

## Myosin Activator Omecamtiv Mecarbil Increases Myocardial Oxygen Consumption and Impairs Cardiac Efficiency Mediated by Resting Myosin ATPase Activity

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**Background**—Omecamtiv mecarbil (OM) is a novel inotropic agent that prolongs systolic ejection time and increases ejection fraction through myosin ATPase activation. We hypothesized that a potentially favorable energetic effect of unloading the left ventricle, and thus reduction of wall stress, could be counteracted by the prolonged contraction time and ATP-consumption.

**Methods and Results**—Postischemic left ventricular dysfunction was created by repetitive left coronary occlusions in 7 pigs (7 healthy pigs also included). In both groups, systolic ejection time and ejection fraction increased after OM (0.75 mg/kg loading for 10 minutes, followed by 0.5 mg/kg/min continuous infusion). Cardiac efficiency was assessed by relating myocardial oxygen consumption to the cardiac work indices, stroke work, and pressure–volume area. To circumvent potential neurohumoral reflexes, cardiac efficiency was additionally assessed in ex vivo mouse hearts and isolated myocardial mitochondria. OM impaired cardiac efficiency; there was a 31% and 23% increase in unloaded myocardial oxygen consumption in healthy and postischemic pigs, respectively. Also, the oxygen cost of the contractile function was increased by 63% and 46% in healthy and postischemic pigs, respectively. The increased unloaded myocardial oxygen consumption was confirmed in OM-treated mouse hearts and explained by an increased basal metabolic rate. Adding the myosin ATPase inhibitor, 2,3-butanedione monoxide abolished all surplus myocardial oxygen consumption in the OM-treated hearts.

**Conclusions**—Omecamtiv mecarbil, in a clinically relevant model, led to a significant myocardial oxygen wastage related to both the contractile and noncontractile function. This was mediated by that OM induces a continuous activation in resting myosin ATPase. (*Circ Heart Fail.* 2015;8:766-775. DOI: 10.1161/CIRCHEARTFAILURE.114.002152.)

**Key Words:** contractility ■ heart failure ■ inotropic agent ■ myocardial metabolism  
■ omecamtiv mecarbil ■ oxygen consumption

Omecamtiv mecarbil (OM) is a novel synthetic cardiac inotrope with a unique mechanism of action. It is classified as a myosin activator, discovered through high-throughput screening with a cardiac myosin ATPase bioassay.<sup>1</sup> The compound has been identified as potentially strengthening of the cardiac muscle and is presently being tested in chronic heart failure patients in a phase 2b trial (www.clinicaltrials.gov NLM identifier: NCT01786512). Initial studies argue that OM works independently of the calcium transient through an increase in the number of myosin heads interacting with the actin filament.<sup>2</sup> Analogously, this has been described as more hands pulling on the rope.<sup>3</sup> OM extends systolic ejection and

augments left ventricular (LV) shortening, thereby increasing LV ejection fraction (EF). This can potentially improve cardiac efficiency through reduction of LV wall stress. Conversely, a prolonged systolic ejection time (SET) may impair efficiency because systole is the primary energy-consuming phase of the cardiac cycle reflecting prolonged myosin ATPase activation. Administration of OM in a canine model of heart failure indicated no increase in myocardial oxygen consumption (MVO<sub>2</sub>) and thus suggested improved cardiac efficiency after OM treatment.<sup>4</sup> In this study, however, MVO<sub>2</sub> was not related to cardiac work, nor was substrate metabolism assessed. Thus, the aim of the present study has been to clarify the cardiac energetic and metabolic profile of OM. Besides healthy pigs, we have used a clinically relevant pig model of postischemic

### Clinical Perspective on p 775

Received November 12, 2014; accepted May 27, 2015.

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*Circ Heart Fail* is available at <http://circheartfailure.ahajournals.org>

DOI: 10.1161/CIRCHEARTFAILURE.114.002152

LV dysfunction,<sup>5</sup> an ex vivo working mouse heart model without neurohumoral influence,<sup>6</sup> and isolated mitochondria from the mouse myocardium. Our hypothesis has been that OM has a neutral effect on myocardial energy consumption because the favorable effects of reduced wall stress potentially can be counteracted by the prolongation of systole through the myosin ATPase activity.

## Methods

### Experimental Animals

The experimental protocols were approved by the local steering committee of the National Animal Research Authority at the Faculty of Health, UIT, The Arctic University of Norway. Twenty-two castrated male domestic pigs weighing 28±6 kg were adapted to the animal department for 5 to 7 days and fasted overnight before experiments, with free access to water. Additionally, 49 female NMRI mice from Charles Rivers Laboratories (Wilmington, MA) were used in this study. All mice received chow and drinking water ad lib and were housed at 23°C.

### Anesthesia and Surgical Preparation

#### In Vivo Pig

The pigs were premedicated with intramuscular injections of 20 mg/kg Ketamine (Pfizer AS, Norway) and 1 mg of atropine (Nycomed Pharma, Norway). Anesthesia was induced by inhalation of isoflurane (Abbot, Norway). After endotracheal intubation, an intravenous injection of 10 mg/kg pentobarbital sodium (Abbott, Sweden) and 0.01 mg/kg fentanyl (Hameln Pharmaceuticals, Germany) were given, and the animals were normoventilated. An introducer sheath catheter was placed in the left internal jugular vein, and anesthesia was maintained throughout the experiment by a continuous infusion of 4.0 mg/kg/h pentobarbital sodium, 0.02 mg/kg/h fentanyl, and 0.3 mg/kg/h midazolam (B. Braun, Germany). The circulating volume was maintained by a 20 mL/kg/h continuous infusion of 0.9% NaCl supplemented with 1.25 g/L glucose. The animals received 2500 IU heparin and 5 mg/kg amiodarone (Sanofi-Synthelabo, Sweden) to avoid blood clotting of catheters and cardiac arrhythmias.

The surgical preparation of the open chest pig model has been described in detail previously.<sup>5</sup> In brief, a 7.5 Fr Swan-Ganz catheter was placed in the pulmonary artery via the jugular vein. A 7 Fr manometer-pressure catheter (Millar MPVS Ultra, Houston, TX) was placed in the left ventricle. Arterial pressure was measured via a vascular catheter in the abdominal aorta. A 7 Fr balloon catheter was introduced to the inferior vena cava for preload reduction. After medial sternotomy, the pericardium was removed, and the coronary arteries and pulmonary trunk were dissected free to place transit-time flow probes (Medi-stim, Norway) for measurements of coronary blood flow and cardiac output. Three sonomicrometry crystals (Sonometrics corporation, trx 4, Canada) were placed in the myocardium to measure long axis and short axis myocardial shortening. Epicardial echocardiography (Vivid I, GE, Fairfield, CT) was used to calibrate the crystal dimensions to ventricular volumes. Myocardial venous blood was sampled from a catheter placed in the great cardiac vein via the coronary sinus (after ligating the hemiazygous vein).

