept in the PVA-MVO_2 relation indicates the myocardial oxygen cost not related to pump function, also referred to as unloaded MVO_2, which is reported to be increased in several models of HF[66]. This work-independent MVO_2 defines the energy used for excitation-contraction coupling (ECC) and basal metabolism. The energy cost of ECC is primarily defined by Ca^{2+} handling by SR ATPase. Suga showed that both catecholamines and Ca^{2+} increased unloaded MVO_2 independent of the slope of the PVA-MVO_2 relation due to increased Ca^{2+} handling during ECC. The energy expenditure of the quiescent myocardium (BM; basal metabolism) can be assessed in KCL-arrested hearts. Korvald et al.[67] demonstrated that the PVA-MVO_2 relationship was affected by metabolic substrates without a change in contractility.

In contrast, the inverse slope (1/slope) of the PVA-MVO_2 relation describes myocardial efficiency (contractile efficiency) independent of basal metabolism and ECC. The work-dependent MVO_2 reflects the energy cost for the mechanical processes and includes the generation of myocardial force and pressure in the ventricular wall (PE) and ejection of blood against an afterload pressure (SW).

Total mechanical efficiency (PVA/MVO_2) is largely dependent on the size of the unloaded MVO_2 because the slope of the PVA-MVO_2 relation has been proven to be quite stable[68,65]. Importantly,

this concept was developed in an in vitro model of cross-circulated dog hearts[69]. Although this model was validated in vivo, some of the determining factors can only be extrapolated, not directly measured. V_o is extrapolated from ESPVR in vivo; thus, unloaded MVO_2 must be extrapolated from the PVA-MVO_2 relationship. Further complicating in vivo analysis is the fact that ESPVR in vivo is curvilinear[70]: it is convex in low-contractility states and concave in high-contractility states. Regardless of the limitations in vivo, the PVA-MVO_2 concept shows a close correlation between MVO_2 and PVA and enables a split between mechanical and non-mechanical energy. This framework is useful for describing changes in myocardial efficiency after interventions (i.e., drug treatment).

5. Aims of the studies

5.1 Overall aim

This thesis focused on investigating two new suggested treatment principles of AHF regarding cardiometabolic function (papers 1 and 2), LV systolic function (papers 1 and 3) and LV diastolic function (paper 3).

5.2 Paper 1

- We set out to investigate whether the inotropic and lucitropic effects of dobutamine were preserved when combined with ivabradine in a clinically relevant model of LV post-ischaemic dysfunction.
- We also assessed to what extent this co-treatment could restore SV and CO, in theory, by prolonging the diastolic time interval.
- Finally, we investigated whether adding ivabradine to dobutamine could improve CE in the post-ischaemic pig. CE was measured as the relation between MVO_2 and external cardiac work.

5.3 Paper 2

- Unlike catecholamines, OM reduces the diastolic time as a consequence of the increase in SET. Thus, the time for diastolic myocardial perfusion and filling are reduced[71] with OM.
- The aim of this study was to clarify the cardiac energetic and metabolic profiles of OM[35].
- In addition to healthy pigs, we used a clinically relevant pig model of post-ischaemic LV dysfunction[72], an ex vivo working mouse heart model without neurohumoral influence[73] and isolated mitochondria from mouse myocardium.
- Our hypothesis was that OM has a neutral effect on myocardial energy consumption, as the favourable effects of reduced wall stress potentially can be counteracted by the prolongation of systole through myosin ATPase activity.

5.4 Paper 3

- In this study, we performed a detailed invasive assessment of diastolic function in a pig model with severe LV ischaemia[74].
- Assessments were conducted at intrinsic HR and during pacing-induced tachycardia.
- Furthermore, we used this model to assess two novel inotropic principles to improve systolic unloading and its impact on diastolic function: the myosin activator OM versus a combination dobutamine and ivabradine (D+I) treatment.

6. Methodological considerations

6.1 The animal models

We used domestic castrated male pigs in all 3 studies (papers 1-3), and NMRI mice were used in papers 1 and 2. All in vivo experiments were conducted under general anaesthesia.

Considerable insight into the molecular and cellular basis of cardiovascular biology has come from small animal models, particularly mice (murine model). However, significant differences exist with regard to cardiac characteristics, such as HR, oxygen consumption and

adrenergic receptor ratios, as well as responses to a loss of regulatory proteins, when mice are compared to humans[75]. Compared to murine models, large animal models of AHF more closely approximate human anatomy, function and pathophysiology. Therefore, large animal models are instrumental in bridging discoveries from murine models into clinical practice[76].

6.1.1 The pig model in cardiovascular research

The phylogenetic development of humans and pigs as omnivorous and their accommodation to a sedentary lifestyle have produced similar metabolism and cardiovascular systems. These characteristics, together with similarities in size, structure and blood composition, make pigs suitable for performing in vivo cardiovascular experiments[77]. Still, some differences between humans and pigs need to be considered when interpreting results from porcine studies. Interestingly, based on our pig studies, we have noted significant discrepancies compared to humans regarding haemodynamic effects of vasoactive drugs. How et al.[74] found noradrenalin (100 ng/kg/min) to be a pure inotrope in pigs, whereas the same dose in humans is a vasopressor. In pigs, low-dose dobutamine (5 μg/kg/min) proved more potent as an inotrope compared to equivalent doses in humans (paper 1). The different expression and function profiles of β–receptors can explain some of these findings. The combination of comorbidity, prior chronic HF and age make patients with HF prone to desensitization of β-receptors in the myocardium[78]; in contrast, young healthy pigs have a hypersensitive autonomic system.

Coronary vessel anatomy is strikingly similar between pigs and humans, and the determinants of MVO_2 are closely related in both species[77]. However, low haemoglobin (7-9 g/dl)/haematocrit ratios in pigs lead to increased blood flow to tissues with high metabolic demand compared to humans[77]. One difficulty of using pigs is their predisposition to arrhythmias[76]. To minimize this, we applied strict control of ventilation, oxygenation, glucose levels and electrolyte levels and administered 5 mg/kg amiodarone prior to instrumentation. Hexamethonium (20 mg/kg) was used (paper 3) to minimize the impact of autonomic reflexes on repeated LV function measurements[79]. We chose an open-chest model[72] in all protocols to facilitate the surgical preparation of the heart. In papers 1 and 2, we placed flow probes on the three main coronary arteries and the pulmonary trunk and a catheter in the great cardiac vein via the coronary sinus (after ligating the hemiazygos vein). In papers 1-3, we installed sonomicrometry crystals on the epicardium for LV strain and dimension measurements. To measure the atrioventricular pressure

gradient in diastole, we inserted a fluid-filled manometer pressure catheter through a small thoracic incision into the left atrium (paper 3).

Large animal models continue to be a mainstay for drug and gene therapy development, for device development and for surgical procedure testing[80]. However, there is still a large gap between pre-clinical studies and clinical studies regarding the reproducibility of positive results. To increase rigor and reproducibility, Steven P Jones et al.[81] have suggested a new paradigm with a multicentre, randomized, controlled, clinical trial-like infrastructure for pre-clinical evaluation of cardioprotective strategies, which may bridge the gap between animal and human studies. This multicentre cooperation is now operative and generates reproducible results of cardioprotection studies using mice, rats and pigs as models.

