

# The $\beta_3$ Adrenergic Receptor Antagonist L-748,337 Attenuates Dobutamine-Induced Cardiac Inefficiency While Preserving Inotropy in Anesthetized Pigs

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## Abstract

Excessive myocardial oxygen consumption ( $MVO_2$ ) is considered a limitation for catecholamines, termed oxygen cost of contractility. We hypothesize that increased  $MVO_2$  induced by dobutamine is not directly related to contractility but linked to intermediary myocardial metabolism. Furthermore, we hypothesize that selective  $\beta_3$  adrenergic receptor ( $\beta_3AR$ ) antagonism using L-748,337 prevents this. In an open-chest pig model, using general anesthesia, we assessed cardiac energetics, hemodynamics and arterial metabolic substrate levels at baseline, ½ hour and 6 hours after onset of drug infusion. Cardiac efficiency was assessed by relating  $MVO_2$  to left ventricular work (PVA; pressure–volume area). Three groups received dobutamine (5  $\mu\text{g}/\text{kg}/\text{min}$ ), dobutamine + L-748,337 (bolus 50  $\mu\text{g}/\text{kg}$ ), or saline for time-matched controls. Cardiac efficiency was impaired over time with dobutamine infusion, displayed by persistently increased unloaded  $MVO_2$  from ½ hour and 47% increase in the slope of the PVA– $MVO_2$  relation after 6 hours. Contractility increased immediately with dobutamine infusion ( $dP/dt_{\text{max}}$ ;  $1636 \pm 478$  vs  $2888 \pm 818$  mmHg/s,  $P < 0.05$ ) and persisted throughout the protocol ( $2864 \pm 1055$  mmHg/s,  $P < 0.05$ ). Arterial free fatty acid increased gradually ( $0.22 \pm 0.13$  vs  $0.39 \pm 0.30$  mM,  $P < 0.05$ ) with peak levels after 6 hours ( $1.1 \pm 0.4$  mM,  $P < 0.05$ ). By combining dobutamine with L-748,337 the progressive impairment in cardiac efficiency was attenuated. Interestingly, this combined treatment effect occurred despite similar alterations in cardiac inotropy and substrate supply. We conclude that the extent of cardiac inefficiency following adrenergic stimulation is dependent on the duration of drug infusion, and  $\beta_3AR$  blockade may attenuate this effect.

## Keywords

inotrope, cardiac energetics, cardiac efficiency, myocardial oxygen consumption,  $\beta_3$  adrenergic receptor

## Introduction

Dobutamine (Dob) is an inotrope used for treating patients with acute heart failure and cardiogenic shock.<sup>1</sup> There has been conflicting evidence on the impact on myocardial oxygen consumption ( $MVO_2$ ) using this inotrope. The general understanding is that sympathomimetic drugs lead to myocardial oxygen wastage because of cAMP-induced increase in calcium handling.<sup>2-4</sup> Contradictory results have shown that Dob does not induce oxygen wastage at clinically relevant doses.<sup>5</sup> A limitation in that study is that the measurements was carried out shortly after the onset of drug infusion. This is relevant since Dob-induced myocardial oxygen wastage was seen in the subsequent dose escalating protocol.<sup>5</sup> Thus, it is difficult to conclude whether the cardiac inefficiency is time dependent, dose dependent, or a combination. Several different protocols investigating Dob-induced changes in cardiac energetics have been done using various doses,<sup>6-12</sup> but with the infusion lasting for a maximum of around 1 hour.

Dob stimulates primarily the  $\beta_1$  adrenergic receptor (AR) which in turn activates hormone-sensitive lipase leading to lipolysis in adipose tissue and higher circulating levels of free fatty acid (FFA). Altered substrate metabolism has been shown to influence  $MVO_2$  and cardiac efficiency. A negative impact on myocardial energetics following increased myocardial FFA utilization has been shown,<sup>13,14</sup> also in the presence of

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isoproterenol.<sup>15</sup> This is due to a higher oxygen use for basal metabolism with increased FFA utilization. The same effect has been described in pigs when infusing FFA intravenously, with a 48% increase in unloaded MVO<sub>2</sub> compared to glucose infusion.<sup>16</sup>

A third AR, the  $\beta_3$ AR, has been identified. The  $\beta_3$ AR agonist BRL 37344 has a lipo-mobilizing effect from adipose tissue<sup>17-19</sup> likely through elevation of intracellular cAMP levels.<sup>20</sup> This is accompanied by a negative inotropic effect.<sup>21,22</sup> Thus, the receptor can have a central role in modulating the metabolic effects of adrenergic stimulation.  $\beta_3$ AR blocking with L-748,337 has been shown to give a positive inotropic effect,<sup>23</sup> and in theory may inhibit lipolysis in adipose tissue during  $\beta_1$ AR activation.

We hypothesize that Dob-induced myocardial oxygen wastage is time dependent and related to higher circulating levels of FFA over several hours, and may be counteracted by blockade of the  $\beta_3$ AR using L-748,337.