#### Ex Vivo Mouse

Isolated perfused mouse hearts were used for assessment of unloaded MVO<sub>2</sub> and myocardial substrate oxidation as described previously.<sup>7</sup> In brief, the mice were anesthetized with 10 mg sodium pentobarbital IP. The hearts were quickly excised, and the aorta was cannulated and initially perfused retrogradely (Langendorff) with recycled Krebs-Henseleit bicarbonate buffer containing 5 mmol/L glucose and 0.4 mmol/L palmitate bound to 3% bovine serum albumin. Subsequently, hearts were perfused either in unloaded retrograde mode using for assessment of unloaded MVO<sub>2</sub><sup>6</sup> or in the working heart mode for assessment of myocardial glucose and fatty acid oxidation rates by the use of radiolabeled isotopes.<sup>7</sup>

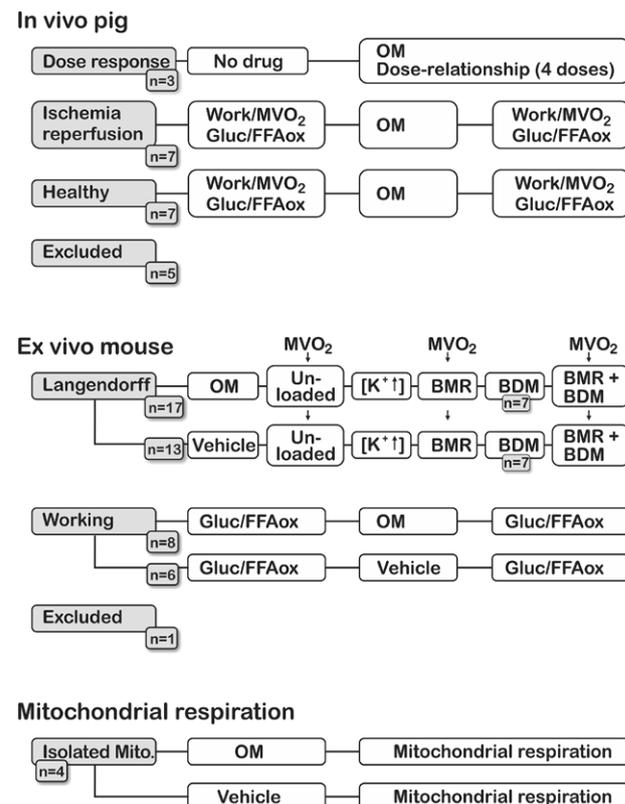
In the retrograde perfused unloaded hearts, the ventricular cavity was vented by inserting a 25 G steel cannula through the apex of the heart, allowing drainage of any perfusate trapped in the LV lumen. In the working heart perfusions, the left atrium was cannulated with a 16 G steel cannula connected to a preload reservoir ensuring forward perfusion through the aortic valve. Aortic and filling pressures were set to column heights of 55 and 8 mmHg, respectively. Electrodes were placed on the right atrium for electric pacing at 7 Hz, and cardiac temperature was maintained at 37°C throughout in both perfusion modes.

### Experimental Protocols

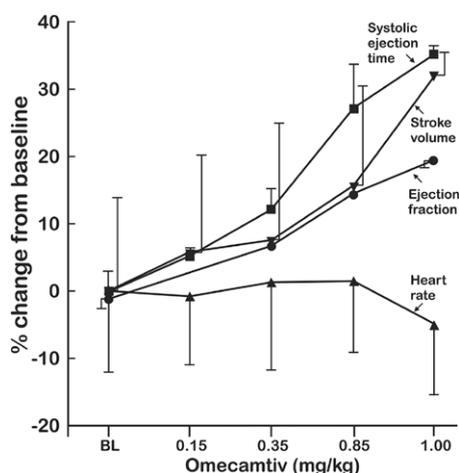
One in vivo pig, 2 ex vivo mouse heart, and 1 isolated mitochondrial protocol were performed (Figure 1). All protocols were conducted with a repeated measures design with measurements at baseline and following the administration of OM or vehicle. OM (Selleck Chemicals, TX) was formulated for all experiments as a solution of 1 mg/mL with 50 mmol/L citrate in sterile water, adjusted to pH 5.0 with NaOH.<sup>8</sup> With reference to animal<sup>2,4</sup> and human studies,<sup>3,8</sup> we decided on a dose targeting a 20% increase in SET, thus being at a clinical relevant level, providing a significant hemodynamic output.<sup>3,8</sup> SET was defined in the pig model as the time between peak positive and peak negative derivatives of LV pressure (dP/dmax and dP/dmin, respectively)<sup>4</sup> and in the mouse model as the time between minimum aortic pressure and the dirotic notch.

#### In Vivo Pig

In a dose-response protocol (n=3), intravenous infusion of OM showed a linear increase in SET, cardiac output, and EF until maximum dose of 1 mg/kg (Figure 2). Based on this data, the following dose was selected for the main protocol; a bolus dose of 0.75 mg/kg over 10 minutes followed by a continuous infusion of 0.5 mg/kg/h. This corresponds to a plasma concentration of 500 to 1000 ng/mL.<sup>3,8</sup>



**Figure 1.** Outline of experimental protocols. BDM indicates 2,3-butanedione monoxide; BMR, basal metabolic rate; Gluc/FFAox, glucose/free fatty acids oxidation; MVO<sub>2</sub>, myocardial oxygen consumption; and OM, omecamtiv mecarbil.



**Figure 2.** Dose-response protocol in pigs (n=3). Omecamtiv (2.25 mg/min) was given continuously and hemodynamic recordings were obtained at different timepoints during a 16 minutes period. The accumulated omecamtiv mecarbil (OM) dose at these timepoints is given on the x-axis. Data (means±SD) are reported as % change from baseline.

The effect of OM on cardiac energetics was then assessed in healthy pigs (n=9) and in pigs with postischemic LV dysfunction (n=10). After surgery and a 30-minute stabilization period, baseline measurements were performed. In the postischemic group, acute heart failure was induced using our ischemia-reperfusion model.<sup>5</sup> In short, this protocol uses repetitive coronary occlusion and reperfusion episodes with a total of ≈20 minutes of accumulated ischemia. The occlusion affects ≈80% of the LV and induces a reproducible acute impairment of LV function, which remains stable for several hours. Then pigs were stabilized for 30 to 60 minutes before performing postischemic measurements. Afterward, OM infusion was initiated, and the final recordings were performed after 20 minutes. The healthy group had an identical experimental protocol without the induction of ischemia-reperfusion injury.