6.1.2 Induction of LV HF in pigs

We applied two different strategies to induce LV dysfunction: ischaemia-reperfusion[72] (papers 1 and 2) and coronary microembolization (CME)[82] (paper 3). The ischaemia-reperfusion model induces moderate LV systolic post-ischaemic dysfunction (stunning) that is reversible after several hours[72]. ROS generation, together with Ca^{2+} overload and EC uncoupling, seem to be the most prominent[24] pathogenic factors. Repetitive ischaemia-reperfusion episodes are also a clinically relevant problem[83]. Since the 1980s, the era of revascularization has generated increased interest in stunning. The rationales to revascularize hypokinetic regions after coronary occlusions are based on the presumption that some, or all, of contractile dysfunction is caused by stunning, which is reversible after timely revascularization. Importantly, the stability of the model makes it feasible to perform reproducible energetic measurements (5-7 preload reductions per intervention)[72]. We also measured an increase in troponin release in this model, indicating a component of irreversible myocardial damage that may be related to direct cardiac compressions performed in ventricular fibrillation prior to DC conversion.

In paper 3, we aimed to generate a severe form of LV dysfunction with a significant increase in LA pressure. Based on previous results from our group[79,84], the CME model allow us to induce a severe stable LV failure to targets of 20 mmHg LA pressure and 30% reduction in SV, respectively. A review by Heusch et al. underpinned the potential clinical relevance of this model[82]. Post-mortem biopsy studies, experimental studies and clinical evidence have revealed

that CME is frequently a component of acute myocardial contractile dysfunction. CME occurs in approximately 25% of all PCI-procedures.

6.1.3 Ex vivo mouse hearts

In papers 1 and 2, we perfused ex vivo mouse hearts[73]. This ex vivo preparation provides a model for assessing cardiometabolic function under controlled loading conditions and is, to a large extent, free of the potentially confounding effects of anaesthesia and neurohumoral influences[85]. The isolated heart is denervated so that values of LV dP/dt_{max} are typically less than 50% of those found in anaesthetized preparations in vivo[85]. The hearts were initially retrograde perfused (Langendorff) with recycled Krebs-Henseleit bicarbonate buffer containing 10 mM glucose and 0.5% palmitate bound to 3% bovine serum albumin.

In the retrograde-perfused hearts used for estimating unloaded MVO_2 (papers 1-2), the ventricular cavity was vented by inserting a 25 G steel cannula through the apex of the heart, allowing drainage of any perfusate trapped in the LV lumen.

The working heart perfusions were used to assess haemodynamics (papers 1-2) and CE (paper 1), and radiolabelled isotopes[86] were used to assess myocardial glucose and fatty acid oxidation rates (paper 2). The left atrium was cannulated with a 16 G steel cannula connected to a preload reservoir to ensure forward perfusion through the aortic valve. Aortic and filling pressures were set to column heights of 55 and 12 mmHg, respectively. HR and temperature were set to fixed values.

In paper 2, we added the myosin ATPase inhibitor 2,3-butanedione monoxide (BDM) (Sigma Aldrich, USA) after basal MVO_2 measurements. The specificity of BDM as a myosin inhibitor is not known; some researchers have found that it also affects ECC [87]. However, in a study of rat myocytes where all membrane-bound ATPase activity was stripped away by Triton X-100 before exposure to BDM, researchers were able to show that 40% of the basal activity remained[88], suggesting that myosin ATPase has a large role in determining the unloaded MVO_2 of the basal metabolic rate.

6.2 Assessment of myocardial oxygen consumption in pigs

When assessing MVO_2; correct assessment of CBF is critical. We used transit–time flow probes (Medi-stim, Norway) placed on the stems of the three main coronary arteries. A flow computer reported continuous mean flow in ml/min.

The arteriovenous difference was determined by arterial and coronary venous samples from the aorta abdominalis and sinus coronarius/vena coronarius sinister, respectively. The vena hemiazygos fuses with vena coronarius sinister in pigs; therefore, we ligated the vena hemiazygos in all animals and introduced a catheter into the sinus coronarius/vena coronarius sinister for sampling.

In hearts with intact autoregulation, the oxygen saturation difference is quite stable, leaving blood flow as the main regulator of MVO_2. In paper 1, the LV coronary blood flow was estimated from the following formula: $LVCBF=CBF \cdot 0.7$ [89] where LVCBF and CBF are the LV and total coronary blood flow, respectively. The weight of the LV was calculated as 3.3 g LV weight/kg pig weight[90]. In paper 2, the LV CBF was calculated from the following formula: $LVCBF=CBF/W \cdot LVW$[89] where LVCBF and CBF are LV and total CBF, respectively. W and LVW are total myocardial and LV myocardial weight, respectively.

6.3 Cardiac volumetry

6.3.1 Available methods on LV volumetry

The ideal tool to evaluate LV volume should be non-invasive and accurate, with high reproducibility and easy application. For decades, two-dimensional (2D) echocardiography has been the main non-invasive imaging modality used to evaluate LV function in the clinical setting. However, there are many limitations to echocardiography. Intra- and inter-observer variations, inadvertent use of foreshortened views of the left ventricle and reliance on geometric modelling have reduced the accuracy and reproducibility of this method.

Three-dimensional (3D) echo is a promising tool to reduce the need for geometrical assumptions. This method depends on stable LV long-axis projections. Unfortunately, this projection is not available in pigs, as the apex is attached to the dorsal side of the distal sternum,

resulting in foreshortened views. In addition, the left lung is interposed between the oesophagus and the heart, making optimal imaging with TEE difficult.

Since the 1970s, cardiac magnetic resonance imaging (CMRI) has been considered the gold standard of cardiac volumetry. Cardiac computer tomography (CCT) is an alternative to CMRI; however, compared to CMRI, CCT overestimates EDVs and ESVs, resulting in a significant bias in the EF[91].

In our lab, it is not feasible to obtain the absolute volumes with CCT or CMRI. Still, the open–chest model allows us to use invasive techniques that are not available to clinicians. CO and SV can be measured with a time-transit flow probe on the pulmonary artery (Medi-stim, Norway). This method slightly underestimates CO because of bronchial veins draining distal to the probe. Nevertheless, flow probe measurement of CO is found to be more accurate than thermodilution techniques in pigs[92].

Unlike time-transit flow and thermodilution techniques, the conductance catheter technique enables real-time assessment of LV volume in vivo. This method employs a multi-electrode catheter that sets up a low-level current field within the ventricle, allowing measurement of time-varying electrical conductivity that changes proportionally with ventricular blood volume. The formula used to convert conductance to volume is as follows: $V(t)=1/\alpha \cdot \rho \cdot L^2 \cdot (G(t)-Gp)$[59] where V(t) is total intraventricular volume, α the slope factor relating conductance volume to an independent volume estimation, L is the inter-electrode distance, ρ is the blood resistivity, and G(t) and Gp are the sums of segmental conductances and the parallel conductance, respectively.

