

# Paper 1

# Reversing dobutamine-induced tachycardia using ivabradine increases stroke volume with neutral effect on cardiac energetics in left ventricular post-ischaemia dysfunction

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## Abstract

**Aim:** Compensatory tachycardia can potentially be deleterious in acute heart failure. In this study, we tested a therapeutic strategy of combined inotropic support (dobutamine) and selective heart rate (HR) reduction through administration of ivabradine.

**Methods:** In an open-chest pig model ( $n = 12$ ) with left ventricular (LV) post-ischaemia dysfunction, cardiac function was assessed by LV pressure catheter and sonometric crystals. Coronary flow and blood samples from the coronary sinus were used to measure myocardial oxygen consumption ( $MVO_2$ ). LV energetics was assessed by comparing  $MVO_2$  with cardiac work at a wide range of workloads.

**Results:** In the post-ischaemia heart, dobutamine ( $5 \mu\text{g kg}^{-1} \text{min}^{-1}$ ) increased cardiac output (CO) by increasing HR from  $102 \pm 21$  to  $131 \pm 16$  bpm (beats per min;  $P < 0.05$ ). Adding ivabradine ( $0.5 \text{ mg kg}^{-1}$ ) slowed HR back to  $100 \pm 9$  bpm and increased stroke volume from  $30 \pm 5$  to  $36 \pm 5$  mL ( $P < 0.05$ ) by prolonging diastolic filling time and increasing end-diastolic dimensions. Adding ivabradine had no adverse effects on CO, mean arterial pressure and cardiac efficiency. Similar findings on efficiency and LV function were also seen using an *ex vivo* working mouse heart protocol.

**Conclusions:** A combined infusion of dobutamine and ivabradine had a neutral effect on post-ischaemia LV efficiency and increased left ventricular output without an increase in HR.

**Keywords** acute heart failure, dobutamine, ivabradine, myocardial oxygen consumption.

Inotropic support is often administered in severe life-threatening acute heart failure (AHF; Hocjman *et al.* 1999), and dobutamine is often the drug of choice (McMurray *et al.* 2012, Steg *et al.* 2012). The SHOCK trial (Jeger *et al.* 2007) indicated that stroke

volume (SV), and not cardiac output (CO), was the best early predictor of mortality in severe AHF in need of inotropic treatment. However, a beta-adrenergic stimulation in AHF patients may not necessarily result in increased SV due to the chronotropic effect

leading to hampered diastolic filling of the ventricles. Chronotropy being mediated by stimulation of If (funny)-channels in the sinus node (Accili *et al.* 2002). A pharmacological intervention separating the inotropic and chronotropic responses has not been available until recently. Also, a heart rate (HR) reduction is by itself suggested to be beneficial in both chronic and acute heart failure (Kjekshus 1987, Böhm *et al.* 2010) as this may reduce myocardial oxygen demand and the risk of subendocardial ischaemia (Heusch 2008). Using ivabradine, an If-channel inhibitor (Sulfi & Timmis 2006), we are now able to attenuate the chronotropic effect of beta-adrenergic drugs without affecting their inotropy (Simon *et al.* 1995, Vilaine *et al.* 2003). Ivabradine reduces HR by selectively decelerating the spontaneous depolarization of the sinoatrial node (Sulfi & Timmis 2006). Recently, two clinical studies have indicated promising outcome of combined dobutamine–ivabradine treatment in severe AHF (Gallet *et al.* 2014, Cavusoglu *et al.* 2015). Both studies used oral ivabradine (5–7.5 mg  $\times$  2). A pre-clinical study with intravenous ivabradine could validate the haemodynamic effect of this drug combination and provide supplemental data on cardiac function and cardiac energetics.

The aim of this study was to investigate whether the inotropic and lusitropic effects of dobutamine were preserved when combined with ivabradine in a clinically relevant model of LV post-ischaemia dysfunction. In addition, we assessed to what extent this cotreatment could restore SV and CO, theoretically by prolonging the diastolic time interval. Finally, we investigated whether adding ivabradine to dobutamine could improve cardiac efficiency in the post-ischaemia pig measured as the relation between myocardial oxygen consumption (MVO<sub>2</sub>) and contractile function. In addition, an *ex vivo* mouse heart perfusion model (How *et al.* 2005) was applied to assess cardiac energetics and haemodynamics under controlled loading conditions and without neurohumoral influences (Grieve *et al.* 2004).

## Material and methods

### Experimental animals

The experimental protocol was approved by the local steering committee of the National Animal Research Authority located at the Faculty of Health, UIT, The Arctic University of Norway. Twelve castrated male domestic pigs weighing  $30 \pm 5$  kg were adapted to the Animal Department for 5–7 days and fasted overnight before experiments, with free access to water. Additionally, 16 female NMRI mice were used in this study. The mice were kept in cages (five

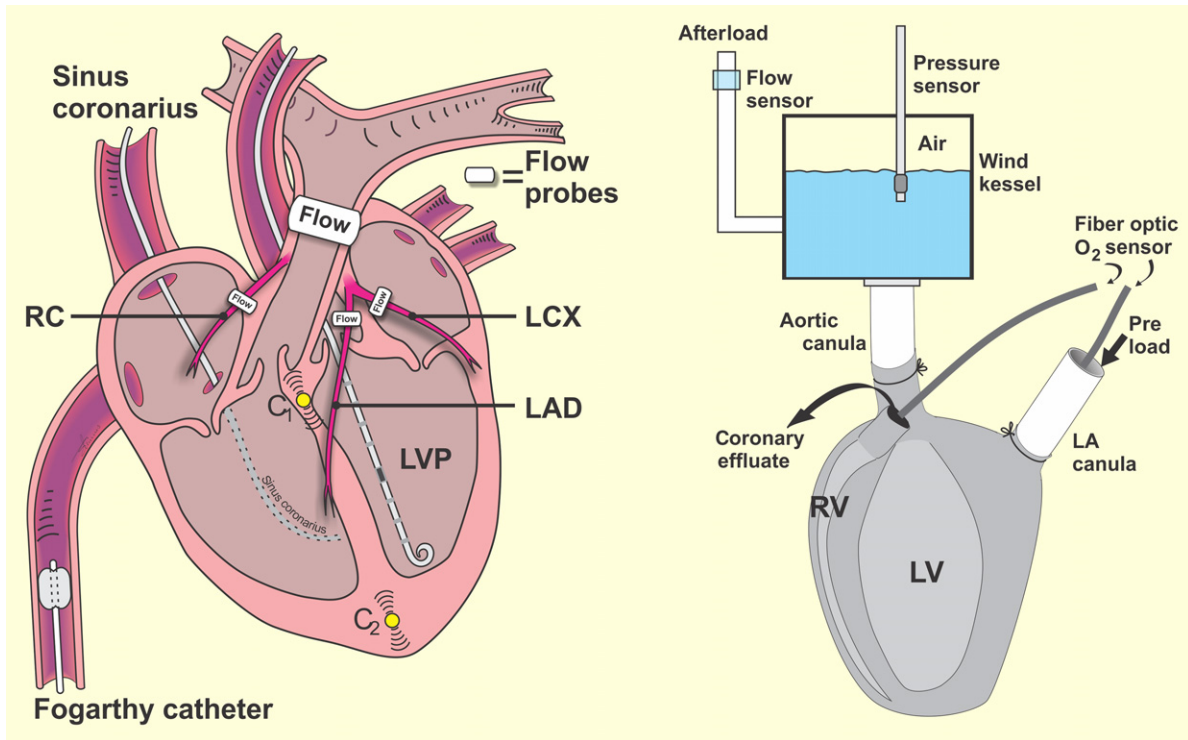
animals per cage) with *ad libitum* access to food and water.