### Ex Vivo Mouse

The dose-response relationship of OM was studied in ex vivo working mouse hearts (n=3), using a range of OM concentrations from 100 to 1200 ng/mL. An increased SET of 15% to 20% was obtained at 400 ng/mL of OM, which was selected for the subsequent protocols.

In the Langendorff perfusions (n=30), baseline unloaded  $MVO_2$  measurements were made after 20 minutes of stabilization before OM (n=17) or vehicle (n=13) was added to the recirculating buffer. After a second stabilization period, new unloaded  $MVO_2$  measurements were performed. Then extracellular  $K^+$  concentration was raised to 16 mmol/L to electrically arrest the heart, which allowed measurement of basal  $MVO_2$ . Oxygen cost of excitation-contraction coupling was calculated as the difference between  $MVO_2$  measured before and after cardioplegia. In a substudy (n=7, each group), 20 mmol/L of the myosin ATPase inhibitor 2,3-butanedione monoxide (BDM; Sigma Aldrich, USA) was added after basal  $MVO_2$  measurements. After stabilization,  $MVO_2$  was measured in the arrested heart with inhibited myosin ATPase activity.

The working heart perfusions (n=14) were used for assessment of myocardial glucose and free fatty acids (FFA) oxidation. Both before and after OM (n=8) or vehicle (n=6) administration, 5 consecutive samples of the perfusion buffer were taken with 7 minutes interval. Hemodynamic values were recorded simultaneously.

### Isolated Mitochondrial Respiration

Mitochondria were extracted from mouse hearts (n=4) using the method of Palmer et al<sup>9</sup> with slight modification. Briefly, the heart was cut in small pieces, homogenized, and treated with trypsin (5 mg/mL and 1 mL/g) in isolation buffer, followed by differential centrifugation. Mitochondria were suspended in preservation buffer (Orboros, Innsbruck, Austria) at a concentration of 4  $\mu$ L/mg

tissue and stored on ice for 1 to 3 hours. Mitochondrial respiration was measured using a Clark-type electrode (Oxygraph-2k Orboros Instruments) in both the absence and presence of 200 nmol/L OM. Malate (2 mmol/L) and pyruvate (5 mmol/L) were used as substrates. adenosine diphosphate (ADP; 200  $\mu$ mol/L) was added to achieve maximal mitochondria oxidative phosphorylation (OXPHOS) capacity. Measurement was performed at 37°C in 2 mL Mir05 mitochondrial respiration medium, adjusted to pH 7.1. Mitochondrial LEAK respiration was measured in presence of substrates, but absence of ADP. State 3 respiration was defined at maximal OXPHOS after adding ADP. State 4 respiration was recorded when all added ADP was converted to ATP and state oligo after adding the ATPase blocker oligomycin (4  $\mu$ g/mL). *P/O* ratio was calculated by measuring the mitochondrial oxygen consumption used to deplete 200  $\mu$ mol/L ADP (ATP produced per oxygen atom reduced by the respiratory chain).

## Left Ventricular Function and Energetics

### In Vivo Pig

LV pressure, sonomicrometric blood flow, and vascular pressure signals were recorded, digitized, and analysed using ADI LabChart Pro software (Dunedin, New Zealand). At baseline, and after interventions, the LV end diastolic volume (EDV) was calculated from epicardial short axis ultrasound data (Vivid i, GE) using the Teicholz formula,  $EDV = [7 / (2.4 + EDD)] \times (EDD^3)$ . The end systolic volume was calculated by subtracting stroke volume (SV; from Time-transit flow probe on the pulmonary artery) from the LV EDV. The short- and long-axis sonomicrometric crystals were converted to a composite output using the Area Length (Bullet) formula,<sup>10</sup>  $Volume = 5/6 \times Area \times Length$ . The composite sonomicrometric output was calibrated against end systolic volume and EDV at each intervention.

To assess cardiac efficiency by the work- $MVO_2$  relationship, 6 to 8 recordings of varying steady-state work levels, hemodynamic parameters, coronary blood flow (CBF), and blood sampling were performed. Preload was reduced stepwise by inflating the balloon catheter in the caval vein to obtain different levels of mechanical work and their corresponding oxygen consumption as described in detail previously.<sup>11</sup> LV mechanical work was calculated as stroke work<sup>12</sup> and pressure-volume area (PVA).<sup>13</sup> In brief, PVA consists of the area bounded by the pressure-volume loop (stroke work) and the triangular area limited by the line of the end-systolic and end-diastolic pressure-volume relations, as obtained by a transient vena cava occlusion. The Y-intercept in the PVA- $MVO_2$  relation indicates the myocardial oxygen cost not related to pump function (unloaded  $MVO_2$ ). 1/slope indicates the energy cost for contractile work (contractile efficiency).  $LVCBF$  was calculated from the formula  $LVCBF = CBF / W \times LVW$ ,<sup>14</sup> where  $LVCBF$  and  $CBF$  are LV and total CBF, respectively.  $W$  and  $LVW$  are total myocardial and LV myocardial weight, respectively. LV  $MVO_2$  was calculated from the formula  $MVO_2 = (LVCBF \times avdO_2 \times Hb \times 1.39) / HR \times 20.2$ , where  $MVO_2$  is LV  $MVO_2$ ,  $avdO_2$  is the difference between aortic and myocardial venous oxygen saturations,  $Hb$  is hemoglobin in grams per liter, 1.39 is a constant (mL  $O_2$ /g Hb), and  $HR$  is heart rate. To convert  $MVO_2$  to mechanical energy equivalents, the factor 20.2 J/mL  $O_2$  was used.