This method has several assumptions. Alpha correction was not critical to us because relative changes in volume would suffice in our model. However, changes in LV volume detected with conductance technique are vulnerable to changes in parallel volume. There are several caveats regarding parallel volume in this model. Both a slow increase in blood/fluid into the mediastinum, and dilatation of the right ventricle following ischaemia-reperfusion increase parallel volume, causing a leftward shift of LV volume (reduction in ESV). In addition, this conductance technique is very sensitive to the position of the LV catheter, in contrast to fixed crystals. Last, accurate assessments of conductance volumetry require multiple injections of hypertonic saline (assessment of parallel conductance) throughout the experiments. This solution ends up in the coronary arteries and transiently impairs cardiac function, a side effect that we wanted to avoid. Based on these shortcomings, we discarded the conductance catheter technique.

As an alternative, we applied sonomicrometry crystals (Sonometrics Corporation, trx 4, Canada) combined with 2D echo (Vivid I, GE, USA) and a geometrical model[93]. Sonomicrometry is the measurement of distance using ultrasound. Transit times of ultrasound between different

crystals are converted to distance (typically 1550 metres per second in biological material). Transit time is measured digitally, typically in steps of 15 nanoseconds, resulting in a resolution of 24 μm. This technique allows us to measure myocardial shortening in different axes with great accuracy, even at high heartrates; thus, we reduced the need for repeated 2D echo. A limitation of this approach is the dependency on geometrical models to convert distances into volumes.

To summarize, SV can be measured quite accurately by time–transit flow probes. In contrast, invasive measurement of absolute volumes is affected by a number of limitations. As a consequence, consistent measurement of relative volume changes was our main objective.

6.3.2 Different LV volumetric models using sonomicrometry

In papers 1-3, we used a combined LV pressure/volume catheter (Millar, Houston, TX, USA) to measure LV pressure. In paper 1, SW was used as a work index to avoid the uncertainties of absolute volume assessment. We used a combination of SVs from time-transit flow measurements on the pulmonary trunk, and sonometric measurements of long-axis movement based on a spherical model of LV. To obtain relative differences in dimensions throughout the experiments, the sonomicrometry crystals were calibrated to end-systolic and end-diastolic diameters (ESD, EDD) at baseline. ESD and EDD were estimated from epicardial echocardiograms (2-D short axis). In this simplistic model, we used ΔLV dimension as a surrogate for ΔLV volume.

In paper 2, we wanted to apply the PVA index of cardiac work and therefore needed a more accurate model of LV volumes to estimate the ESV, EDV and Vo. Thus, we improved the LV volumetric model by combining LV short- and long-axis sonomicrometry. At baseline and after interventions, the LV EDV was calculated from epicardial short-axis ultrasound data using Teicholz's formula $EDV = [7/(2.4+EDD_{endo})] \cdot (EDD_{endo}^{3})$. The ESV was calculated by subtracting SV (from a time-transit flow probe on the pulmonary artery) from the EDV. The short- and long-axis sonomicrometry crystals were converted to a composite output using the area-length (Bullet) formula [94]: $Volume = 5/6 \cdot A_{endo} \cdot L_{endo}$. The composite sonomicrometric output was calibrated against ESV and EDV at each intervention.

In paper 3, we further modified the LV volumetric model from paper 2. The dyskinesia/akinesia observed in HF induced by ischaemia-reperfusion (paper 1-2) are even more profound in the CME (paper 3). These segmental wall motion abnormalities are detected by short-axis (Cx) and anterior long-axis (LAD) sonometric input. To improve LV volumetric accuracy in

the CME model a prolate ellipsoid model by Zile ($V= \pi \cdot (EDD_{endo})^2/6 \cdot L_{endo}$)[95] was used. This formula has been validated in normally shaped and sized ventricles, whereas only the biplane (Simpsons) method has been validated in ventricles with segmental wall motion abnormalities[96]. Additionally, the sonometric signals were corrected to measurements from epicardial short-axis ultrasound data (Vivid i, GE) at baseline. The echocardiographic L_{endo} (endocardial length) was calculated from EDD_{endo} (endocardial diameter) at a ratio of 1.37[97]. The SV from the time-transit flow probe on the pulmonary artery was reported independent of the estimated sonometric volumes.

6.3.3 Assessment of preload-recruitable stroke work

PRSW is usually obtained by abrupt VCO. To plot SW versus EDV we need accurate beat-to-beat SV. This measurement cannot be obtained by the time-transit flow probe; therefore, we chose to use sonomicrometry instead. Thus, the uncertainties connected to LV volumetry also apply for PRSW.

However, in paper 1, the post-processing of data obtained by abrupt VCO was inadequate because of a low regression coefficient. Therefore, to evaluate PRSW, we used the sequential steady state measurements obtained by different preload reductions from the LV energetic assessment, which allowed us to use SV obtained from the time-transit flow probe.

To estimate PRSW in paper 3, we performed an abrupt VCO and used the volume from the conductance catheter because the sonomicrometric model used in steady state measurements seemed to be increasingly inaccurate with VCOs. We theorized that the large degree of LV conformational change associated with the VCO in the CME-model was not adequately reflected by the two sonomicrometric axes.

7. Summary of results

7.1 Paper 1

In this study, we investigated haemodynamic and energetic effects of D+I cotreatment in an acute pig model of post-ischaemic LV dysfunction. After 20 minutes of accumulated ischaemia, the HR (100 bpm) and ESD increased, while the external mechanical energy output (SW) and SV were reduced by 44% and 27% from baseline, respectively. After dobutamine infusion, a further increase in HR occurred (130 bpm), and ESD was reduced to baseline levels with unchanged SV. After ivabradine was added, we observed a reversal of the dobutamine-induced tachycardia (24% reduction). SW and SV increased significantly, by 23% and 20%, respectively, compared with dobutamine alone (Figure 6). There was no significant change in CO or MAP after adding ivabradine, and there was no significant change in LV function after adding ivabradine to dobutamine. Ivabradine had no impact on the SW-MVO_2 relations at a broad range of workloads, resulting in maintained CE[65] (Figure 7).