### In vivo pig study

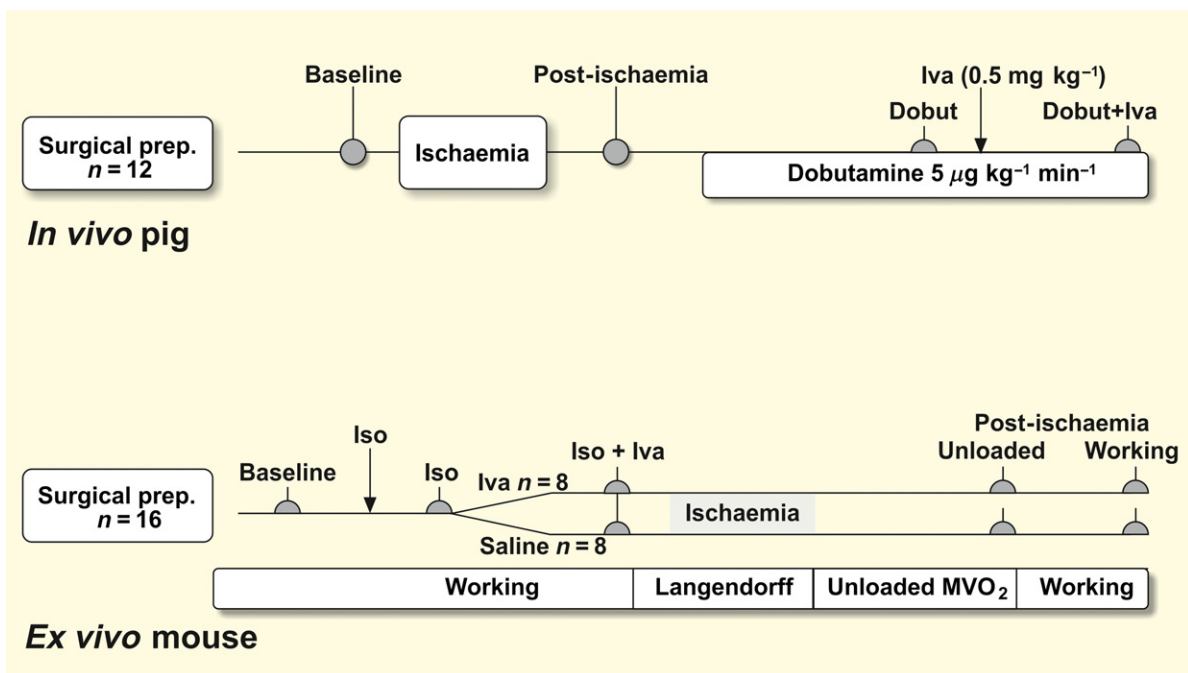
**Surgical preparation and instrumentation.** The pigs were premedicated with an intramuscular injection of 20 mg kg<sup>-1</sup> ketamine (Pfizer AS, Oslo, Norway) and 1 mg of atropine (Nycomed Pharma, Oslo, Norway). Anaesthesia was induced by intravenous injection of 10 mg kg<sup>-1</sup> pentobarbital sodium (Abbott, Stockholm, Sweden) and 0.01 mg kg<sup>-1</sup> fentanyl (Hameln Pharmaceuticals, Hameln, Germany), and the animals were normoventilated after tracheostomy. Normoventilation was defined as arterial PaCO<sub>2</sub> of  $40 \pm 2$  mmHg. A central venous catheter was placed through the left internal jugular vein, and anaesthesia was maintained throughout the experiment by a continuous infusion of 4 mg kg<sup>-1</sup> h<sup>-1</sup> pentobarbitone sodium, 0.02 mg kg<sup>-1</sup> h<sup>-1</sup> fentanyl and 0.3 mg kg<sup>-1</sup> h<sup>-1</sup> midazolam (B. Braun, Melsungen, Germany). The circulating volume was maintained by a 20 mL kg<sup>-1</sup> h<sup>-1</sup> continuous infusion of 0.9% NaCl supplemented with 1.25 g L<sup>-1</sup> glucose. The animals received 2500 IU of heparin and 5 mg kg<sup>-1</sup> amiodarone (Sanofi-Synthelabo, Stockholm, Sweden) to avoid blood clotting of catheters as well as to prevent cardiac arrhythmias.

The surgical preparation of the animals has been described in detail previously (Korvald *et al.* 2000). See Figure 1 for an illustration of the set-up. In brief, a 7-Fr manometer pressure–volume catheter (Millar MPVS Ultra, Houston, TX, USA) was inserted through an introducer sheath into the left ventricle. The arterial pressure was measured via a vascular catheter in the abdominal aorta. A 7-Fr balloon catheter was introduced into the inferior vena cava for preload reduction. Following medial sternotomy, the pericardium was removed and the coronary arteries and pulmonary trunk were dissected free to place transit-time flow probes (Medi-stim, Oslo, Norway) for measurements of the coronary blood flow and CO. Two sonomicrometry crystals (Sonometrics Corporation, trx 4, London, ON, Canada) were placed in the myocardium to measure long axis myocardial shortening. Epicardial echocardiography (Vivid I, GE, Fairfield, CT, USA) was used to calibrate the sonomicrometry crystals to the LV dimensions. Myocardial venous blood was sampled from a catheter placed in the great cardiac vein via the coronary sinus (after ligating the hemiazygos vein).