### Ex Vivo Mouse

Coronary flow was derived from timed measurements of coronary effluent.<sup>6</sup> Fiber optic probes (FOXY-AL 300; Ocean Optics, Duiven, Netherlands) were used to measure partial oxygen pressure ( $P_{O_2}$ ) in the arterial and venous coronary buffer. These were placed proximally in the aortic line and in the pulmonary trunk, respectively.  $MVO_2$  was then calculated by the following equation:  $MVO_2 = [P_{O_2}(\text{coronary inflow}) - P_{O_2}(\text{coronary effluent})] \times \text{Bunsen solubility coefficient of } O_2 \times \text{coronary flow}$ .<sup>6</sup>

## Metabolism

### In Vivo Pig

Methods for assessing myocardial glucose and FFA oxidation by isotopic tracers are described in details previously.<sup>11</sup> In brief, isotope-labeled oleic acid and glucose was dissolved in 50 mL of

**Table 1. Hemodynamics**

	Healthy Hearts Group			Postischemic LV Dysfunction Group		
	Baseline	Healthy	OM	Baseline	Postischemia	OM
Mean arterial pressure, mmHg	86±7	85±9	83±8	84±6	71±6*†	65±5*†
Mean pulmonary arterial pressure, mmHg	21±2	22±2	22±2	20±1	25±2*	25±4 *
Heart rate, beats/min	81±14	91±14	94±15	82±13	120±31*†	118±33*†
Cardiac output, L/min	2.56±0.29	2.80±0.37	2.83±0.52	2.72±0.37	2.85±0.46	3.10±0.53*
Systemic vascular resistance, dyn s/cm <sup>5</sup>	2509±309	2273±332	2190±323*	2321±423	1880±569*	1570±509*†‡
Stroke work, mmHg mL	2770±531	2686±652	2626±791	2828±465	1752±339*†	1800±458*†
Stroke volume, mL	32±5	31±6	31±8	34±6	25±7*	28±8*
End-diastolic volume, mL	69±10	70±10	61±12*†	68±4	68±16	56±17*†
End-systolic volume, mL	37±5	39±6	30±8‡	34±3	42±12	28±9‡
Ejection fraction, %	46±2	44±4	51±8	50±5	39±7*	54±12‡
Coronary blood flow, mL/beat	1.25±0.29	1.28±0.27	1.68±0.53*†	1.30±0.33	1.39±0.35	1.65±0.31*†
Tau, ms	31±2	31±2	37±4*†	32±1	35±8	46±12*†
Diastolic filling time, ms	512±110	442±88	390±75*†	494±112	311±106*†	280±107*†
Systolic ejection time, ms	242±8	229±14	266±22‡	255±15	224±30*	270±45‡
End-diastolic pressure, mmHg	13±3	11±2	10±2*	13±3	13±7	9±4*†
dP/dtmax, mmHg/s	1523±344	1773±613	2157±630*†	1500±249	1460±429	1918±734
dP/dtmin, mmHg/s	2656±510	2912±738	3173±903	2743±416	2114±881	1929±587*

Hemodynamic data were assessed in both the healthy and the postischemic LV dysfunction group (n=7 in both groups, except dP/dtmax/min, and tau (both n=6)). There were 3 hemodynamic measurements in each group. Baseline, with or without postischemia and subsequently after administration of omecamtiv mecarbil (OM). dP/dtmax/min denotes peak positive and negative derivative of left ventricular pressure. Systolic ejection time denotes time between dP/dt max and min. Diastolic filling time refer to cardiac cycle time minus systolic ejection time.

\*P<0.05 vs baseline, analysed by 1-way repeated measures ANOVA.

†P<0.05 between healthy and postischemic LV dysfunction group measurements, analysed by linear mixed model analysis.

‡P<0.05 vs before drug, analysed by 1-way repeated measures ANOVA.

plasma obtained from the pig to give a final radioactivity of 0.126 and 1.014 MBq/mL of [U-<sup>14</sup>C] glucose and [9,10-<sup>3</sup>H] oleic acid, respectively. Infusion of isotopes was started 30 minutes before administration of OM with a bolus of 30 mL/h for 15 minutes, continued by steady-state infusion at 8 mL/h throughout the experiment. Arterial and coronary sinus blood samples were drawn simultaneously before and 20 minutes after OM administration. Five 1-mL samples for <sup>14</sup>CO<sub>2</sub> determination were transferred to airtight <sup>14</sup>CO<sub>2</sub> trapping vials. Four 1.25-mL aliquots were cold-centrifuged, and the plasma was immediately frozen. Plasma was stored at -70°C and analysed later for determination of <sup>3</sup>H<sub>2</sub>O and substrate levels. The content of <sup>3</sup>H<sub>2</sub>O in plasma was determined by Folch extraction.<sup>15</sup> The <sup>14</sup>CO<sub>2</sub> content of the blood was assessed by a diffusion method, as described by Wisneski and associates.<sup>16</sup> Aliquots of blood (with trapped <sup>14</sup>CO<sub>2</sub>) or plasma water (with <sup>3</sup>H<sub>2</sub>O) were then mixed with scintillation fluid, and the radioactivity was determined

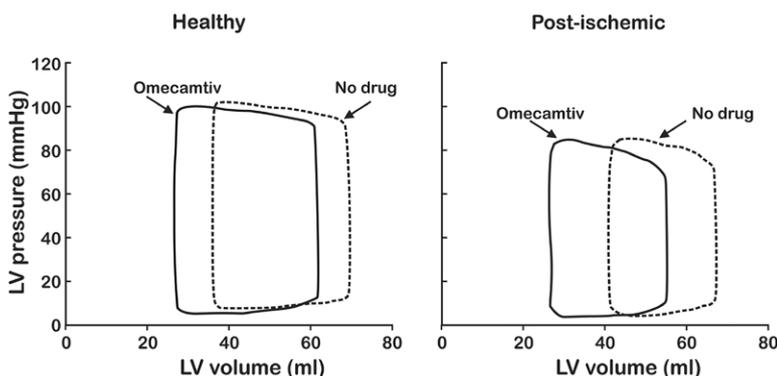
on a β-scintillation counter (Packard 1900 TR Liquid Scintillation Analyzer; Packard Instruments BV-Chemical Operations, Groningen, the Netherlands).

### Ex Vivo Mouse

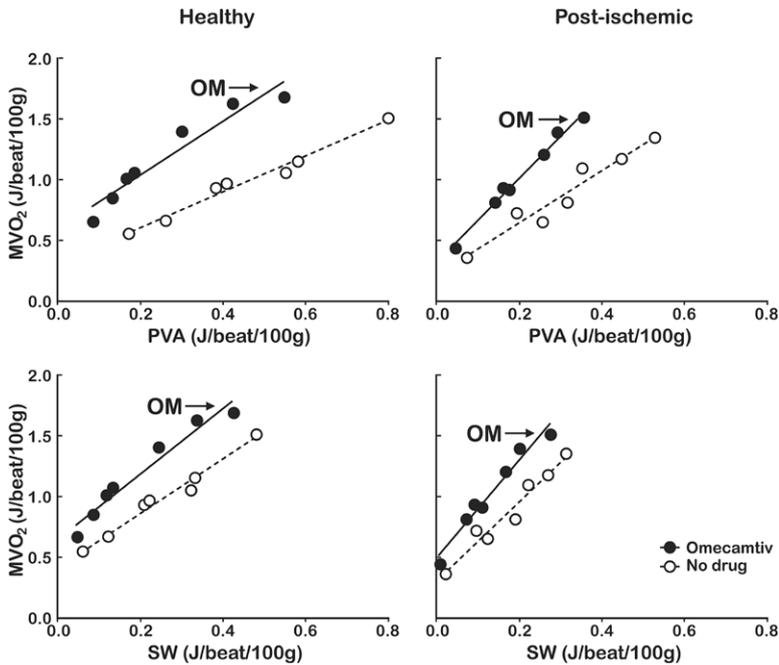
Glucose and FFA oxidation in the ex vivo working hearts were measured simultaneously as described previously.<sup>17</sup> Glucose oxidation was determined by measuring <sup>14</sup>CO<sub>2</sub> released by the metabolism of [U-<sup>14</sup>C] glucose and FFA oxidation by measuring <sup>3</sup>H<sub>2</sub>O released from [9,10-<sup>3</sup>H] palmitate. Cardiac output was obtained as the sum of aortic perfusate flow and coronary flow. At the end of the perfusion, hearts were frozen and total dry mass was determined.