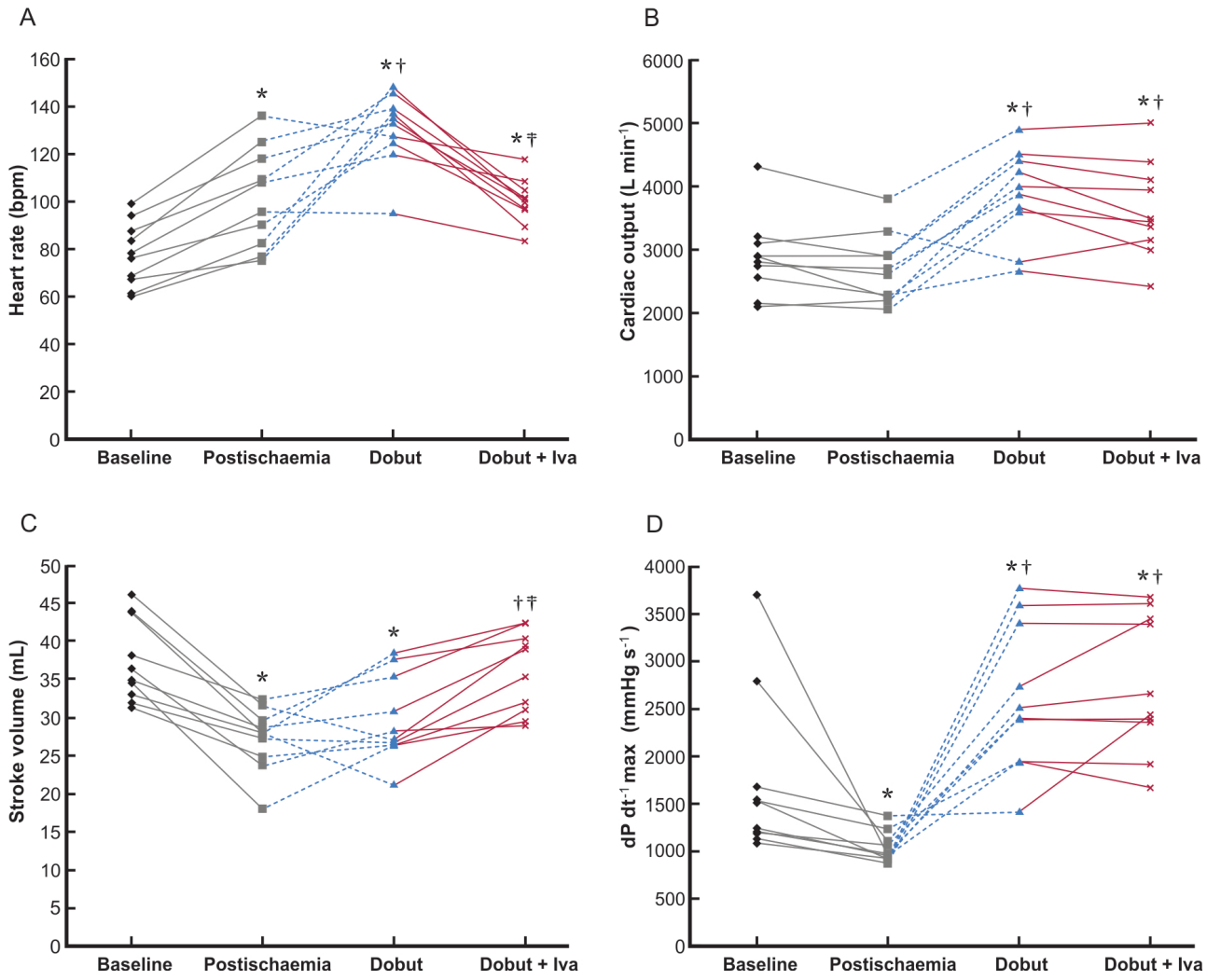

Figure 6. Individual data (n=10) of the selected main haemodynamic indices. The data are presented as connected dot-plots from 4 consecutive measurement periods: Baseline, post-ischaemia, dobutamine (dobut) and dobutamine+ivabradine (dobut+iva). Panel A, heart rate; Panel B, cardiac output; Panel C, stroke volume; Panel D, dP/dt_{max}. Between timepoint differences:* P<0.05 vs baseline,† P<0.05 vs post-ischaemia, ‡ P<0,05 vs dobutamine.

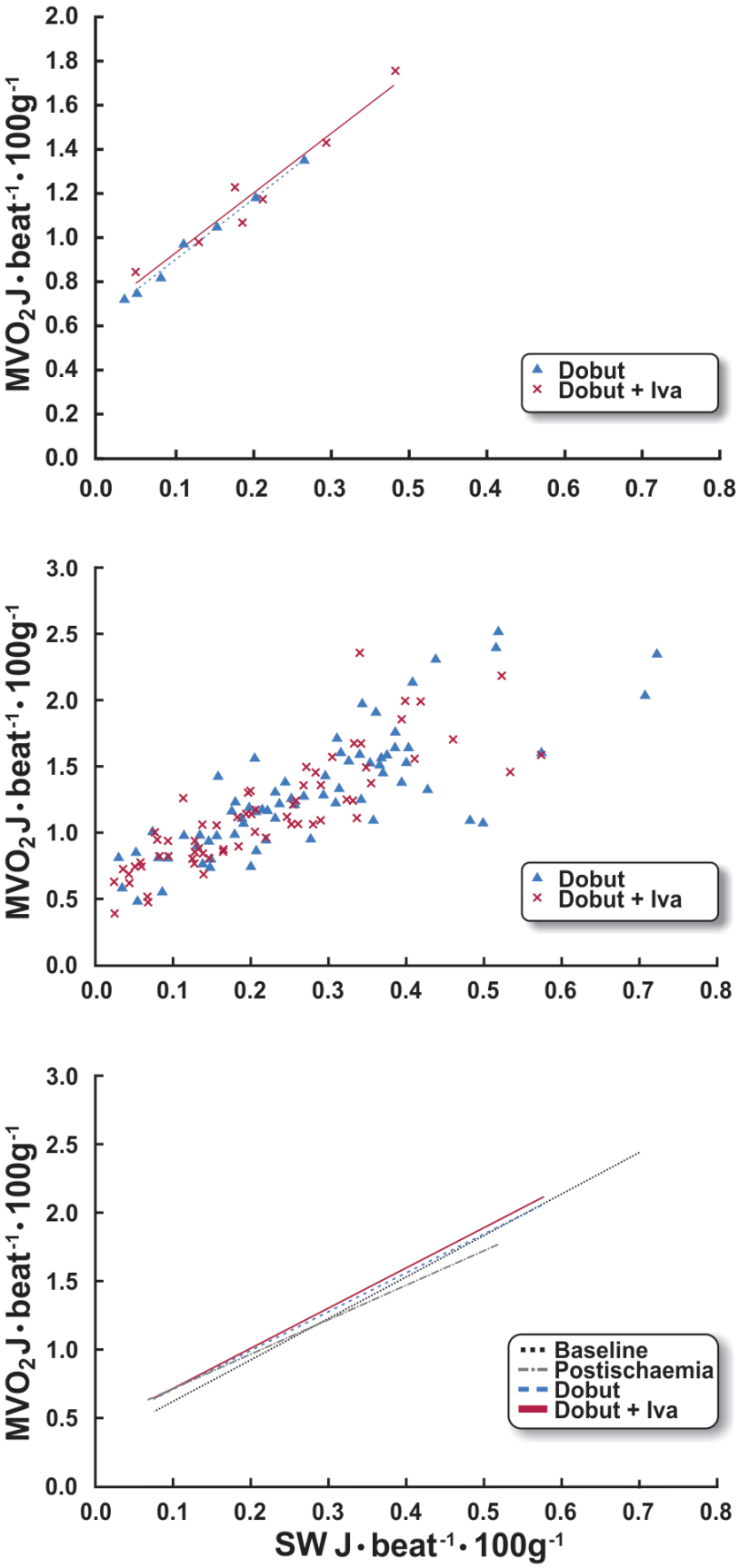

Figure 7. All panels show left ventricular (LV) mechanical work-myocardial oxygen consumption (MVO_2) relationship. LV mechanical work is presented as stroke work (SW). The top panel displays the data from a single experiment, whereas the middle panel displays pooled scatter data for all of the experiments following dobutamine and dobutamine/ivabradine infusion. The bottom panel displays the regression line based on pooled data of the SW-MVO_2 relationships between all interventions in all experiments. Here, the individual scatters are removed for simplicity. No significant differences were detected between the dobutamine and dobutamine/ivabradine timepoints. Iva, ivabradine; Dobut, dobutamine.