**Experimental protocol.** See Figure 2 for a schematic illustration. We used our established ischaemia–reperfusion model (Korvald *et al.* 2001) to assess the haemodynamic and energetic effects of the



**Figure 1** Illustration of surgical preparation and setup. *In vivo* pig heart (left) and *ex vivo* mouse heart (right). Pig: LCX, left circumflex artery; LAD, left anterior descending artery; RC, right coronary artery; and C1 and C2, sonometric crystal pair; LVP, left ventricular pressure. Mice: RV, right ventricle; LV, left ventricle; LA, left atrium.



**Figure 2** Experimental protocol. *In vivo* pig (top) and *ex vivo* mouse heart studies (bottom). *In vivo* pig: Iva, ivabradine; Dobut, dobutamine. *Ex vivo* mouse: Iso, isoproterenol; Iva, ivabradine.

combination of dobutamine and ivabradine. In short, this protocol uses repetitive simultaneous coronary occlusion (rubber bands) and reperfusion of the left

anterior descending artery (LAD) and the circumflex artery (LCX). We induced 21.3 ± 6.5 min of accumulated ischaemia. The occlusion affects ~80% of the

LV and induces a reproducible acute impairment of global LV function that remains stable for several hours (Korvald *et al.* 2001). In cases when ventricular fibrillation/ventricular tachycardia occurred, we administered biphasic DC shock (10–20 joules) combined with direct manual cardiac compressions.

The experiment was conducted with a repeated measures design. Following surgery, the pigs were allowed to rest for 30 min before baseline measurements (T1). Then, we induced LV ischaemia and allowed the pigs to stabilize for another 30–60 min before recording the post-ischaemia measurements (T2). Dobutamine was subsequently administered continuously at  $5 \mu\text{g kg}^{-1} \text{min}^{-1}$ . New measurements were taken after 30 min at a haemodynamically steady state (T3). Finally, a bolus infusion of ivabradine (ivabradine hydrochloride, ChemOKI Synthesi-TECH, Jiangsu, China),  $0.5 \text{ mg kg}^{-1}$  (Vaillant *et al.* 2008), was added and the final measurements were conducted after 30 min (T4).

**Obtaining data and analysis.** Sonomicrometry, flow and pressure data were sampled, digitized and analysed (using ADI Labchart Pro software, Dunedin, New Zealand) to obtain various indices of LV function and haemodynamics. SV was measured with time-transit flow probe on the pulmonary artery. To obtain relative differences in dimensions throughout the experiments, the sonomicrometry crystals were calibrated to end-systolic and end-diastolic diameter (ESD, EDD) at baseline. ESD and EDD were estimated from epicardial echocardiogram (2-dimensional short axis).

The inflation of a balloon catheter in the vena cava produced a stepwise reduction in preload, enabling cardiac work and  $\text{MVO}_2$  to be obtained at 5–7 various steady state workloads (Fig. 2). The total LV mechanical work was calculated as the stroke work (SW; Baxley *et al.* 1977). In brief, the SW consists of the area bounded by the pressure–volume loop (external work) obtained at steady state preload reduction.

The LV coronary blood flow was estimated from the formula;  $\text{LVCBF} = \text{CBF} \times 0.7$  (Domenech & Hoffmann 1969); where LVCBF and CBF are the left ventricular and total coronary blood flow respectively. The weight of the LV was calculated as  $3.3 \text{ g LV weight kg}^{-1} \text{ pig weight}$  (Misare *et al.* 1992). The LV myocardial oxygen consumption was calculated from the formula;  $\text{MVO}_2 = (\text{LVCBF} \times \text{AVdO}_2 \times \text{Hb} \times 1.39) / \text{HR} \times 20.2$ ; where  $\text{MVO}_2$  is the LV myocardial oxygen consumption and  $\text{AVdO}_2$  is the arteriovenous difference in oxygen saturation in blood drawn from the aorta and coronary sinus respectively. Hb is the haemoglobin concentration in  $\text{g mL}^{-1}$ , 1.39 is a constant ( $\text{mL O}_2 \text{ g}^{-1} \text{ Hb}$ ), and HR is the heart rate. To

convert  $\text{MVO}_2$  to mechanical energy equivalents, the factor  $20.2 \text{ J mL}^{-1} \text{ O}_2$  was used.  $\text{MVO}_2$  was normalized to  $\text{Joule beat}^{-1} 100 \text{ g}^{-1} \text{ LV}$ .

### Ex vivo mouse study

Isolated perfused mouse hearts were used for assessment of haemodynamics, and energetic data described previously (How *et al.* 2005). In brief, the mice were anaesthetized with 10 mg of sodium pentobarbital intraperitoneally. The hearts were quickly excised, and the aorta was cannulated and initially perfused retrogradely (Langendorff) with recycled Krebs–Henseleit bicarbonate buffer containing 10 mM glucose and 0.5% palmitate bound to 3% bovine serum albumin.

In the working mode, the left atrium was cannulated with a 16 G steel cannula connected to a preload reservoir ensuring forward perfusion through the aortic valve. Aortic and filling pressures were set to column heights of 55 and 12 mmHg respectively. In the retrograde-perfused unloaded mode, ischaemia was induced by 40 min of low flow ( $3.1 \text{ mL min}^{-1} \text{ mg}^{-1} \text{ dW}$ ) followed by 5 min of reperfusion. The cardiac temperature was maintained at  $37^\circ \text{C}$  throughout both perfusion modes.

Coronary flow (CF) was measured by timed collections of the effluent dropping from the heart, and aortic flow (AF) was determined by a drop counter at the outlet of the afterload line, whereas cardiac output (CO) was calculated as the sum of AF and CF. Mean stroke volume (SV) was defined as CO divided by heart rate.  $\text{MVO}_2$  was then calculated by the following equation:  $\text{MVO}_2 = [\text{pO}_2 (\text{coronary inflow}) - \text{pO}_2 (\text{coronary effluent})] \times \text{Bunsen solubility coefficient of O}_2 \times \text{CF}$  (How *et al.* 2005). At last, cardiac efficiency is calculated as the ratio between SV and  $\text{MVO}_2 \text{ beat}^{-1}$ .