### Statistical Analysis

All data are presented as means±standard deviation (SD), unless stated otherwise. Within-group effects of energetic data and



**Figure 3.** Illustration of typical pressure–volume loops showing the effect of omecamtiv on left ventricular (LV) function. The loops are based on mean volumes and pressure given in Table 1. **Left panel** shows loop from healthy animals and **right panel** from animals with postischemic LV dysfunction.

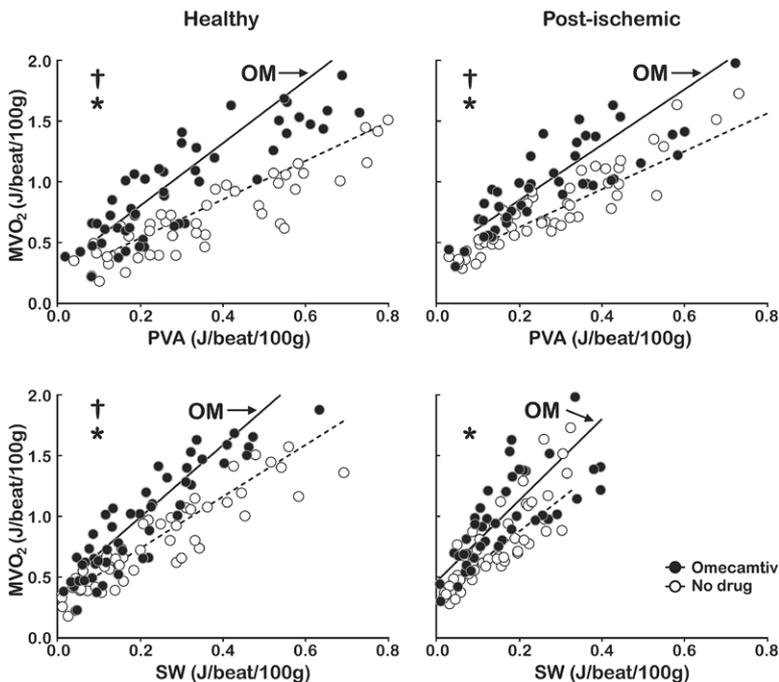


**Figure 4.** Example of 2 experiments showing the relation between left ventricular (LV) mechanical work and myocardial oxygen consumption. LV mechanical work is presented in the **upper panel** as pressure–volume area (PVA) and in the **lower panel** as stroke work (SW). **Left panels** are data from 1 healthy pig and **right panels** are data from 1 pig with postischemic LV dysfunction. Data are obtained at various workloads before (○) and after (●) infusion of omecamtiv mecarbil (OM). Y-intercept represents unloaded myocardial oxygen consumption ( $MVO_2$ ), that is, energy used for excitation–contraction coupling and basal metabolism.  $1/\text{slope}$  of the regression line represents the contractile efficiency of the heart.

between-group effects of hemodynamic data were analyzed using a linear mixed-models approach with a restricted maximum likelihood method and the subject identifier as the random effect. Within-group effects of hemodynamic data were assessed by 1-way repeated measures analysis of variance. We performed Wilcoxon signed-rank test to compare means of metabolic data from pigs and substrate metabolism in working mouse hearts. Measurements of  $MVO_2$  in unloaded retrograde perfused hearts and measurements of mitochondrial respiratory data were assessed using the Mann–Whitney Wilcoxon test. Multiple comparisons were adjusted for by Bonferroni correction.  $P$  values  $<0.05$  were regarded as statistically significant, and all analyses were conducted in SPSS 22.0 (Chicago).

## Results

A total of 19 pigs were used in the in vivo study. Fourteen pigs were included for analysis of energetics and hemodynamics in the healthy ( $n=7$ ) and the postischemic LV dysfunction ( $n=7$ ) group. Three pigs were excluded because of hemodynamic collapse after induction of myocardial ischemia with sustainable need of vasopressor and 2 pigs were excluded because of surgical complications. Forty-nine mice were used in the ex vivo study. A calibration error led to exclusion of 1 heart. Thirty mice were included in the retrograde perfusion (Langendorff) protocol for assessment of unloaded  $MVO_2$ . In the



**Figure 5.** Pooled scatters of left ventricular (LV) mechanical work–myocardial oxygen consumption ( $MVO_2$ ) relationship from all experiments. **Left panels** are from the healthy pigs ( $n=7$ ) and **right panels** are from pigs with postischemic LV dysfunction ( $n=7$ ). Omecamtiv mecarbil (OM) impairs cardiac efficiency as displayed by a significant increase in Y-intercept and slope in all panels, except for only increased Y-intercept in the lower **right panel**.  $*P < 0.05$  vs no drug for Y-intercept, and  $†P < 0.05$  vs no drug for slope (linear mixed model analysis). See Figure 4 for an extended legend. SW indicates stroke work.