## Methods and Materials

### Experimental Animals

Twenty-one castrated domestic pigs (*Sus scrofa domesticus*) weighing  $30 \pm 5$  kg (mean  $\pm$  standard deviation) were included. The animals were in quarantine for 5-7 days for environmental adaptation with free access to water and food, with only water available overnight before experiments. The use of experimental animals in this protocol was approved by the Norwegian Food Safety Authority, following Norwegian legislations for the use of animals in experimental research. The animal experiments were conducted in accordance with the Consensus Author Guidelines on Animal Ethics and Welfare for Veterinary Journals published by the International Association of Veterinary Editors, and all the investigations adhere to the ARRIVE Guidelines.

### Anesthesia and Surgical Instrumentation

Premedication using intramuscular injections of ketamine (20 mg/kg) (Pfizer AS, Norway), midazolam (1 mg/kg) (B.Braun, Germany) and atropine (1 mg) (Nycomed Pharma, Norway) was administered to all animals. Thereafter, anesthesia was induced through inhalation of 5% isoflurane gas (Abbot, USA) prior to endotracheal intubation. Intravenous injections of pentobarbital sodium (10 mg/kg) (Abbot, USA) and fentanyl (0.01 mg/kg) (Hameln Pharmaceuticals, Germany) were given through an ear vein cannula. Mechanical ventilation with an air-oxygen mixture (FiO<sub>2</sub>) of 60% was used and ventilation was monitored by capnography and arterial blood gases taken regularly. Through a central venous catheter placed in the left internal jugular vein, anesthesia was maintained throughout the experiments by continuous infusion of pentobarbital sodium (4.0 mg/kg/h), fentanyl (0.02 mg/kg/h) and midazolam (0.3 mg/kg/h). Heparin (2500 IU) (Leo, Denmark) and amiodarone (5 mg/kg) (Sanofi-Synthelabo, Sweden)

were injected to prevent blood clotting on catheters and cardiac arrhythmia, respectively. The urinary bladder was drained by cystostomy. Introducer sheaths were placed in: 1) the right common carotid artery to allow placement of a 7 Fr multisegment Millar MPVS Ultra pressure-volume catheter (Millar, USA) in the left ventricle (LV), 2) the right femoral vein for placement of a 7 Fr balloon catheter (Sorin, Italy) to conduct preload reductions by inferior vena cava occlusions, and 3) the right internal jugular vein for placement of a Swan-Ganz catheter in the pulmonary artery via the right atrium and ventricle. A central arterial catheter (BD Secalon-T<sup>TM</sup>, Argon, Netherlands) was placed in the abdominal aorta through the left femoral artery for mean arterial pressure measurements. Median sternotomy was performed followed by removal of the pericardium and ligation of the hemiazygos vein. The left anterior descending coronary artery, the circumflex coronary artery, the right coronary artery and the pulmonary artery were dissected free from connective tissue to place transit-time ultrasonic flow probes (Medi-stim, Norway) to measure coronary blood flow (CBF) and cardiac output. Sonomicrometric crystals (Sonometrics Corp., Canada) for collection of dimension data were sutured in 3 regions of the myocardium: apically, and anteriorly and posteriorly in the base of the LV wall. A pediatric central venous catheter (Arrow 24G, eSutures, USA) placed in the great cardiac vein via the superior vena cava was used for blood sampling. The pressure-volume catheter was placed in the LV lumen via the carotid introducer, and its position verified by visualization of the pressure-volume loop. The 7 Fr balloon catheter was placed in the inferior vena cava via the femoral vein introducer, and vena cava occlusions were obtained to verify its position.

### Drug Dosage

Dobutamine (Sigma Aldrich) was administered as a continuous intravenous infusion (5  $\mu$ g/kg/min). L-748,337 (Sigma Aldrich) was given as a bolus dose (50  $\mu$ g/kg) based on previous studies to selectively block the  $\beta_3$ AR.<sup>23-25</sup>

### Experimental Protocol

Following surgical preparation and positioning of instruments, all animals underwent approximately 45 minutes of hemodynamic stabilization. To maintain intravascular volume, sodium chloride solution 0.9% (20 ml/kg/h) (Fresenius Kabi) was infused throughout experiments.

Short axis end-systolic and end-diastolic endocardial diameter measurements were done by epicardial echocardiography (GE Vivid-I, KPI Healthcare, USA) for calibration of the sonomicrometric crystals dimension data. At baseline; venous, arterial and cardiac venous blood samples, and recordings of general hemodynamics were obtained. Thereafter, 6-8 recordings of cardiac function and MVO<sub>2</sub> at different workloads were obtained by stepwise inflation of the vena cava balloon catheter. Finally, an abrupt vena cava occlusion was done for assessment of preload-recruitable stroke work. After baseline

recordings, the animals were given either Dob ( $n = 9$ ), Dob in combination with L-748,337 (Dob + L;  $n = 5$ ), or vehicle substance (sodium chloride 0.9%;  $n = 7$ ). The L-748,337 bolus in the Dob + L group was given simultaneously as Dob infusion was started. Stable hemodynamics, defined by stable heart rate and mean arterial pressure, was present at 30 minutes after start of drug infusions, and all measurements and recordings were repeated as described above. Six hours after initiation of drug infusion the final recordings were carried out. All animals were euthanized with an overdose of pentobarbital sodium.