**Experimental protocol.** After baseline measurements, isoprenaline (10 nM) was added to the recycled buffer. After 5 min, new measurements were conducted. Sixteen mice were randomly split into two groups with  $n = 8$ . One group receiving ivabradine ( $3 \mu\text{M}$ ) and the other vehicle. New measurements were conducted after 15 min. Thereafter, both groups were subjected to low-flow ischaemia in Langendorff mode. The aortic line was clamped, and coronary flow reduced to  $3.1 \text{ mL min}^{-1} \text{ mg}^{-1} \text{ dW}$  (which is  $\sim 3\%$  of their baseline coronary flow) for 40 min controlled by a peri-staltic pump (Hafstad *et al.* 2007). The aortic line was then reopened, and the coronary was reperfused for 5 min in unloaded mode followed 10 min in working mode before the final

measurements. See Figure 2 for a schematic illustration.

### Statistical analysis

The data are expressed as mean  $\pm$  standard deviation (SD). A one-way repeated-measurements analysis of variance (ANOVA) was used to determine haemodynamic and LV function differences between baseline, post-ischaemia, dobutamine and dobutamine + ivabradine time points (Kusuoka & Hoffman 2002). Myocardial energetic data ( $MVO_2/SW$ ) were obtained at four different measurement time points, T1–T4. These data were compared by linear (repeated effects) mixed model analysis (Fitzmaurice & Ravichandran 2008) with  $MVO_2$  as the dependent variable,  $SW$  as the covariate, T1–T4 as repeated effects and subject id as random effect. In the isolated mouse hearts, within-group effects were analysed using one-way repeated-measurements ANOVA. Between-group effects were analysed using a linear mixed model analysis with

subject id as random effect. Multiple comparisons were adjusted for by Bonferroni correction.  $P$ -values  $<0.05$  were regarded as statistically significant, and all of the analyses were conducted in SPSS 22.0 (Chicago, IL, USA).

### Results

A total of 12 pigs were used in this study, of which ten were included in the analysis of the energetics and haemodynamics. Exclusions were based on the following: abrupt haemodynamic collapse following induction of ischaemia (one pig) and rapid supraventricular tachycardia after the administration of ivabradine (one pig).

#### Ischaemia–reperfusion protocol

The accumulated ischaemia time was  $21.3 \pm 6.5$  min composed of  $12.9 \pm 4.6$  occlusion periods (mean  $\pm$  SD). Ventricular fibrillation/ventricular

**Table 1** Hemodynamic, metabolic and left ventricular functional parameters

	Baseline	Post-ischaemia	Dobutamine	Dobutamine + Ivabradine
Heart rate, bpm	78 $\pm$ 14	102 $\pm$ 21*	131 $\pm$ 16* <sup>†</sup>	100 $\pm$ 9* <sup>‡</sup>
Cardiac output, L min <sup>-1</sup>	2.9 $\pm$ 0.6	2.7 $\pm$ 0.5	3.7 $\pm$ 0.7* <sup>†</sup>	3.6 $\pm$ 0.7* <sup>†</sup>
Mean arterial pressure, mmHg	84 $\pm$ 12	69 $\pm$ 10*	75 $\pm$ 10*	72 $\pm$ 11*
Cardiac power output, W	0.55 $\pm$ 0.2	0.42 $\pm$ 0.1*	0.65 $\pm$ 0.2 <sup>†</sup>	0.59 $\pm$ 0.2
Stroke work, mmHg $\times$ mL	43 $\pm$ 10	24 $\pm$ 6*	31 $\pm$ 9*	38 $\pm$ 9* <sup>†,‡</sup>
Stroke volume, mL	37 $\pm$ 5	27 $\pm$ 4*	30 $\pm$ 5*	36 $\pm$ 5 <sup>†,‡</sup>
Coronary blood flow, mL min <sup>-1</sup>	119 $\pm$ 40	128 $\pm$ 28	192 $\pm$ 39* <sup>†</sup>	167 $\pm$ 38
Systemic vascular resistance, dyn $\times$ s cm <sup>-5</sup>	2112 $\pm$ 266	1767 $\pm$ 300	1450 $\pm$ 357*	1466 $\pm$ 358
Central venous pressure, mmHg	8.8 $\pm$ 2.6	9.2 $\pm$ 2.7	8.5 $\pm$ 2.8	8.8 $\pm$ 2.8
Myocardial oxygen consumption, J min <sup>-1</sup> 100 g <sup>-1</sup> LV	140 $\pm$ 48	144 $\pm$ 40	218 $\pm$ 50* <sup>†</sup>	192 $\pm$ 56
Systolic ejection time, ms	261 $\pm$ 34	254 $\pm$ 31	182 $\pm$ 15* <sup>†</sup>	195 $\pm$ 13* <sup>†</sup>
Diastolic filling time, ms	532 $\pm$ 109	360 $\pm$ 101*	283 $\pm$ 61*	411 $\pm$ 53* <sup>‡</sup>
Preload recruitable stroke work, mmHg	60 $\pm$ 18	48 $\pm$ 14*	67 $\pm$ 29 <sup>†</sup>	67 $\pm$ 13 <sup>†</sup>
End systolic long axis dimension, mm	32.0 $\pm$ 3	34.5 $\pm$ 3*	31.5 $\pm$ 3 <sup>†</sup>	32.0 $\pm$ 3 <sup>†</sup>
End diastolic long axis dimension, mm	40.0 $\pm$ 3	40.5 $\pm$ 4	38.5 $\pm$ 4 <sup>†</sup>	40.5 $\pm$ 4 <sup>‡</sup>
dP dt <sup>-1</sup> max, mmHg s <sup>-1</sup>	1606 $\pm$ 768	1037 $\pm$ 161*	2608 $\pm$ 771* <sup>†</sup>	2755 $\pm$ 724* <sup>†</sup>
dP dt <sup>-1</sup> min, mmHg s <sup>-1</sup>	-2978 $\pm$ 904	-1819 $\pm$ 352*	-2839 $\pm$ 569 <sup>†</sup>	-3178 $\pm$ 658 <sup>†</sup>
Tau, ms	33 $\pm$ 5	37 $\pm$ 6	21 $\pm$ 2* <sup>†</sup>	22 $\pm$ 3* <sup>†</sup>
End systolic pressure, mmHg	99 $\pm$ 12	83 $\pm$ 10*	88 $\pm$ 11*	91 $\pm$ 9*
End diastolic pressure, mmHg	15 $\pm$ 4	17 $\pm$ 3	12 $\pm$ 6 <sup>†</sup>	14 $\pm$ 5