**Table 2. Left Ventricular Energetics**

	Healthy		Postischemic LV Dysfunction	
	Healthy	Healthy+OM	Postischemia	Postischemia+OM
<b>A</b>				
Pressure–volume area, J/beat/100 g	0.78±0.15	0.53±0.15	0.49±0.11	0.46±0.16
MVO <sub>2</sub> , J/beat/100 g	1.25±0.29	1.38±0.39	1.07±0.29	1.33±0.30
Y-Intercept	0.22±0.12	0.29±0.14*	0.31±0.09	0.38±0.12*
Slope	1.58±0.38	2.58±0.60*	1.57±0.36	2.30±0.78*
R <sup>2</sup>	0.97±0.02	0.95±0.02	0.95±0.01	0.96±0.02
<b>B</b>				
Stroke work, J/beat/100 g	0.49±0.11	0.40±0.13	0.27±0.04	0.28±0.08
MVO <sub>2</sub> , J/beat/100 g	1.25±0.29	1.38±0.39	1.07±0.29	1.33±0.30
Y-Intercept	0.32±0.09	0.40±0.16*	0.36±0.11	0.47±0.12*
Slope	2.11±0.37	2.98±0.42*	2.63±0.61	3.32±1.00
R <sup>2</sup>	0.98±0.01	0.96±0.02	0.96±0.02	0.96±0.03

The data show the relationship between myocardial oxygen consumption (MVO<sub>2</sub>) and left ventricular (LV) mechanical work over a large range of workloads for both the healthy (n=7 pigs) and the postischemic LV dysfunction (n=7) group. LV mechanical work is presented in A as pressure–volume area and in B as stroke work. Y-intercept indicates MVO<sub>2</sub> for myocardial processes not related to pump function (unloaded MVO<sub>2</sub>), whereas the 1/slope indicates the energy cost for contractile work (contractile efficiency). R<sup>2</sup> is the coefficient of determination. OM indicates omecamtiv mecarbil

\*P<0.05 vs before drug, analysed by linear mixed model analysis.

working heart perfusion protocol for assessment of substrate oxidation, 14 mice were included, whereas in the mitochondrial respiratory assessment, 4 mice were included.

## LV Function

### In Vivo Pig Hearts

Cardiac effects of the ischemia-reperfusion protocol are shown in Table 1. The effects of the accumulated 17±5 minutes of ischemia are compatible with postischemic LV stunning<sup>5,18</sup> with reduced SV, stroke work, and EF and a concomitant increase in HR and mean pulmonary arterial pressure. The pigs received a OM dose targeting a clinically relevant increase in SET of 20%.<sup>3,8</sup> This dose resulted in a 16%±4% and 20%±6% increase in SET in healthy and postischemic hearts, respectively. At the same time, the HR was unaffected (Table 1). OM reduced diastolic filling time with ≈11%. These changes in the cardiac cycle can be illustrated by comparing the ratio of SET/diastolic filling time before and after OM, resulting in a 31% and 35% increase in the healthy and the postischemic group, respectively. Simultaneously, OM induced a substantial increase in CBF in both healthy and postischemic pigs (Table 1).

OM caused the left ventricle to work at lower volumes in both healthy and postischemic hearts (Figure 3) coupled with an increased EF (Table 1). However, the enhanced LV emptying was accompanied by an impaired ventricular filling as seen by a reduced EDV and an increased tau. Thus, the net result of these alterations was an unchanged SV after OM treatment in both healthy and postischemic hearts. Also, perfusion pressure in the systemic and pulmonary circulation was unchanged by OM.

### Ex Vivo Mouse Hearts

In ex vivo working mouse hearts, SET increased 16%±4% after OM administration. Cardiac output was unchanged in these hearts.