### Calculations

LV end-systolic- and end-diastolic volume (ESV and EDV, respectively) at baseline, steady state hemodynamics, were calculated using the ellipsoid formula  $V = \frac{\pi(S_{\text{endo}})^2}{6 \cdot L_{\text{endo}}}$ ,<sup>26</sup> where  $V$  is volume, and  $S_{\text{endo}}$  and  $L_{\text{endo}}$  are endocardial short axis diameter in end-systole and end-diastole, respectively, measured from epicardial echocardiography. To measure the ESV and EDV, the short- and long-axis sonomicrometric crystal signals were calibrated against ESV and EDV at baseline, and converted to a composite output using the Area Length (Bullet) formula,<sup>27</sup> Volume =  $\frac{5}{6} \cdot \text{Area} \cdot \text{Length}$ . Stroke work (SW) was calculated as  $\text{SW} = \left( \frac{P_{\text{max}} + \text{ESP}}{2} \right) - \text{EDP} \cdot \text{SV}$ , where  $P_{\text{max}}$  (mmHg) is the maximum pressure in the LV, ESP and EDP (mmHg) are end-systolic- and end-diastolic pressure in the LV, respectively, and SV is LV stroke volume. Pressure–volume area (PVA, J/beat/100 g) was calculated as  $\text{PVA} = \frac{\text{SW} + \text{PE}}{\text{LVW}} \cdot 100$ , where LVW is LV weight and PE is potential energy calculated as  $\text{PE} = \left( \frac{\text{ESP} \cdot (\text{ESV} - V_0)}{2} - \frac{\text{EDP} \cdot (\text{EDV} - V_0)}{4} \right) \cdot 0.000133$ , where  $V_0$  is the LV volume at zero pressure and 0.000133 (J/mmHg/ml) is a constant for energy production. MVO<sub>2</sub> (J/beat/100 g) was calculated using the formula  $\text{MVO}_2 = \left( \frac{\text{LVCBF} \cdot \text{O}_2\text{sat}_A + \text{Hb} \cdot 1.39}{\text{HR}} \cdot \frac{\text{SW} + \text{PE}}{\text{LVW}} \cdot 100 \right)$ , where LVCBF is LV CBF (ml/min), O<sub>2</sub>sat<sub>A</sub> is the arteriovenous difference in arterial and great cardiac vein oxygen saturations, Hb is hemoglobin (g/ml), 1.39 is a constant for ml O<sub>2</sub>/g Hb, HR is heart rate (beats/min), and 20.2 is a constant for energy production (J/ml O<sub>2</sub>).

### Data Recording and Analyses

LV function, hemodynamics, and dimension data from sonomicrometric crystals were recorded and analyzed using ADI LabChart (ADInstruments; Dunedin, New Zealand). Coronary artery and pulmonary trunk blood flow was recorded using transit-time flow probes (Medi-stim, Norway) with connecting software. LVCBF was estimated from global CBF (GCBF) and adjusted to LV weight (LVW) using the formula  $\text{LVCBF} = \text{GCBF} \cdot 0.7 \cdot \left( \frac{100}{\text{LVW}} \right)$ .<sup>28</sup>

### Statistical Analyses

Calculations and statistical analyses were conducted using spreadsheet (Excel, Microsoft Office 2017), SPSS software

statistics package (IBM, New York, USA) and GraphPad Prism (GraphPad, USA). Hemodynamics and metabolic data were analyzed within groups using a two-way mixed analysis of variance for repeated measures, and multiple comparisons were adjusted for by Bonferroni correction. The effects of drug interventions on the pool of linear regression lines of the PVA–MVO<sub>2</sub> relation were evaluated using linear mixed model both for covariance analysis and to test if the slopes and y-intercepts differ between timepoints. The linear mixed model was conducted with MVO<sub>2</sub> as the dependent variable, PVA as covariate, and animal identification, PVA, time and PVA\*time as random and fixed effects.  $P < 0.05$  was considered statistically significant.