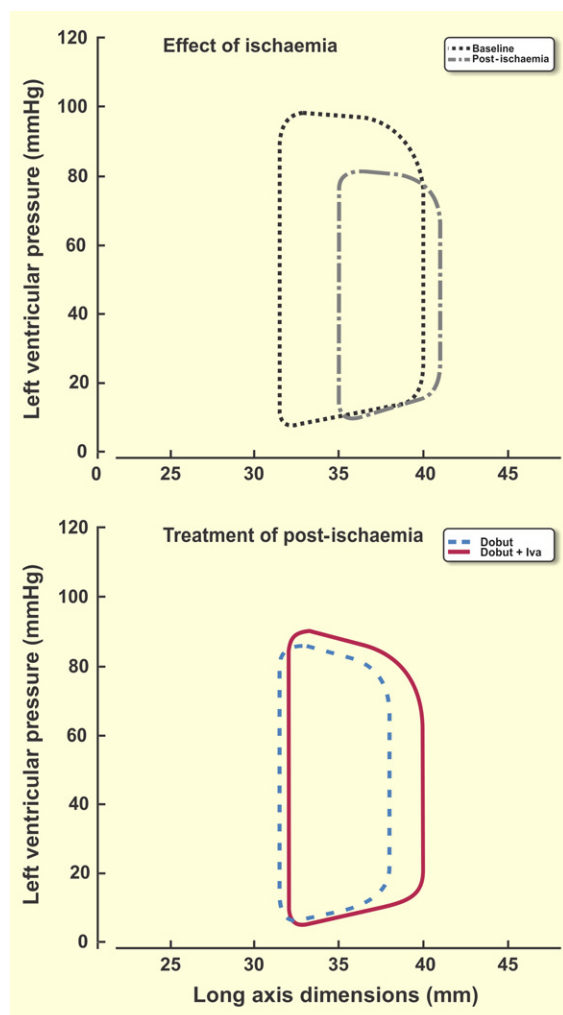
Haemodynamic, metabolic and left ventricular (LV) function parameters were assessed in pigs ( $n = 10$ ) with four repetitive measurements. Baseline, post-ischaemia, after administration of dobutamine and subsequently after dobutamine + ivabradine cotreatment.  $MVO_2$ , myocardial oxygen consumption (Joule min<sup>-1</sup> 100 g<sup>-1</sup> left ventricular weight), is derived from time-transit flow measurements and oximetry from the coronary sinus (see details in text). End-systolic- and end-diastolic pressures are derived from the LV pressure catheter measurements. End-systolic- and end-diastolic diameters are derived from the sonomicrometry measurements. Preload recruitable stroke work is derived from steady-state data at different workloads. dP dt<sup>-1</sup> max and dP dt<sup>-1</sup> min (the maximum and minimum rate of pressure change in the ventricle), and Tau is derived from pressure–volume data (LV-catheter). Systolic ejection time denotes time between dP dt<sup>-1</sup> max and min. Diastolic filling time refers to cardiac cycle time minus systolic ejection time. All other indexes are derived from flow and pressure measurements. Between time point differences: \* $P < 0.05$  vs. baseline, <sup>†</sup> $P < 0.05$  vs. post-ischemia, <sup>‡</sup> $P < 0.05$  vs. dobutamine.



tachycardia necessitating internal defibrillation occurred on average 1.6 times  $\text{pig}^{-1}$ . Troponin T analysis showed an increase from  $50.3 \mu\text{g L}^{-1}$  at T1 (baseline) to  $225.4 \mu\text{g L}^{-1}$  (mean) at T4 (last point of measurement). The haemodynamic effects of the accumulated ischaemia–reperfusion are compatible with post-ischaemia LV dysfunction as summarized in Table 1. The HR and end-systolic diameter (ESD) increased, while the external mechanical energy output (SW) and stroke volume (SV) were depressed by 44% and 27% from baseline respectively.

### Haemodynamics and left ventricular function

Haemodynamic data are presented in Table 1 and Figures 3 and 4. Dobutamine was administered to reverse



**Figure 3** Illustration of a typical left ventricular pressure-long axis dimension loop at each time point. Pressure-dimension loops are calibrated against the mean end-systolic-, end-diastolic dimensions, and end-systolic-, end-diastolic pressure values as shown in Table 1; Iva, ivabradine and Dobut, dobutamine.

the haemodynamic effects of post-ischaemia LV dysfunction. After dobutamine infusion, a further increase in HR occurred, and ESD was reduced to baseline levels with unchanged SV. After adding ivabradine, 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. There was no significant change in CO or MAP after adding ivabradine.

The effects of ischaemia, dobutamine and dobutamine + ivabradine on LV contractile function are shown in Table 1. The ischaemia–reperfusion protocol produced a 20% reduction in global LV function as measured by the preload recruitable stroke work (PRSW).  $dP dt^{-1}$  max and min were reduced by 35% and 39% respectively. Dobutamine infusion increased the  $dP dt^{-1}$  max by 151%, and Tau was reduced by 42%. There was no significant change in LV function after adding ivabradine to dobutamine.