In ex vivo mouse hearts, we assessed the combination of a β-agonist (isoproterenol) and ivabradine. Isoproterenol addition led to increased MVO_2 and CO due to an elevated HR. Adding ivabradine returned the HR and CO to their baseline values, with a proportionate reduction in MVO_2. There was no significant alteration in SV, but the trends were similar to the findings in vivo. Adding ivabradine had no effect on CE (stroke work/MVO_2) in neither the pre- nor the post-ischaemic working heart.

7.2 Paper 2

The haemodynamic, energetic and metabolic effects of OM were assessed in an open-chest model of healthy and post-ischaemic pigs and in ex vivo mouse heart models. OM was administered in a dose targeting a 20% increase in SET, which is considered a clinically relevant level. This treatment reduced LV volumes, resulting in increased EF, without changes in SV and HR. OM addition increased unloaded MVO_2 (*y*-intercept of the PVA-MVO_2 relationship) in both healthy and post-ischaemic pigs (Figure 8) and in ex vivo mouse hearts (Figure 9). Furthermore, contractile efficiency was impaired (increase in the slope of the PVA-MVO_2 regression) in healthy and post-ischaemic pigs. Myocardial substrate oxidation was minimally affected in pigs but resulted in a significant metabolic switch to glucose oxidation in the mouse protocol. However, adding the myosin ATPase inhibitor BDM to arrested mouse hearts abolished the surplus MVO_2 in the OM group (Figure 9). Thus, increased basal metabolism explains the increase in unloaded MVO_2 after OM administration.

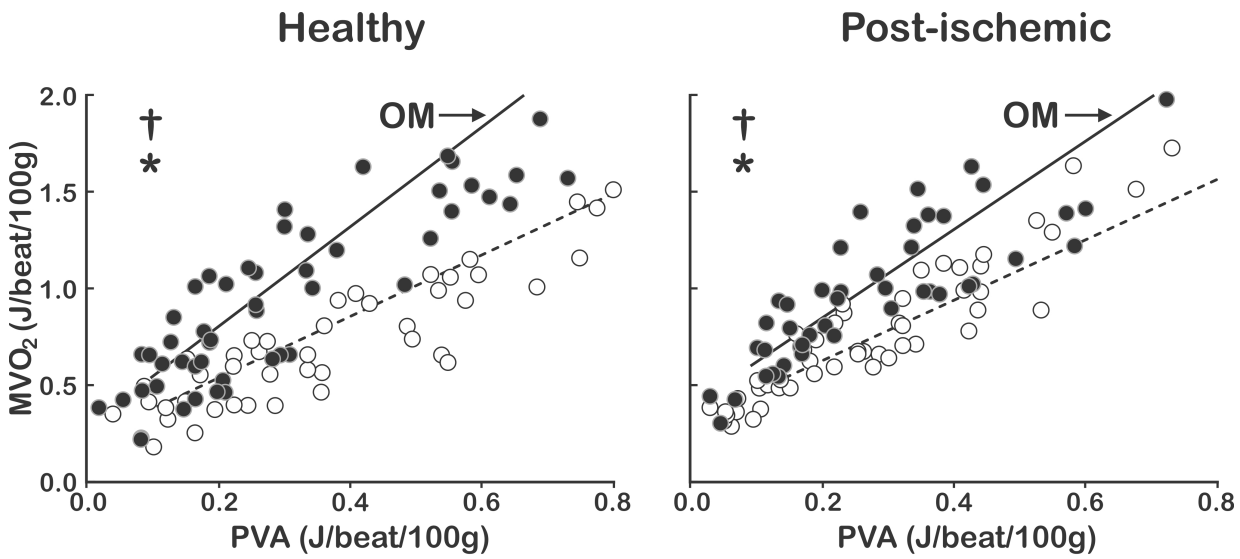

Figure 8. Pooled scatters of left ventricular (LV) mechanical work–myocardial oxygen consumption (MVO_2) relationships from all experiments. LV mechanical work is presented as pressure–volume area (PVA). Left panel are from healthy pigs (n=7), and right panels are from pigs with post-ischaemic LV dysfunction (n=7). The data were obtained at various workloads before (○) and after (•) infusion of omecamtiv mecarbil (OM). The *y*-intercept represents unloaded MVO_2, that is, energy used for excitation–contraction coupling (ECC) and basal metabolism. 1/slope of the regression line represents the contractile efficiency of the heart. OM impairs cardiac efficiency, as indicated by a significant increase in *y*-intercept and slope values in all panels, except for only an increased *y*-intercept value in the lower right panel. **P*<0.05 vs no drug for *y*-intercept; †*P*<0.05 vs no drug for slope (linear mixed model analysis).