## Results

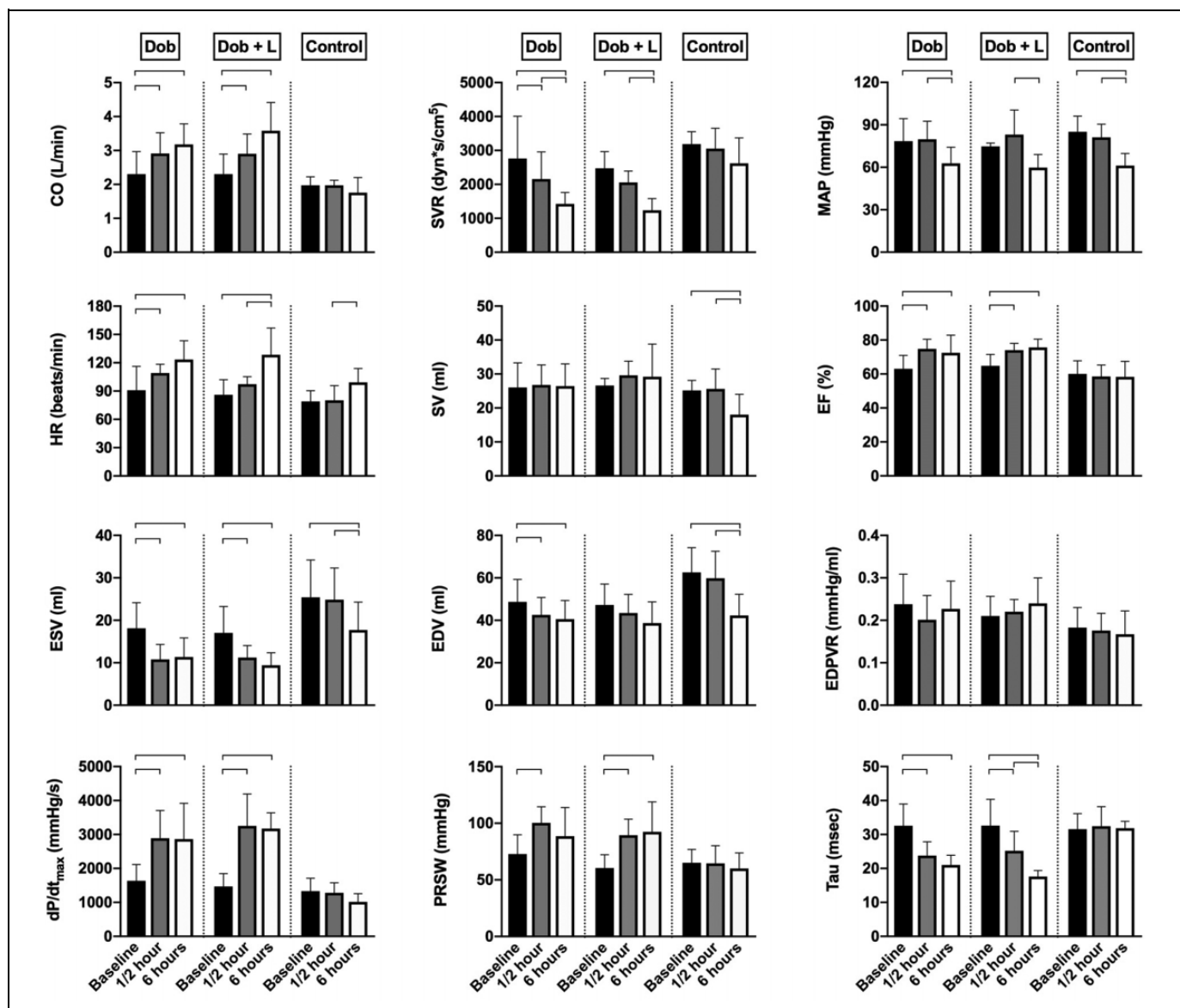
### Hemodynamic Effects of Drug Infusion

Hemodynamic data are displayed in Figure 1. Baseline cardiac volumes varied between the groups, possibly because absolute volumetry is limited by the assumptions of accurate positioning of the sonomicrometric crystals and a perfect ellipsoid shape of the ventricle.

Dob gave an immediate increased cardiac output of 26% due to increased contractility, increased heart rate of 20% and a vasodilation with lowering of the systemic vascular resistance. Combining Dob with L-748,337 did not change cardiac inotropy and general hemodynamics. These immediate effects of the drugs were not diminished after 6 hours. Hemodynamics in time-matched controls showed preserved contractility and cardiac output. A progressive decline in mean arterial pressure throughout the protocol occurred in all 3 groups, which may have resulted from the cumulative effects of approximately 9 hours of deep general anesthesia in an open-chest experimental model.

### Cardiac Energetics and Metabolism

Cardiac energetics are shown in Figure 2 and Table 1, and metabolic data in Figure 3. Cardiac efficiency was impaired over time with Dob infusion, displayed by persistently increased unloaded MVO<sub>2</sub> from ½ hour and 47% increase in the slope of the PVA–MVO<sub>2</sub> relation after 6 hours. When adding L-748,337 to Dob, y-intercept was higher after ½ hour compared to 6 hours, but at the end of the protocol neither y-intercept nor slope were significantly changed from baseline. Arterial FFA concentration was immediately elevated and consistently rose throughout 6 hours of Dob infusion, independently of the presence of the β<sub>3</sub>AR blocker. Both with Dob alone and in combination with L-748,337, steady state MVO<sub>2</sub> increased over time of drug infusion. In time-matched controls, steady state LV work and MVO<sub>2</sub> were matched during the entire protocol, and arterial FFA levels increased. When analyzing scatterplots for PVA–MVO<sub>2</sub> there was no observed oxygen wastage in controls.



**Figure 1.** Hemodynamic data from healthy pigs. CO indicates cardiac output, measured using a flow probe on the pulmonary trunk; SVR, systemic vascular resistance, calculated from mean arterial pressure, central venous pressure in the vena cava and CO; MAP, mean arterial pressure measured using a vascular catheter in the abdominal aorta; HR, heart rate; SV, stroke volume calculated from CO and HR; EF, ejection fraction; End-systolic volume (ESV) and end-diastolic volume (EDV) calculated from sonomicrometric crystal signals calibrated by endocardial short axis diameter from epicardial echocardiography; EDPVR, end-diastolic pressure–volume relation given as the ratio of pressure divided by volume;  $dP/dt_{max}$ , peak positive derivative of left ventricular pressure; PRSW, preload-recruitable stroke work, the slope of the relation between end-diastolic volume and stroke work; Tau, time constant of left ventricular isovolumetric relaxation calculated by Weiss method. Dob, dobutamine 5  $\mu\text{g}/\text{kg}/\text{min}$  ( $n = 9$ ); L, L-748,337 bolus 50  $\mu\text{g}/\text{kg}$  ( $n = 5$ ); Control, saline 0.9% ( $n = 7$ ). Data were analyzed within groups using a two-way mixed ANOVA for repeated measures, and multiple comparisons were adjusted for by Bonferroni correction. Bars indicate mean values  $\pm$  standard deviation. Brackets indicate statistically significant difference ( $P < 0.05$ ).