## Cardiac Efficiency and Metabolism

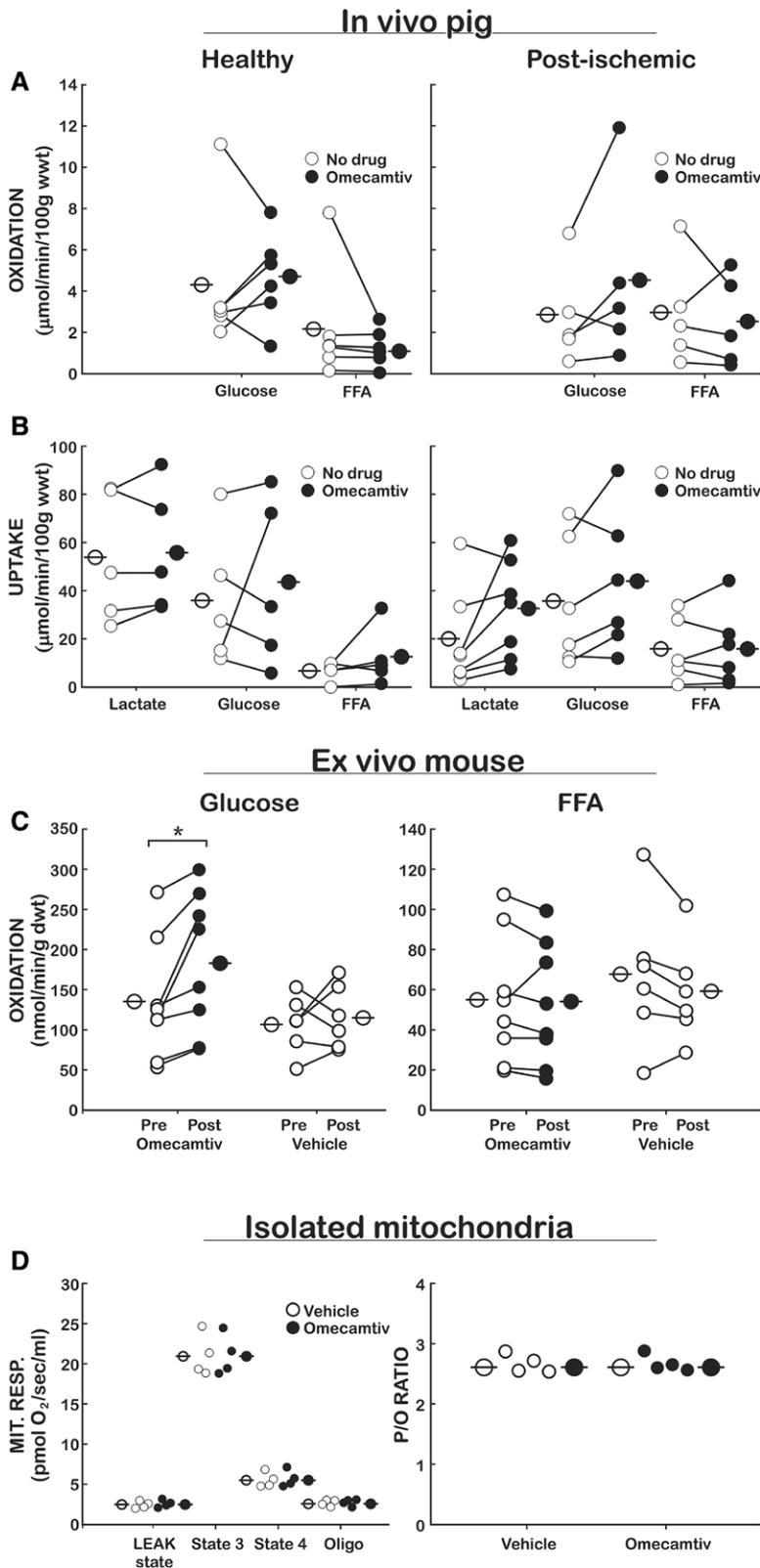
### In Vivo Pig Hearts

Figure 4 shows the effect of OM on the relation between mechanical work and MVO<sub>2</sub> in typical experiments with healthy and postischemic hearts. Figure 5 presents all data points used in the statistical analysis of the OM effect on the work–MVO<sub>2</sub> relationship. In therapeutically relevant doses, OM had a negative effect on cardiac efficiency as measured by increased MVO<sub>2</sub> relative to cardiac work (Table 2). The impaired energetic state by OM was observed in both healthy and postischemic hearts over a large range of workloads (Figure 5). Unloaded oxygen cost (y-intercept of the PVA–MVO<sub>2</sub> relation) increased by 31% and 23% in healthy and postischemic pig hearts, respectively. OM also impaired contractile efficiency as seen by a 63% (healthy) and 46% (postischemic) increase in the slope of the PVA–MVO<sub>2</sub> regression (Figure 5; Table 2).

OM only marginally affected the relative substrate consumption with a trend toward more glucose utilization at the expense of FFA (Figure 6A). The uptake of lactate, glucose, and FFA were not affected by OM (Figure 6B).

### Ex Vivo Mouse Hearts

OM increased unloaded MVO<sub>2</sub> in ex vivo mouse hearts similar to the extrapolated Y-intercept in the PVA–MVO<sub>2</sub> relation in pig protocols. This was attributed to a 63%±31% increase in basal metabolic rate, whereas oxygen cost of excitation–contraction coupling was unaffected. Adding the myosin ATPase inhibitor, BDM, abolished all surplus basal MVO<sub>2</sub> in the OM-treated hearts (Figure 7). There was a small increase in the rate of glucose oxidation with OM in the ex vivo working hearts (Figure 6C), whereas the mitochondrial respiration in isolated mitochondria from mouse hearts was unaffected by OM (Figure 6D). There was no difference in any of the respiratory states or mitochondrial efficiency shown by unchanged P/O ratio.

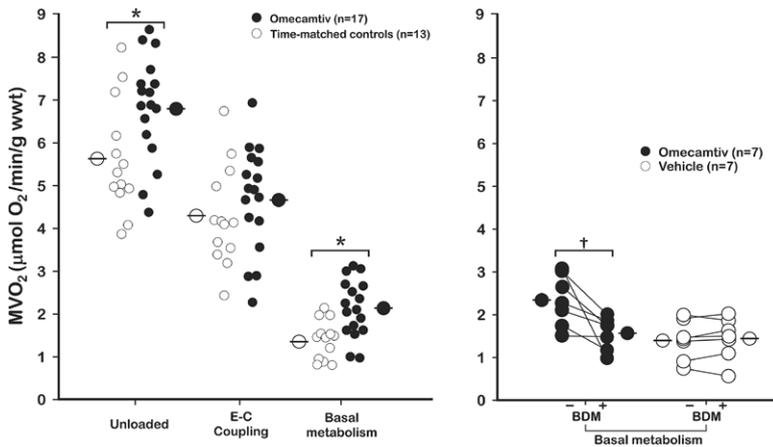


**Figure 6.** Dot plots presenting data in all panels. Mean values are presented as circles crossed by a horizontal line. **A**, Substrate oxidation rate of glucose and free fatty acids (FFA) before and after omecamtiv mecarbil (OM) in healthy pig hearts to the left and postischemic pig hearts to the right. Means compared by Wilcoxon signed-rank test. **B**, Myocardial uptake rate of glucose, lactate, and FFA in the same animals as in panel A (Wilcoxon signed-rank test). **C**, Glucose and FFA oxidation rate assessed in ex vivo working mice hearts treated with OM (n=8) and in time-matched controls (n=6). White dots are baseline/vehicle; black dots are OM. \* $P < 0.05$  vs preadministration of OM or vehicle (Wilcoxon signed-rank test). **D**, In vitro respiration of mitochondria isolated from mice hearts incubated with OM to the left and adenosine diphosphate/oxygen ratio (P/O ratio) to the right. Means compared by Mann-Whitney Wilcoxon test.

### Discussion

The main finding of this study is that OM contributes to a significantly increased myocardial oxygen cost in both healthy and postischemic stunned hearts. OM increases oxygen

consumption because of energetically inefficient LV function and increased oxygen consumption by noncontractile processes. The increase in  $\text{MVO}_2$  was mediated by hyperactivity in myosin ATPase.



**Figure 7.** Myocardial oxygen consumption ( $MVO_2$ ) in ex vivo mice 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**, There is significant increase in unloaded  $MVO_2$  and oxygen consumption from basal metabolism in hearts treated with omecamtiv ( $n=17$ ) compared with time-matched controls ( $n=13$ ). Oxygen cost for excitation–contraction (E–C) coupling was unaffected by omecamtiv mecarbil (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 seen in the controls ( $n=7$ ). \* $P<0.05$  vs omecamtiv, † $P<0.05$  vs without BDM, analysed by Mann–Whitney Wilcoxon test.

### Effect on Cardiac Function

OM's mechanism of action on the contractile apparatus is difficult to classify. An important characteristic of inotropy is accelerating the contraction and relaxation (measured as an increase in  $dP/dt$  max and min), indices that are only marginally affected by OM because it extends the systolic contraction rather than reinforcing it. On the other hand, OM increases EF similarly to classic inotropes by improved emptying in systole. Such reduction of wall stress is considered energetically favorable as systolic wall stress is a main determinant of  $MVO_2$ .<sup>19</sup> However, the oxygen cost of a prolonged systolic phase may outweigh the beneficial effect of a reduced wall stress. We found no significant changes in SV and cardiac output. This can potentially be explained by a reduced LV diastolic filling and increased Tau. Shen et al<sup>4</sup> found a small increase in SV in dogs given OM, and this could be caused by a reduced afterload as seen by a concomitant reduction in vascular resistance in these dogs.

The reduced contractile efficiency observed may be caused by an unfeasible increase of SET at the expense of diastolic filling time. The fact that EDV becomes smaller, despite unchanged preload and HR, suggest that OM induces myocardial constrain in late diastole. However, our stunning model of acute heart failure does not have the characteristics of diastolic dysfunction. Thus, the interpretation that OM impairs diastolic performance demands caution. Studies that address potential effects of OM in tachycardia and reduced coronary reserve should be performed. It is unclear how the LV filling and coronary perfusion are affected in the heart when there is a shortened diastole and concomitantly elevated HR. Thus, a thorough assessment of the impact of OM in a relevant model of diastolic dysfunction seems warranted.