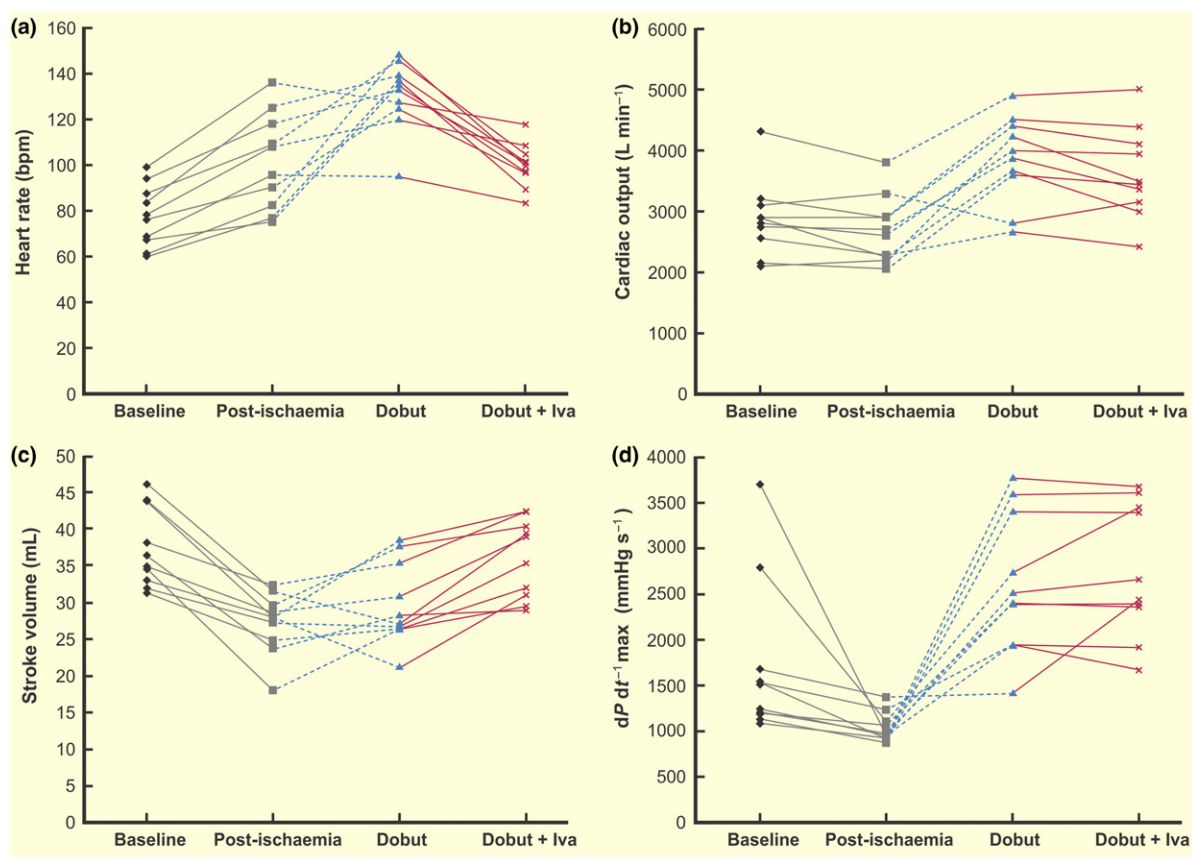
### Cardiac energetics

The drug-induced effects on the relation between mechanical work and oxygen consumption are summarized in Table 2 and Figure 5. A similar relation between the SW and the  $MVO_2$  was seen between dobutamine and dobutamine+ivabradine over a broad range of workloads. Normalizing the  $MVO_2$  to a fixed CO (3 L) and LV weight ( $100 \text{g}^{-1}$ ) showed a non-significant trend towards a myocardial oxygen-sparing effect with adding ivabradine.

In isolated working mouse hearts, isoproterenol (iso) led to an increased  $MVO_2$  and cardiac output due to an elevated HR (Table 3). 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. Oxygen cost for non-contractile purposes as measured by unloaded  $MVO_2$  (equivalent to the Y-axis intercept from the in vivo studies in Table 2) was not altered by adding ivabradine ( $1.85 \pm 0.54 \text{ nmol O}_2 \text{ beat}^{-1} \text{ ISO}$  vs.  $1.74 \pm 0.36 \text{ nmol O}_2 \text{ beat}^{-1}$  ivabradine + iso,  $n = 8$ ). Adding ivabradine had no effect on cardiac efficiency (stroke volume/ $MVO_2$ ) in neither the pre- nor the post-ischaemia working heart.

### Discussion

This study has two important observations; reversing dobutamine-induced tachycardia in post-ischaemia using ivabradine was haemodynamically well tolerated and restored left ventricular pump function to pre-ischaemia levels. On the other hand, adding ivabradine to dobutamine did not improve cardiac efficiency as assessed by the work– $MVO_2$  relationship.



**Figure 4** Individual data ( $n = 10$ ) of selected main haemodynamic indices. Data presented as connected dot-plots from four consecutive times of measurement: Baseline, postischaemia, dobutamine (dobut) and dobutamine + ivabradine (dobut + iva). Panel (a), heart rate. Panel (b), cardiac output. Panel (c), stroke volume. Panel (d),  $dP dt^{-1} \max$ . Mean values are given in Table 1.

**Table 2** Cardiac energetics and plasma metabolites

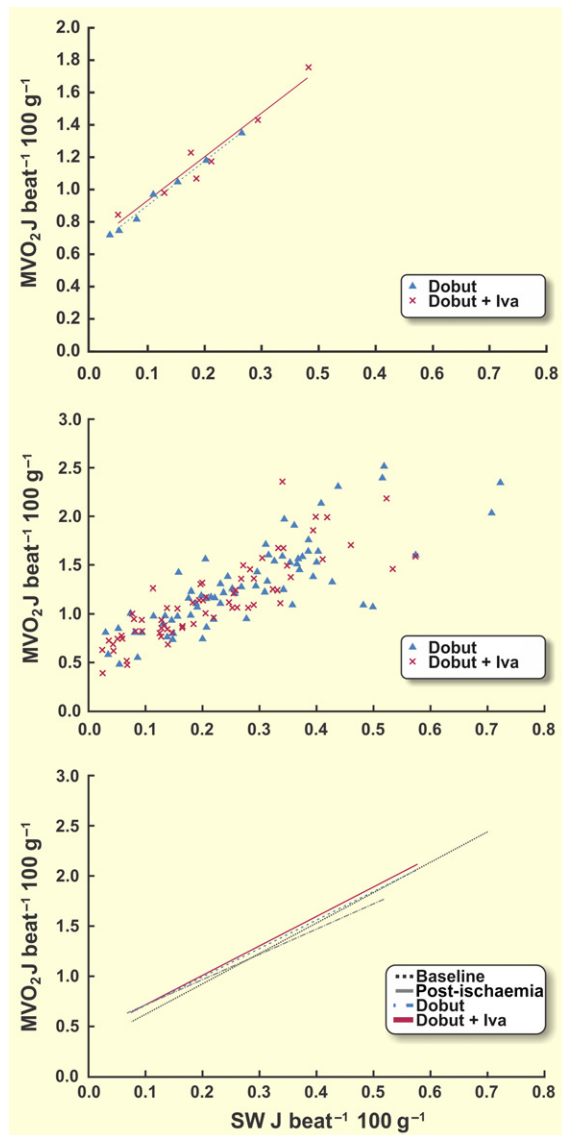
	Baseline	Post-ischaemia	Dobutamine	Dobutamine + Ivabradine
Stroke work, y-intercept	$0.46 \pm 0.1$	$0.52 \pm 0.1$	$0.54 \pm 0.1$	$0.55 \pm 0.2$
Stroke work, slope	$2.77 \pm 0.8$	$2.93 \pm 0.6$	$2.80 \pm 0.6$	$2.68 \pm 0.8$
Stroke work, $R^2$	$0.94 \pm 0.1$	$0.93 \pm 0.1$	$0.94 \pm 0.1$	$0.91 \pm 0.1$
Troponin T, $\mu\text{g L}^{-1}$	$50 \pm 28$	n/a	n/a	$225 \pm 129$
Free fatty acids, $\mu\text{mol L}^{-1}$	$233 \pm 66$	$239 \pm 126$	$503 \pm 283$	$622 \pm 267$
Glucose, $\text{mmol L}^{-1}$	$5.84 \pm 1.0$	$5.89 \pm 1.5$	$5.90 \pm 1.0$	$5.08 \pm 1.0$
Lactate, $\text{mmol L}^{-1}$	$1.12 \pm 0.5$	$1.54 \pm 0.7$	$0.88 \pm 0.3$	$0.98 \pm 0.3$
$MVO_2$ , $\text{J min}^{-1} 100 \text{ g}^{-1} 3\text{L CO}^{-1}$	$146 \pm 43$	$151 \pm 36$	$169 \pm 19$	$158 \pm 26$