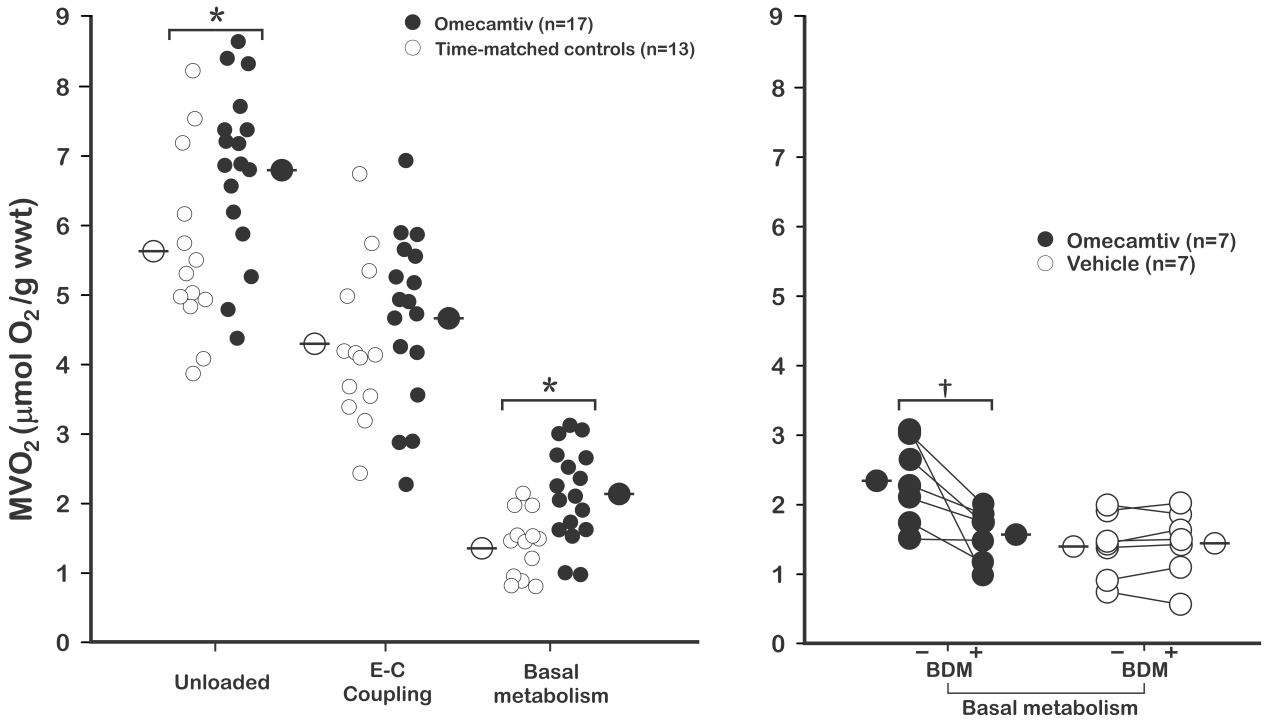

Figure 9. MVO_2 in ex vivo mouse hearts retrograde perfused in Langendorff mode. Dot plots presenting data in all panels. Mean values are presented as circles crossed by a horizontal line. Left: Significant increases in unloaded MVO_2 and oxygen consumption from basal metabolism in hearts treated with OM (n=17) compared with time-matched controls (n=13). Oxygen cost for excitation–contraction coupling (ECC) was unaffected by OM. Right: Addition of 2,3-butanedione monoxime (BDM) abolished surplus basal MVO_2 in the OM-treated hearts (n=7), whereas no effect of BDM was observed in the controls (n=7). **P*<0.05 vs omecamtiv; †*P*<0.05 vs without BDM; analysed by the Mann–Whitney-Wilcoxon test.

7.3 Paper 3

In a pig model of severe LV ischaemia, we performed an invasive assessment of the haemodynamics and LV diastolic function of two novel inotropic treatment principles, the myosin activator OM versus a combination D+I treatment. Assessments were conducted at intrinsic HR and during pacing-induced tachycardia. Left CME was followed by decreases in the SV, EF, CO, dP/dt_{max}, and dP/dt_{min} and by increases in LAP and EDP. HR was only slightly elevated. CO and EF increased in the D+I group but not in the OM group (Figure 10). SET increased from 42 to 54% of the cardiac cycle in the OM group but decreased from 45 to 39% in the D+I group.

The two treatment principles had opposite effects on early diastole. Tau increased significantly in the OM group, while it was significantly decreased in the D+I group. The rate of

relaxation of the myocardium (negative strain rate), as measured by long-axis ultrasonic crystal movement, was significantly faster in the D+I-treated animals, while in the OM-treated animals, we observed a nonsignificant slowing of relaxation. dP/dt_{max} and dP/dt_{min}, parameters of myocardial contraction and relaxation, respectively, were decreased in the OM group but increased in the D+I group. The L V-A_{max} and the L V-$A_{integral}$ were increased in the D+I group but reduced in the OM group at spontaneous HR. These differences are illustrated in Figure 11.

The animals were paced at 120 and 160 before and after treatment. The lengthening effect of OM on the SET diminished with higher HR. In D+I-treated animals, we observed a decreased SET. The CO and MAP diminished at higher pacing rates in the OM-treated group, while they were sustained in the D+I-treated group.

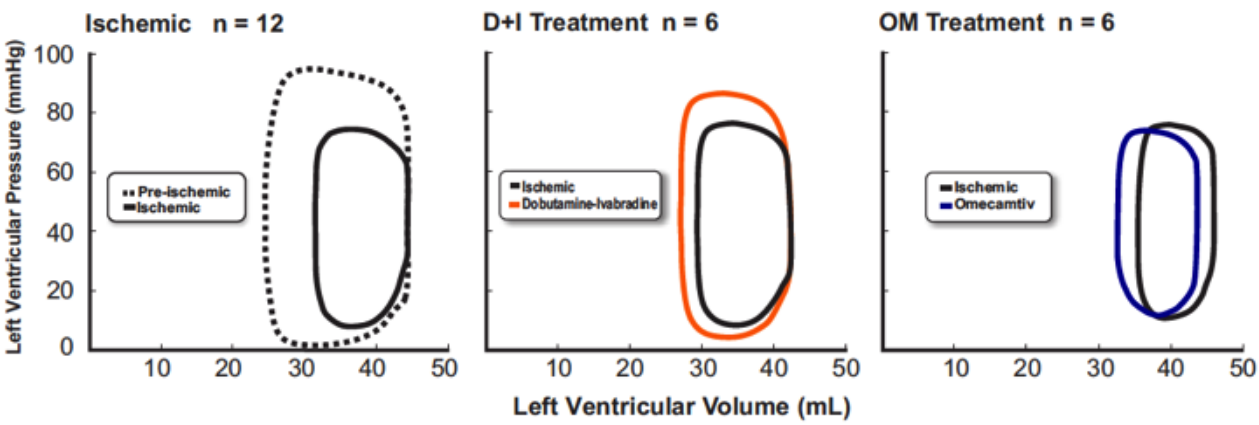

Figure 10. Representative PV recordings. Left panel shows pre- (dotted line) and ischemia (solid line). The middle panel shows treatment with dobutamine combined with ivabradine (D+I, yellow line) compared with untreated ischemia. The right panel shows treatment with omecamtiv (OM, blue line) compared with untreated ischemia. Steady-state loops are calibrated against mean values of ESV, EDV,EDP and ESP.