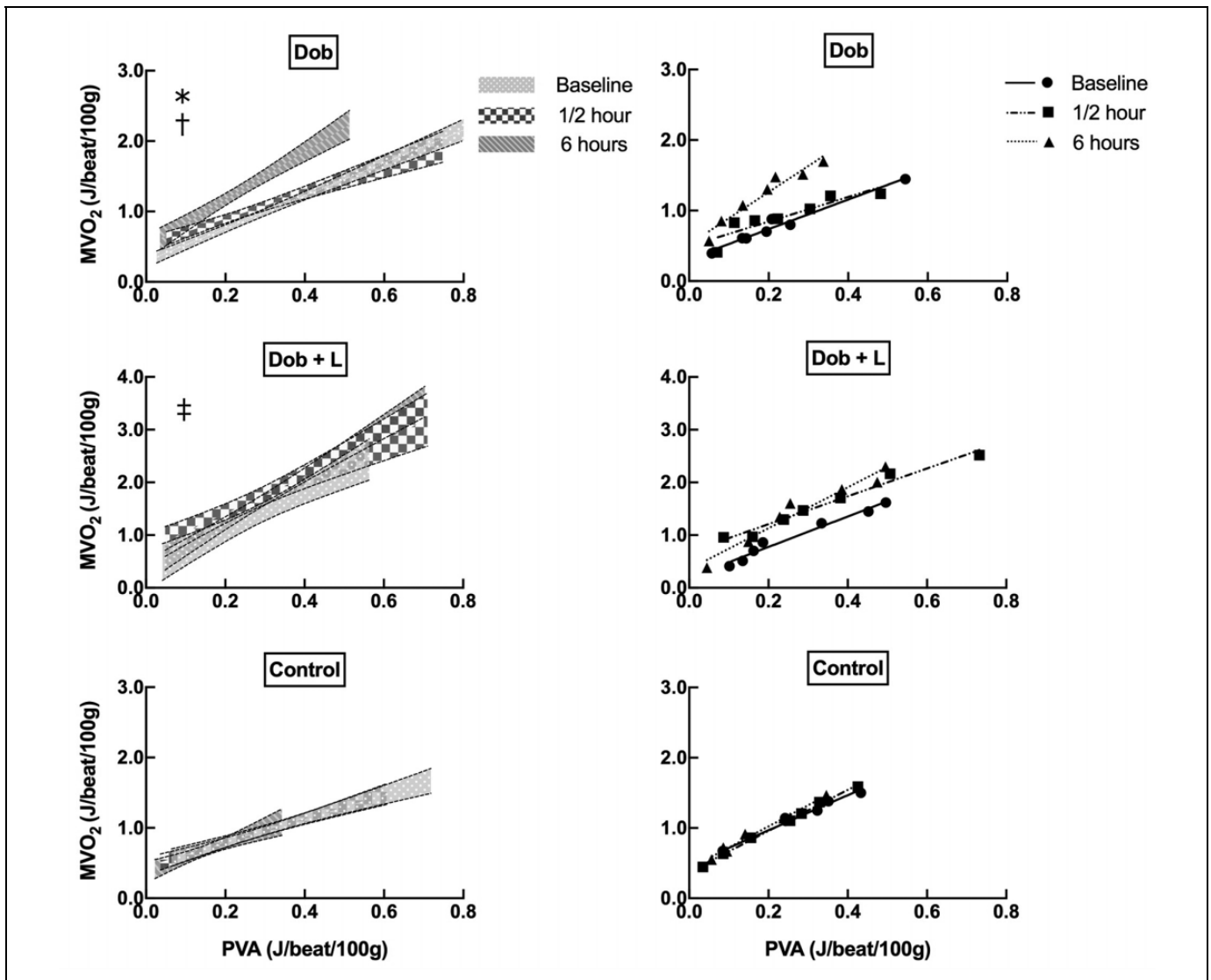
## Discussion

### Main Finding

In this study, 6 hours of Dob infusion impaired cardiac efficiency as measured by a leftward shift of the PVA–MVO<sub>2</sub> relationship (Figure 2). As cardiac inefficiency progressed throughout the protocol, this suggests a time dependent myocardial oxygen wastage by sympathomimetic drugs that is not

related to the inotropic state of the heart. Furthermore, the results suggest that specific blockade of the  $\beta_3\text{AR}$  attenuates this inefficiency without affecting the inotropic effect of Dob.