The data show the relationship between left ventricular stroke work (SW) and myocardial oxygen consumption ( $MVO_2$ ) and plasma metabolites ( $n = 10$  pigs). SW consists of the area bounded by the pressure–volume loop (external work) obtained at steady state preload reductions. The Y-intercept and slope are values of individual experiments. The Y-intercept denotes the unloaded  $MVO_2$ . The slope denotes the contractile efficiency.  $R^2$  is the coefficient of determination. Troponin T was analysed at baseline and at the end of experiment. The free fatty acids, lactate and glucose levels are sampled from the femoral artery. The  $MVO_2$  in  $\text{Joule min}^{-1} 100 \text{ g}^{-1}$  left ventricular weight at 3 L in cardiac output.

Results from the SHIFT (Böhm *et al.* 2010) and the BEAUTIFUL (Fox *et al.* 2008) trials revealed better clinical outcome and a well-tolerated treatment,

respectively, in patients with chronic heart failure receiving ivabradine. In AHF, the significance of HR reduction is uncertain. That said, reducing tachycardia





**Figure 5** 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 upon 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 and Dobut, dobutamine.

in AHF management is potentially beneficial for two main reasons; lowering of myocardial energy demand and prolongation of coronary perfusion time. Digitalis is the only inotropic drug with a concomitant negative chronotropic effect. Unfortunately, the drug is not

feasible in treating critically ill ICU patients due to a narrow therapeutic spectrum and long elimination half-time. Alternatively, HR control could be obtained with  $\beta$ -adrenergic blockers. However, their negative inotropic effect and potential uncovering of the  $\alpha$ -adrenergic vasoconstriction (Heusch *et al.* 2000) limit its use in AHF patients (Chen *et al.* 2005, Van Diepen *et al.* 2014), and  $\beta$ -adrenergic blockers should be withheld until stabilization and/or discontinuation of inotropic agents (McMurray *et al.* 2012). In a critical condition where inotropic support is needed, dobutamine is the first agent of choice, a therapy that may raise concern due to a potentially increased  $MVO_2$  (Suga 1990).  $\beta$ -3-stimulated lipolysis elevates plasma free fatty acid levels, which causes a metabolic switch in the myocardium towards inefficiency (Korvald *et al.* 2000). However, we have previously shown that there is no excess  $MVO_2$  with the use of clinically relevant doses of dobutamine ( $2 \mu\text{g kg}^{-1} \text{min}^{-1}$ ) in pigs with post-ischaemia AHF. Myocardial oxygen wastage was only observed at suprathreshold levels ( $10 \mu\text{g kg}^{-1} \text{min}^{-1}$ ) and was associated with a complete offset in haemodynamics (Müller *et al.* 2010). Dobutamine infusion ( $5 \mu\text{g kg}^{-1} \text{min}^{-1}$ ) in this study caused a substantial and proportional increased CO, HR and  $MVO_2$ , resulting in maintained cardiometabolic efficiency.

Another concern related to dobutamine treatment in AHF is the exaggeration of the tachycardia response with a potential for inducing malignant arrhythmias. According to case reports (Link *et al.* 2009, Franke *et al.* 2011), ivabradine exerts a favourable haemodynamic effect in counteracting dobutamine-induced tachycardia, with further facilitated weaning from dobutamine. This has led to formulation of hypothesis (Roubille *et al.* 2013) and further clinical studies of ivabradine as an adjunct to inotropic treatment in AHF (Gallet *et al.* 2014, Cavusoglu *et al.* 2015). Selective HR reduction by ivabradine is shown to reduce  $MVO_2$  in a dose-dependent manner with preserved systolic function (Colin *et al.* 2004). In a canine model of exercise-induced ischaemia LV dysfunction, ivabradine increased systolic wall thickening and improved coronary blood flow (Monnet *et al.* 2001, Vilaine *et al.* 2003). Our model of ischaemia-reperfusion-induced LV dysfunction has many of the haemodynamic features seen in post-ischaemia AHF with impaired contractile function, mild tachycardia and a reduced SV. Inotropic support ( $5 \mu\text{g kg}^{-1} \text{min}^{-1}$  dobutamine) increased cardiac output (CO) by a further elevation of HR but did not restore SV. Adding ivabradine abolished this chronotropic effect of dobutamine, resulting in a 'pure inotropy' strategy that increased SV to pre-ischaemia levels without a significant reduction in MAP or CO. Ivabradine did not