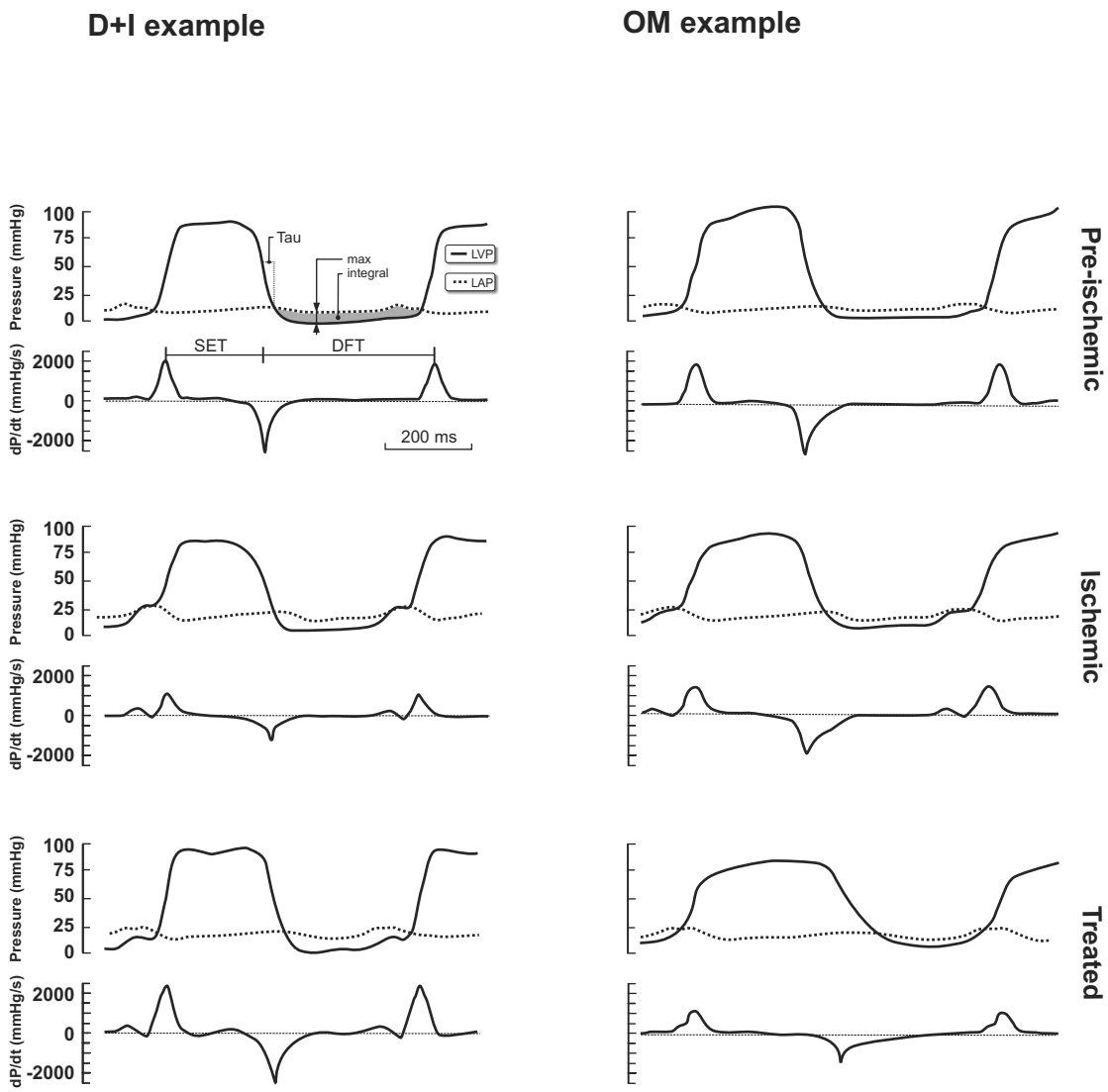

Figure 11. Modified Wigger's diagram showing examples of actual tracings of the left atrial-ventricular pressure interplay. Left column: dobutamine-ivabradine (D-I) treatment, pre-ischemia, ischemia, treatment. Right column: omecamtiv (OM) treatment, pre-ischemia, ischemia, treatment. LVP, left ventricular pressure; LAP, left atrial pressure; dP/dtmax and dP/dtmin, maximal and minimal derivative of the LV pressure, respectively; Tau, isovolumetric relaxation constant; Pmin, minimal pressure in the LV during the cardiac cycle; L V-A $_{MAX}$, maximal left ventricular-atrial pressure difference in diastole; L V-A $_{INTG}$,left ventricular-atrial pressure-time-integral during diastole; DFT, diastolic filling time; SET, systolic ejection time.

8. Discussion

Clinical experience and knowledge of the distinct pathophysiological syndromes is vital to applying optimal pharmacologic therapies in AHF. Accordingly, intravenous diuretics and vasodilators are recommended in patients with pulmonary and/or systemic venous congestion, as well as in patients with signs of elevated filling pressures. In patients with signs of malperfusion, or shock, intravenous inotropic support should be considered to maintain the peripheral perfusion by increasing the CO and the blood pressure. Jeger et al. reported[28] SV to be the best early haemodynamic predictor of mortality in CS. ESC guidelines suggest dobutamine as the first drug of choice[29] based on pre-clinical studies and expert opinion (class of recommendation, IIB; level of evidence, C), despite lack of evidence from randomized controlled trials (RCTs)[29]. In contrast, clinical studies have reported a lack of, or even negative, effects of these drugs when used long term[98]. There are multiple reasons for this gap between clinical practice and scientific evidence. First, inotropic drugs are frequently used as short-term rescue therapy in conditions with malperfusion, for example, pre-revascularization or post-CABG HF. Thus, designing controlled studies to represent these conditions is not feasible for ethical reasons. Second, different combinations of vasoactive drugs are often combined to tailor the patients' haemodynamic profiles. Third, studies have failed to enrol only patients in absolute need of inotropic support[99]. As a consequence of heterogeneity, a large study may contain subgroups of individuals with haemodynamic profiles who benefit from treatment, although the overall results are indifferent.

Therefore, ongoing research on inotrope drugs should follow two courses. One, RCTs with clearly defined aetiology and pathophysiological profiles that more closely represent clinical reality are needed. Two, pre-clinical studies assessing new drugs or new combinations of drugs are needed. The ideal drug should improve both systolic and diastolic properties without compromising, and maybe even improving CE.

When assessing the adjuvance of ivabradine to dobutamine in LV post-ischaemic dysfunction, both systolic and diastolic function was unchanged; thus, the enhanced ventricular relaxation[100] (Tau and peak filling rates) caused by dobutamine was maintained. Ivabradine abolished the chronotropic effect of dobutamine, resulting in prolonged diastolic filling time (by 45±19%) and increased SV. Taken together, these findings suggest that this drug combination, with simultaneously contractile enhancement and prolongation of diastole, provides an optimized pump function for the left ventricle.

Unlike the above-mentioned treatment, OM represents a new mechanism for improving myocardial performance. Classic inotropes increase myocardial contractility, while OM exhibits systolic unloading by prolonging SET[101], thus leading to an increase in the EF similar to other inotropes by improved emptying in systole. In contrast, classic inotrope indices such as dP/dt_{max} and dP/dt_{min} are only marginally affected by OM. We found no significant changes in SV and CO in the LV post-ischaemic dysfunction model, despite an increased EF. Shen et al. [102] found a small increase in SV in dogs with pacing-induced HF given OM, and this increase could be explained by a reduced afterload as observed by a concomitant reduction in vascular resistance in these dogs. In addition, the lack of time controls failed to address the reversibility of their rapid-ventricular pacing HF model. Notably, OM proved promising with increases in the SET and SV in a phase 2 study of systolic HF[103]. Further afield in the pipeline, a randomized and controlled phase II B trial (ATOMIC HF) was undertaken to investigate the effect of OM on hospitalized patients with AHF; this study showed a significant increase in SET, but no change in the LV shortening fraction, EDV or SV, and did not meet the primary end point of reduced dyspnea[104].