### Effect on Cardiac Energetics

Our findings are in contrast with a previous study on dogs by Shen et al.<sup>4</sup> These authors found that OM significantly improved cardiac efficiency by enhancing LV function without a corresponding change in  $MVO_2$ .<sup>4</sup> They reported a nonsignificant increase in  $MVO_2$  after 24 hours of OM infusion ( $MVO_2$  from 3 to 4 mL/min). However, a formal

evaluation of cardiac efficiency require measurements of  $MVO_2$  and total cardiac work (ie, PVA) over a wide range of workloads.<sup>13</sup> Such an analysis can separate the energy consumption ( $MVO_2$ ) in unloaded  $MVO_2$  (the y-intercept of the regression line), reflecting the oxygen cost of excitation–contraction coupling and basal metabolism and contractile efficiency (increased slope of the regression line). In both the healthy and the posts ischemic pig hearts, we observed a pronounced impairment in cardiac efficiency after OM infusion. This was evident by increase of both the y-intercept and the slope of the PVA- $MVO_2$  regression.

To further elucidate the energetics, we measured unloaded  $MVO_2$  in ex vivo mouse hearts. Here we confirmed that the elevated y-intercept as seen in the pigs also was evident by direct measurements of unloaded  $MVO_2$  in mice. When the hearts were arrested by cardioplegia (ie, blocking the cyclic depolarization of the myocyte membrane), we observed that the surplus unloaded oxygen could be attributed to an increased basal metabolic rate and not because of the calcium handling in excitation–contraction coupling. This observation is compatible with the proposed action of OM; activation of myosin ATPase used in the sliding contraction of the myofilaments and not the traditional increased inotropy-controlled intracellular calcium transients.<sup>20</sup> Changes in substrate metabolism do not seem to be the explanation for the increased oxygen consumption in hearts perfused with OM. Only a marginal switch toward glucose oxidation after administration of OM was seen in both the pig and mouse heart, reaching significance only in the mouse protocol. However, glucose is in fact a more oxygen sparing substrate compared with fatty acids because of an increased  $P/O$  ratio.<sup>21</sup> Such a switch should therefore potentially counteract the observed inefficient energetic state in OM-perfused hearts. An increased basal metabolic rate could also be caused by an altered mitochondrial respiration, that is, mitochondrial uncoupling in the oxidation phosphorylation.<sup>22</sup> However, we did not observe any effect on the efficiency in isolated myocardial mitochondria that was incubated with OM. Combined with the missing alteration in substrate metabolism on both the organ and subcellular levels, this warrants

another explanation for the increased oxygen cost in non-contracting hearts. As OM is a nonbiological compound without integrated receptor, signal molecule, and ionic effectors, a distinct possibility is that the myosin ATPase is activated with no relation to the electrophysiological cycling of the myocardium and therefore is still active in noncontracting and arrested hearts.

The oxygen waste caused by the drug is likely to be a by-product of its interaction with myosin. A chemical precursor of OM was discovered through a high-throughput screening aimed at compounds increasing cardiac sarcomere ATPase activity.<sup>1</sup> This compound was refined to reduce its toxicity, and the fully developed OM showed a 40% increased activity of the myosin ATPase at only 0.58  $\mu\text{M}$ ,<sup>1</sup> demonstrating that OM is a potent activator of myosin ATPase. The contribution of the resting rate of myosin ATPase to basal metabolism is generally regarded as small or nonexistent in normal myocardium.<sup>23</sup> Most of these studies were done with the cross-bridge inhibitor BDM. The specificity of BDM as a myosin inhibitor is not known, and some researchers have found that it also affects the excitation–contraction coupling.<sup>23</sup> Ebus and Stienen examined saponin-skinned cardiac trabeculae from rats without BDM.<sup>24</sup> They were able to show that 40% of the basal activity remained after removing the contribution of all membrane-bound ATPase activity by stripping away membranes with Triton X-100. This suggests that myosin ATPase has a large role in determining unloaded  $\text{MVO}_2$  of basal metabolic rate. This is in line with our observation that BDM abolished all surplus  $\text{MVO}_2$  caused by OM treatment. As this assessment was conducted on arrested hearts with no oxygen cost for excitation–contraction coupling suggests that OM induces myocardial oxygen waste mediated by hyperactivity in resting myosin ATPase.

### Conclusions

This study shows that OM leads to a significant oxygen waste in the myocardium independent of neurohumoral factors. The oxygen waste can be explained by a combination of a reduced contractile efficiency and increased energy consumption in the noncontracting muscle, as mediated by hyperactivity in resting myosin ATPase. An assessment of OM's effect on cardiac energetics in humans is of major interest given the ongoing clinical trials of the drug in heart failure patients. A clinical trial ([www.clinicaltrials.gov](http://www.clinicaltrials.gov) NLM identifier: NCT00748579) addressing this issue was terminated with only 2 patients included.

### Acknowledgments

We acknowledge Trine Lund for assisting with ex vivo mouse hearts and mitochondrial respiration protocols.

### Sources of Funding

This work was financially supported by the health authorities of North Norway and the Norwegian Council of Cardiovascular Diseases.

### Disclosures

None.

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### CLINICAL PERSPECTIVE

Despite their potential negative side effects, that is, arrhythmias and increased cardiac metabolic activity, inotropic drugs have an important role in everyday clinical medicine. Most guideline recommendations find an indication for these drugs in patients with severely compromised circulation, for instance, patients in cardiogenic shock. Also, they are in routine application in intensive care units for patients with circulatory critical states of noncardiac etiology. Their recognized potentially life-threatening side effects and negative large clinical trials have set off an active search for alternative drugs to increase cardiac contractile function. Through a large-scale screening using a myosin-ATPase assay measuring the consumption of ATP, the drug omecamtiv mecarbil has been brought to clinical trials through a series of systematic preclinical and clinical model-testing. Of interest, this compound has an alternative contractile effect by prolonging systolic ejection time and, contrary to traditional inotropes, does not increase intracellular calcium levels and fluxes. Of particular interest, preclinical testing has found no increase in myocardial oxygen consumption based on this drug application, and this observation supports a particular interest for its use in patients with myocardial dysfunction or ongoing ischemia. In the present study, however, we found a relatively increased oxygen consumption in various experimental myocardial models, including postischemic in vivo pig hearts. This is compatible with the theoretical increase in oxygen consumption that should be initiated by the ATPase-stimulatory effect of the drug, and such an effect is of concern if omecamtiv mecarbil should find an indication in acute cardiac dysfunction.