**Table 3** *Ex vivo* mouse: cardiac energetics and haemodynamics

	Baseline	Isoprenaline	Vehicle/Ivabradine	Post-ischaemia
Heart rate (bpm)				
Control	384 ± 39	479 ± 21*	489 ± 21 <sup>§,*</sup>	371 ± 27 <sup>§,†,‡</sup>
Ivabradine	394 ± 38	487 ± 45*	397 ± 67 <sup>§,†</sup>	261 ± 56 <sup>§,*†,‡</sup>
Cardiac output (mL min <sup>-1</sup> )				
Control	14.0 ± 1.4	17.9 ± 1.6*	18.0 ± 1.4*	10.0 ± 2.9 <sup>*,†,‡</sup>
Ivabradine	14.1 ± 1.2	17.9 ± 1.0*	15.4 ± 3.0	9.2 ± 2.0 <sup>*,†,‡</sup>
Stroke volume (μL)				
Control	36.9 ± 5.1	37.5 ± 2.8	36.7 ± 3.1	26.8 ± 8.2 <sup>*,†,‡</sup>
Ivabradine	36.2 ± 3.3	36.8 ± 2.7	38.0 ± 6.2	34.3 ± 5.7
MVO <sub>2</sub> (μmol O <sub>2</sub> min <sup>-1</sup> )				
Control	1.4 ± 0.2	2.1 ± 0.4*	2.1 ± 0.4*	1.0 ± 0.2 <sup>*,†,‡</sup>
Ivabradine	1.4 ± 0.2	2.0 ± 0.3*	1.5 ± 0.4 <sup>*,†</sup>	1.0 ± 0.3 <sup>†,‡</sup>
Cardiac efficiency (%)				
Control	14.1 ± 1.3	12.1 ± 2.3	12.2 ± 1.7	12.7 ± 1.6
Ivabradine	14.4 ± 1.9	13.0 ± 1.8	14.0 ± 1.6	12.8 ± 2.5

Haemodynamic and cardiometabolic data from isolated working mouse hearts in controls ( $n = 8$ ) and ivabradine-treated mice ( $n = 8$ ). The myocardial oxygen consumption (MVO<sub>2</sub>) is calculated as the arteriovenous oxygen difference in the buffer multiplied by the coronary flow (CF). Cardiac efficiency is calculated as the ratio between stroke volume (SV) and MVO<sub>2</sub> beat<sup>-1</sup>. \* $P < 0.05$  within group compared to baseline. † $P < 0.05$  within group compared to isoprenaline. ‡ $P < 0.05$  within group compared to vehicle/ivabradine. § $P < 0.05$  between groups.

affect LV contractility. Diastolic function was also maintained following ivabradine infusion. Thus, the enhanced ventricular relaxation (Cheng *et al.* 1990) (i.e. Tau and peak filling rates.) caused by dobutamine was maintained. Importantly, ivabradine prolonged diastolic filling time by (45 ± 19%), resulting in a significant increase in the SW and SV. Taken together, this drug combination with simultaneously contractile enhancement and prolongation of diastole suggests an optimized pump function for the left ventricle. The heart spends limited time in the energy-consuming systolic phase (short ejection time), rapidly relaxes (short tau) and prolongs the time in a complete relaxed state allowing optimal diastolic filling. A study addressing the therapeutic potential of ivabradine in a severe form of AHF with diastolic dysfunction including pulmonary congestion seems warranted.

No previous study has reported on the effect of ivabradine on cardiac efficiency assessed by a work–MVO<sub>2</sub> relationship. Lauzier *et al.* (2011) found no change in energy substrate metabolism in *ex vivo* working hearts perfused in the presence of ivabradine. In chronic heart failure, ivabradine has been suggested to reduce MVO<sub>2</sub> and preserve contractility (De Ferrari *et al.* 2008). This could potentially improve cardiac efficiency based on the notion that HR is a major determinant of MVO<sub>2</sub>. However, HR is not the sole determinant of MVO<sub>2</sub> (Braunwald 1971). Colin *et al.* (2004) found the HR to be relatively more reduced by ivabradine compared to MVO<sub>2</sub> in exercising dogs. They proposed that the increase in SW partially

counteracted oxygen-sparing effect of HR reduction. This is in line with our observation that ivabradine had no impact on the SW–MVO<sub>2</sub> relations at a broad range of workloads, resulting in a maintained cardiac efficiency (Baxley *et al.* 1977).

To circumvent potential neurohumoral effects on the energetics, we measured MVO<sub>2</sub> in isolated *ex vivo* working and unloaded mouse hearts perfused with isoproterenol and ivabradine. These data confirm the energetic neutrality of ivabradine in combination with a  $\beta$ -agonist.

The therapeutic potential of ivabradine+dobutamine cotreatment is not limited to post-ischaemia AHF. Dobutamine is administered in patients with AHF over a broad range of aetiologies, that is post-cardiac surgery AHF, post-resuscitation AHF, septic cardiomyopathy and acute ischaemia AHF. That said, ivabradine is also an alternative to  $\beta$ -blocker in other critically ill patients with tachycardia, as suggested by De Santis *et al.* (2013). Not least, the energetic neutrality of this cotreatment expands the clinical relevance.

#### Limitations

The experiments are performed in general anaesthesia and with amiodarone 5 mg kg<sup>-1</sup>. This causes reduced chronotropy and inotropy compared to wake animals (Gelissen *et al.* 1996, van Erven & Schlij 2010). The ischaemia–reperfusion protocol in our study produces a cardiac dysfunction where cardiometabolic- and haemodynamic parameters remain stable for several

hours (Sun *et al.* 1995, Korvald *et al.* 2001). This is mandatory to properly evaluate myocardial energetics. However, clinical application of this treatment is intended to patients suffering severe AHF/cardiogenic shock. An energetic study in an unstable and deteriorating circulation is difficult because of the downloading protocol. Therefore, further studies should be undertaken to address the combination of dobutamine and ivabradine in severe AHF with haemodynamic instability. Also, the protocol used is a short evaluation. A minimal invasive model of post-ischaemia acute heart failure that follows the multi-systemic adaptations the first critical days is needed. This would make it feasible to assess the impact on systemic inflammation, multi-organ dysfunction, including kidney and heart metabolism caused by the combination of dobutamine and ivabradine.

## Conclusions

Adding ivabradine reverses the chronotropic effect of dobutamine and restores the SV and SW by increasing diastolic filling time while maintaining the MAP and CO. Also, adding ivabradine partly counteracted the dobutamine-induced increase in myocardial oxygen consumption. However, the work–MVO<sub>2</sub> relationship was unaffected, suggesting a maintained cardiac efficiency by the combined use of dobutamine and ivabradine.

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