The key to explaining the increased SV with D+I and the lack of increased LV output by OM seems to be the diastolic performance of the heart. Therefore, we compared the two principles (D+I vs OM) in a model with severe systolic and diastolic dysfunction induced by LV CME. Based on previous work from our group[74], we know that CME induces a restrictive pattern of profound LV diastolic dysfunction with reduced early (Tau and dP/dt_{min}) and late (EDPVR) diastolic function and a corresponding increase in the LV EDP. In contrast, the ischaemia-reperfusion model we used for energetic assessments reduces early diastolic function (Tau, dp/dt_{min}) without an increase in LV EDP, suggesting a less severe LV relaxation dysfunction[34].

Both animal groups receiving OM or D+I were able to enhance systolic unloading in the ischaemic LV. Thus, both treatments should increase the restoring forces that subsequently facilitate suction in early diastole[105]. However, regarding diastole, the two principles work in opposition. D+I increases SV by concomitantly accelerating contraction and relaxation through enhanced Ca^{2+} flux, which results in improved early relaxation (reduced Tau, increased dP/dt_{min}) and an increased driving force across the mitral valve (L V-A_{max} pressure gradient). In contrast, the direct effect of OM on myosin ATPase unloads the heart by prolonging the SET at the expense of impaired early relaxation (increased Tau, reduced dP/dt_{min} and L V-A_{max} pressure gradient) and DFT. OM increase the force of contraction, without increase in rate of contraction, leading to increase in SET[71]. Hypothermia mimics the actions of OM on the heart, namely, an increase in SET, combined with unchanged or reduced HR[106]. Both OM and hypothermia prolong myocardial relaxation (increased Tau, reduced dP/dt_{min}), without a change in calcium transients. Hypothermia

is hypothesized to slow the calcium-troponin reaction rate, leading to deceleration of contraction and relaxation[106]. At present, there is no indication that OM acts by modulating calcium handling[71]. However, in both conditions, the prolonged relaxation may simply mirror the prolonged time to maximum force of contraction.

Investigating LV myocardial function is necessary, but not sufficient, to evaluate drug interventions on cardiac performance. Normally, the heart is a fine-tuned aerobic machine that produces mechanical work from energy consumption at a ratio of 20-25% (CE). Potential drug effects on CE must be determined. In 1971, Braunwald described the major determinants of MVO_2; HR, contractile state and afterload [16]. These determinants are integrated in the indices of cardiac work, SW and PVA, which provide us with a framework for evaluating CE.

Dobutamine (and other catecholamines or PDE inhibitors) are Ca^{2+} mobilizer inotropic agents that load cardiomyocytes with Ca^{2+} to improve cardiac contractility. Ca^{2+} loading is associated with impaired CE caused by increased work-independent MVO_2 for ECC. The enhanced MVO_2, increased HR, and greater risk of arrhythmias have been suspected to contribute to the higher morbidity and mortality rates[107,108]. Data from our group show no excess MVO_2 compared to cardiac work with the use of clinically relevant doses of dobutamine (2 μg/kg/min) in pigs with post-ischaemic AHF. However, myocardial oxygen waste was observed at supratherapeutic levels (10 μg/kg/min) and was associated with a complete offset in haemodynamics [34]. In our D+I experiments, an infusion of 5 μg/kg/min dobutamine caused substantial and proportional increases in the CO, HR and MVO_2 while maintaining cardio-metabolic efficiency[109]. Attenuating the tachycardia by ivabradine did not improve CE. Perhaps the increase in SW by ivabradine counteracts the oxygen-sparing effect of HR reduction. Similar findings were reported with ivabradine monotherapy in exercising dogs [110].

However, MVO_2 is not only determined by haemodynamic variables. In theory, the metabolic profile can explain up to a 15% difference in O_2 consumption if you compare pure FFA metabolism versus strictly glucose-dependent metabolism[111]. Importantly, in 1971, Mjøs et al. reported that increased uptake of FFA reduced CE in intact dogs. Of note, we observed a gradual increase in FFA during infusion of dobutamine; thus, the short dobutamine infusion time (2 hours) and corresponding CE in our experiments may not be representative of the effects of long-term infusion (days).

The evaluation of the energetic properties of OM gave results that were more surprising. Earlier studies suggested that OM treatment improved CE [102]; however, our evaluation using the PVA-MVO_2 framework [65] gave conflicting results. In both healthy and post-ischaemic pig hearts, we observed a pronounced impairment in CE following OM infusion. This impairment was

evidenced by increases in both the *y*-intercept and the slope of the PVA-MVO_2 regression. This observation is compatible with the proposed action of OM; activation of myosin ATPase was used in the sliding contraction of the myofilaments; the traditional increased inotropy-controlled intracellular calcium transients were not used[112]. The pronounced deterioration in CE with OM is a rare observation in our laboratory. During the 20 years of experience with this model, only the synthetic NO blocker L-NAME has demonstrated myocardial oxygen wastage to the same extent [113]. Both L-NAME and OM are synthetic compounds that have been introduced in an evolutionary fine-tuned biological system.

In contrast to Ca^{2+}-mediated inotropes, OM increased work-independent MVO_2 related to increased basal metabolism. The possible contributors to this increase are changes in mitochondrial function, myosin ATPase or substrate utilization. We found only a marginal metabolic switch towards glucose utilization and thus a tendency towards an improved P:O ratio. Adding OM to mouse heart mitochondria did not change the P:O ratio or respiratory state, indicating that OM did not have a direct effect on mitochondrial function. However, adding BDM to arrested OM mouse hearts abolished surplus MVO_2, indicating that myosin ATPase is the culprit of OM-induced oxygen waste.

We have identified two important shortcomings of OM. The combination of reduced efficiency and impaired diastolic function seem incompatible with clinical use. Nevertheless, a new phase 2 study is now recruiting patients[114]. In clinical studies with healthy volunteers [36], subjects with mild HF [103] and subjects with AHF [104], OM is reported to be safe. Still, in the clinical reality outside of protocols, the patient's condition is a moving target. Arrhythmias, coronary insults, and decompensation episodes all complicate the clinical course. Based on our findings, conditions with impaired diastolic function and/or CBF could potentially unmask detrimental effects of OM, leading to aggravation of ischaemia.

9. Conclusions

Paper 1. Adding ivabradine reversed the chronotropic effect of dobutamine and restored the SV and SW by increasing the DFT while maintaining the MAP and CO. Additionally, adding ivabradine partly counteracted the dobutamine-induced increase in MVO_2. However, the work-MVO_2 relationship was unaffected, suggesting that CE was maintained by the combined use of dobutamine and ivabradine.

Paper 2. The main finding of this study was that OM contributed to a significantly increased myocardial oxygen cost in both healthy and post-ischaemic stunned hearts. OM increased oxygen consumption due to energetically inefficient LV function and increased oxygen consumption by non-contractile processes. The increase in MVO_2 was mediated by overactive myosin ATPase.

Paper 3. Our data showed that combined D+I treatment was able to improve diastolic function in ischaemic hearts. In contrast, OM treatment impaired diastolic function; thus, we hypothesized that altering the intracellular calcium flux is probably mandatory for improving diastolic function in ischaemic hearts.

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11. Papers