The PVA–MVO<sub>2</sub> framework to assess cardiac energetics was introduced by Suga.<sup>2,29</sup> This framework allows to differentiate work-dependent and work-independent energy consumption in the LV. The cost of basal metabolism and excitation-contraction coupling make up the work-independent MVO<sub>2</sub>, given as the



**Figure 2.** Left ventricular work–myocardial oxygen consumption relationship from healthy pigs. Left panels show 95% confidence intervals for pooled scatterplots from all experiments. Right panels show example data from one individual pig in each group. All data was obtained by preload reductions by vena cava occlusions using a balloon catheter. Dobutamine (Dob, 5  $\mu\text{g}/\text{kg}/\text{min}$ ,  $n = 9$ ) impairs cardiac efficiency over time as displayed by an increase in y-intercept after 1/2 hour and increased slope after 6 hours (upper panel). Combining Dob with L-748,337 (L, bolus 50  $\mu\text{g}/\text{kg}$ ,  $n = 5$ ) shows an increase in y-intercept after 1/2 hour compared to 6 hours, but after 6 hours y-intercept and slope is unchanged from baseline (middle panel). In time-matched controls (Control, saline 0.9%,  $n = 7$ ) there is no change in cardiac efficiency for 6 hours (lower panel). MVO<sub>2</sub>, myocardial oxygen consumption; PVA, pressure–volume area. \*  $P < 0.05$ , 6 hours vs baseline and 1/2 hour for slope; †  $P < 0.05$ , baseline vs 1/2 hour and 6 hours for y-intercept; ‡  $P < 0.05$ , 1/2 hour vs 6 hours for y-intercept (linear mixed model analysis).

y-intercept. The present study confirms the Suga concept of oxygen cost of contractility<sup>30</sup> as seen by a concomitant elevation of inotropy and the y-intercept after 1/2 hour in both groups receiving Dob (Figure 1 and Table 1, respectively). This finding suggests that specific  $\beta_3\text{AR}$  blockade does not interact with the inotrope signaling pathway and, thus, does not alter the oxygen cost of contractility in the healthy pig. However, the slope which represents work-dependent MVO<sub>2</sub> was only increased after 6 hours in the Dob group. Thus, only Dob as monotherapy showed a clinically relevant impairment in cardiac efficiency at prolonged drug infusion. This decrement in efficiency is evident in Figure 2 as a substantial leftward shift in the PVA–MVO<sub>2</sub>

relationship after 6 hours. Measuring cardiac energetics using the PVA–MVO<sub>2</sub> over a broad range of workloads is of great importance. This because the relationship of cardiac work (e.g. PVA) and energy consumption (MVO<sub>2</sub>) always has a positive y-intercept. Thus, a crude ratio of these 2 indexes as a measure of efficiency will erroneously favor the heart with the highest workload.<sup>31</sup>

Activation of the  $\beta_1\text{AR}$  increases arterial levels of FFA, and the subsequent disproportional increased MVO<sub>2</sub> due to increased FFA utilization for energy production has been described earlier.<sup>13</sup> A theoretical total metabolic shift from 100% glucose oxidation to 100% FFA oxidation will increase

**Table 1.** Left Ventricular Energetics.<sup>a</sup>

	Baseline	½ hour	6 hours
Dobutamine 5 µg/kg/min, n = 9			
Slope	2.31 ± 0.13 <sup>b</sup>	1.89 ± 0.20 <sup>b</sup>	3.40 ± 0.33 <sup>c</sup>
y-intercept	0.30 ± 0.04 <sup>b</sup>	0.52 ± 0.06 <sup>c</sup>	0.49 ± 0.09 <sup>c</sup>
R <sup>2</sup>	0.94 ± 0.03	0.90 ± 0.08	0.95 ± 0.04
Dobutamine 5 µg/kg/min + L-748,337 50 µg/kg bolus, n = 5			
Slope	3.49 ± 0.58	3.39 ± 0.50	4.51 ± 0.31
y-intercept	0.35 ± 0.19	0.71 ± 0.16 <sup>b</sup>	0.29 ± 0.10
R <sup>2</sup>	0.94 ± 0.05	0.97 ± 0.01	0.93 ± 0.04
Control, saline 0.9%, n = 7			
Slope	1.71 ± 0.20	1.76 ± 0.20	2.18 ± 0.45
y-intercept	0.47 ± 0.07	0.45 ± 0.06	0.36 ± 0.08
R <sup>2</sup>	0.95 ± 0.04	0.96 ± 0.05	0.93 ± 0.06

<sup>a</sup>Slope and y-intercept of the left ventricular pressure–volume area (PVA)–myocardial oxygen consumption (MVO<sub>2</sub>) relationship over a broad range of workloads in healthy pigs. 1/slope is a measure of contractile efficiency (work-dependent oxygen consumption), whereas the y-intercept indicates MVO<sub>2</sub> (l/beat/100 g) in the unloaded ventricle used for excitation-contraction coupling and basal metabolism. R<sup>2</sup> is the regression coefficient from the individual pigs. The data is presented as mean ± standard deviation.

<sup>b</sup>P < 0.05, vs 6 hours (linear mixed model analysis).

<sup>c</sup>P < 0.05, vs baseline (linear mixed model analysis).

unloaded MVO<sub>2</sub> by 11%.<sup>32</sup> But Mjøs O.D.<sup>13</sup> showed 26% increase in MVO<sub>2</sub> when infusing intralipid in intact dogs, suggesting additional mechanisms for the high oxygen consumption. An unbalanced energy production with high intracellular FFA levels with an increased palmitate oxidation to oxygen consumption ratio<sup>33</sup> and a futile triacylglycerol-fatty acid cycle<sup>34</sup> have been proposed. Also, Borst et al<sup>35</sup> reported fatty acid-induced uncoupling of oxidative phosphorylation without increased ATP production despite higher oxygen consumption. There is a marked energetical advantage with glucose as substrate for energy production,<sup>14,16,36</sup> with several studies supporting the detrimental effects on myocardial energetics following increased use of FFA as substrate.<sup>15,37</sup> Thus, therapies that lower the plasma FFA levels seems expedient, especially in the ischemic heart where CBF is considered a limiting factor. It is important to notice that serum glucose levels progressively declines throughout the protocol in all groups (Figure 3), which may have contributed to the decrease in cardiac efficiency by favoring myocardial FFA utilization via the Randle cycle.<sup>38</sup>

Myocardial oxygen wastage induced by inotropes has several potential causes. Increased calcium transients in myocardial cells has been shown to cause wastage due to higher energy expenditure for excitation-contraction coupling such as that reported using catecholamines as well as another inotrope (OPC 8212; phosphodiesterase 3 inhibitor).<sup>30</sup> The changes in intracellular calcium occur immediately after β<sub>1</sub>AR stimulation as reflected by the *dp/dt*<sub>max</sub> value in the present study. This may explain the surplus MVO<sub>2</sub> seen after ½ hour of Dob infusion, but not the progressive disproportionate increase in work-dependent MVO<sub>2</sub> as seen in the following 6 hours.

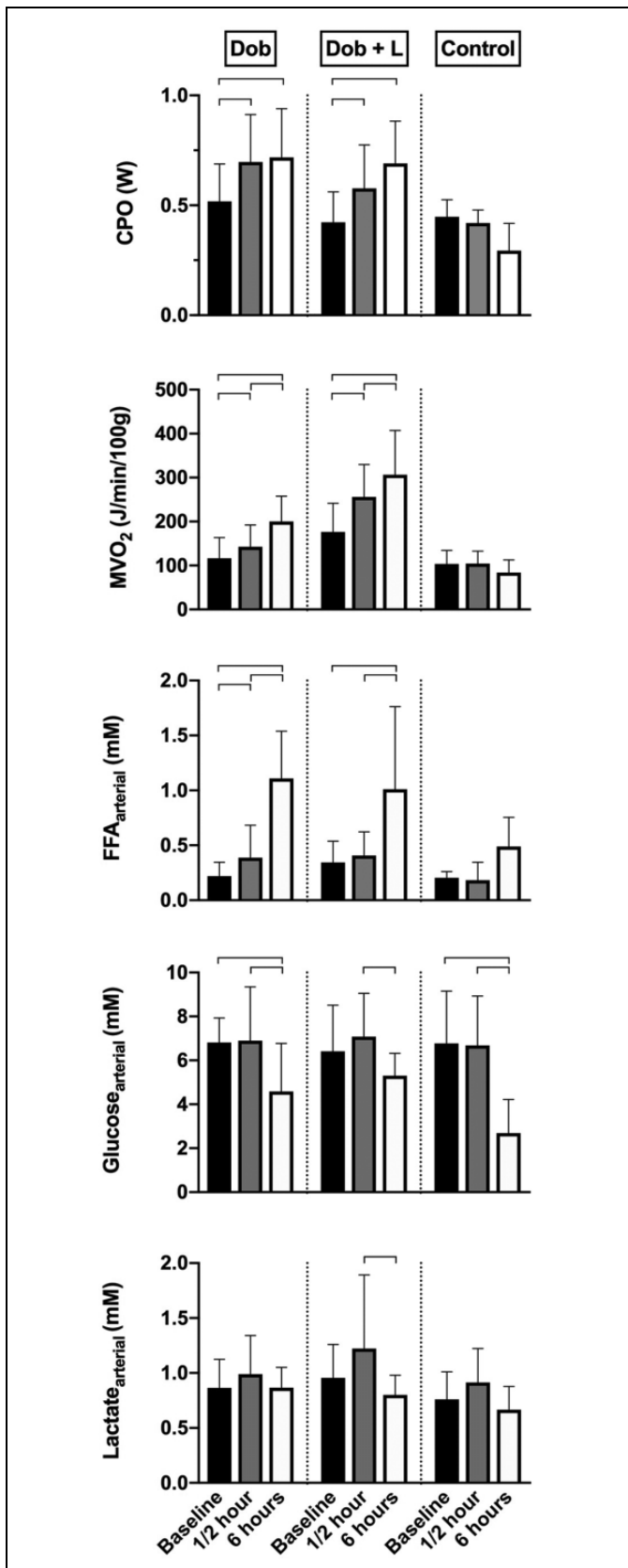
The progressive myocardial oxygen wastage is not linked to contractile state, but our data do not show whether it is

inotropic, increased plasma concentrations of FFA, both those variables, or other factors that is causing the impaired cardiac efficiency following Dob infusion. Korvald et al<sup>16</sup> showed acute myocardial oxygen wastage with a parallel upward shift of the PVA–MVO<sub>2</sub> relation after high dose FFA giving around 3-fold the plasma concentrations induced by Dob in the present study. This was compared with glucose-insulin-potassium infusion. Further studies are warranted to investigate the effect of a moderate to high increase in plasma FFA on cardiac efficiency for 6 hours or more.

### β<sub>3</sub> Adrenergic Receptor Antagonism

The L-748,337 dose was chosen to selectively block the β<sub>3</sub>AR, and a clear inotropic effect is not seen in this present study. Although the percentage increase in *dp/dt*<sub>max</sub> during the first ½ hour of dobutamine infusion was larger when combined with L-748,337 treatment, the study was not designed to test the effect of L-748,337 alone. In heart failure the β<sub>1</sub>AR is desensitized by chronic adrenergic stimulation, while the lack of β<sub>3</sub>AR desensitization may contribute to worsening of cardiac function.<sup>39</sup> Thus, a positive inotropic effect of L-748,337 is not expected in this model using healthy juvenile pigs when dobutamine is present. Systemic vascular resistance similarly was unaffected by L-748,337, which is surprising considering the compound is thought to inhibit the NO system.<sup>40</sup> In particular since a previous study using the same strain of animals has shown high sensitivity to NOS blockade by L-NAME.<sup>41</sup>

The changes in blood levels of FFA, glucose and lactate (Figure 3) are similar within the 2 groups with Dob infusion. This may suggest a minimal effect on blocking lipolysis at the chosen doses of L-748,337. Considering that the myocardial oxygen wasting effect after 6 hours was observed only when



**Figure 3.** Metabolism data from healthy pigs. CPO, cardiac power output calculated as left ventricular developed pressure\*cardiac output/45 l; MVO<sub>2</sub>, myocardial oxygen consumption; FFA, free fatty acid;

Dob was the sole drug, and that the metabolic substrate supply was similar in both those groups, the question of a direct cardiac effect of L-748,337 arises. We were not able to detect differences in myocardial uptake of the metabolic substrates due to large variations in the samples. Thus, changes in myocardial substrate utilization using these drugs should be further explored. The  $\beta_3$ AR has different characteristics than the  $\beta_1$ AR and the  $\beta_2$ AR, i.e. on G-proteins.<sup>42</sup> Also, it is upregulated in failing myocardium<sup>21</sup> and in cells exposed to chronic adrenergic stimulation,<sup>43</sup> which is opposite to the  $\beta_1$ AR and  $\beta_2$ AR lower density in failing human hearts,<sup>44</sup> suggesting that a protective role is feasible.

### Further Implications

The open-chest model is gold standard methodology to investigate cardiac energetics. In general, the clinical perspective is more applicable to a closed-chest situation, and further studies of this combination treatment for acute heart failure are warranted. Our main finding with substantial myocardial oxygen wastage after 6 hours of Dob infusion is of importance due to the use of this inotrope in patients to treat acute heart failure<sup>1</sup> and septic shock.<sup>45</sup> Recommended doses to treat acute heart failure range from 1 to 20 µg/kg/min<sup>46</sup> with the infusion often lasting for several days.<sup>47,48</sup> Myocardial oxygen wastage is considered an adverse effect when administering Dob in patients, and  $\beta_1$ AR stimulation in adipose tissue increases arterial FFA levels which is associated with worsened mechanical function in ischemic hearts.<sup>49</sup> In one study of patients with dilated cardiomyopathy, carvedilol, a non-selective  $\beta$ AR antagonist and  $\alpha_1$ AR antagonist, increased ejection fraction from 26% to 37% after 3 months of treatment.<sup>50</sup> This improvement of LV pump function was accompanied by a 57% reduction in myocardial use of FFA in these patients. Other clinical studies have shown beneficial effects of treatment with glucose-insulin-potassium infusion in post-operative cardiac failure.<sup>51,52</sup>

Based on the evidence of the detrimental effect of relatively high FFA utilization in the heart under certain conditions, the  $\beta_3$ AR antagonists can potentially improve cardiac function and increase contractile indexes without increased lipolysis when treating ischemic acute heart failure. In the present study,  $\beta_3$ AR blocking was unable to restrict the Dob-mediated increase in plasma FFA and did neither change hemodynamics or contractility in healthy pigs. Thus, effects of  $\beta_3$ AR blocking on hemodynamics and cardiac energetics in ischemic acute heart failure needs to be investigated.

**Figure 3.** (Continued). Dob, dobutamine 5 µg/kg/min (n = 9); L, L-748,337 bolus 50 µg/kg (n = 5); Control, saline 0.9% (n = 7). Data were analyzed within groups using a two-way mixed ANOVA for repeated measures, and multiple comparisons were adjusted for by Bonferroni correction. Bars indicate mean values ± standard deviation. Brackets indicate statistically significant difference (P < 0.05).

## Conclusions

These data suggest that the extent of dobutamine-induced myocardial oxygen wastage is time dependent for a minimum of 6 hours. This finding supports earlier studies showing that myocardial oxygen wastage seen as an adverse effect of sympathomimetic drugs can be related to a higher exposure of FFA to the cardiomyocytes, but our data does not show any causality regarding this. The  $\beta_3$ AR antagonist L-748,337 attenuates the progressive dobutamine-induced cardiac inefficiency, without affecting inotropy, general hemodynamics or substrate supply.

## Authors' Note

This work was done at UiT–The Arctic University of Norway. Supplemental material will be made available by the corresponding author upon request.

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## Author Contributions

Lars Rødland: Planned protocol, executed experiments, analyzed data, drafted figures and table, drafted and finalized the manuscript. Leif Rønning: Planned protocol, executed experiments, revised the manuscript. Anders Benjamin Kildal: Evaluated data, revised the manuscript. Ole-Jakob How: Produced initial idea, planned protocol, evaluated data, revised and finalized figures. All authors: revised and accepted the final version of the manuscript, figures and table.


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