The effect of Riociguat on cardiovascular function and efficiency in healthy, juvenile pigs

Torvind Næsheim1,2 | Ole-Jakob How3 | Truls Myrmel1,4

1Cardiovascular Research Group, Department of Clinical Medicine, UiT, The Arctic University of Norway, Tromsø, Norway
2Department of Anesthesiology, University Hospital North Norway, Tromsø, Norway
3Department of Medical Biology, Faculty of Health Sciences, UiT, The Arctic University of Norway, Tromsø, Norway
4Department of Cardiothoracic and Vascular Surgery, University Hospital North Norway, Tromsø, Norway

Abstract

Introduction: Riociguat is a soluble guanylate cyclase stimulator approved for the treatment of pulmonary hypertension. Its effect on cardiometabolic efficiency is unknown. A potential cardiac energy sparing effect of this drug could imply a positive prognostic effect, particularly in patients with right heart failure from pulmonary hypertension.

Method: We infused Riociguat in six healthy juvenile pigs and measured the integrated cardiovascular effect and myocardial oxygen consumption. To assess the interplay with NO-blockade on cardiac function and efficiency we also administered the NO-blocker L-NAME to the animals after Riociguat.

Results and Discussion: Infusion of 100 µg/kg Riociguat gave modest systemic vasodilatation seen as a drop in coronary and systemic vascular resistance of 36% and 26%, respectively. Right and left ventriculoarterial coupling index (Ees/Ea), stroke work efficiency (SWeff), and the relationship between left ventricular myocardial oxygen consumption (MVO2) and total mechanical work (pressure–volume area; PVA) were unaffected by Riociguat. In contrast, systemic and pulmonary vasoconstriction induced by L-NAME (15 mg/kg) shifted the Ees/Ea ratio toward reduced SWeff in both systemic and pulmonary circulation. However, there was no surplus oxygen consumption, that was measured by the MVO2/PVA relationship after L-NAME in Riociguat-treated pigs. This suggests that Riociguat can reduce the NO-related cardiometabolic inefficiency previously observed by blocking the NO pathway.

1 | INTRODUCTION

Myocardial oxygen consumption (MVO2) is influenced by substrate metabolism, heart rate, wall tension and contractility (Braunwald, 1969). Under normal physiological conditions, these parameters are determined by the hormonal state of the individual. Importantly, adrenergic hormones and drugs increase contractility and relative oxygen consumption (Müller, How, & Jakobsen, 2010; Vasu et al., 1978). Previous studies in large animal models indicate that an elevated NO-tone will improve myocardial efficiency, i.e., reduces MVO2 related to mechanical work (Suto et al., 1998). Riociguat, a soluble guanylate cyclase (sGC) stimulator, exhibits a vasodilatory and NO-like effect by direct stimulation of the sGC enzyme, thus circumventing the need for NO stimulation and compensating for decreased NO sensitivity in vascular pathology. Riociguat could therefore have a favorable energetic profile equal to direct NO effects in the myocardium. This would be particularly beneficial in patients with pulmonary hypertension and right heart failure treated with Riociguat (Adempas®). To assess...
this possibility, we used a well-established metabolic and energetic effect model (Korvald, Elvenes, & Myrmel, 2017) to determine the myocardial oxygen consumption and function during Riociguat infusion in healthy pigs. Studies have shown that there is a basal oxygen-sparing level of NO in the myocardium (Loke, McConnell, & Tuzman, 1999; Recchia et al., 1999), and blocking this NO-tone by infusion of L-NAME has been shown to induce surplus MVO₂ (Nordhaug, Steensrud, Aghajani, Korvald, & Myrmel, 2004). We hypothesized that Riociguat added to normal hearts would reduce MVO₂ relative to mechanical work in the left ventricle and that the NO-blocker L-NAME would reverse this oxygen-sparing effect.

2 | METHODS

2.1 | Animals

Six castrated male domestic pigs weighing 30 ± 5 kg were adapted to the animal department for 5–7 days. The pigs were fasted overnight before experiments, with free access to water. The experimental protocol was approved by the Norwegian Animal Research Authority according to the FDF reference; 2012/55972.

2.2 | Surgical preparation

The pigs were premedicated with an intramuscular injection of 20 mg/kg ketamine (Pfizer AS, Oslo, Norway) and 1 mg of atropine (Nycomed Pharma, Oslo, Norway). Anesthesia was induced by intravenous injection of 10 mg/kg pentobarbital sodium (Abbott, Stockholm, Sweden) and 0.01 mg/kg fentanyl (Hameln Pharmaceuticals, Hameln, Germany). The animals were ventilated after intubation. Normal ventilation was defined as a PaCO₂ of 40 ± 2 mmHg. A central venous catheter was placed through the left internal jugular vein and anesthesia was maintained throughout the experiment by a continuous infusion of 4 mg·kg⁻¹·hr⁻¹ pentobarbitone sodium, 0.02 mg·kg⁻¹·hr⁻¹ fentanyl, and 0.3 mg·kg⁻¹·hr⁻¹ midazolam (B. Braun, Melsungen, Germany). The circulating volume was maintained as an NaCl 0.9% supplemented with 1.25 g·L⁻¹ glucose. Following sternotomy, the pericardium was removed, and the coronary arteries and pulmonary trunk were dissected free from connective tissue. The hemiazygos vein was then ligated.

2.3 | Instrumentation

A 7-Fr pressure catheter (Millar MPVS Ultra, Houston, TX, USA) was inserted through an introducer sheath via the carotid artery into the left ventricle. A 5 Fr Swan-Ganz catheter (Edwards Lifescience Corp. Irvine, USA) was advanced into the pulmonary artery. A 5 fr balloon catheter was floated from the superior caval vein into the right ventricle for pressure measurements. Central venous pressure was measured in the right atrium. The systemic arterial pressure was assessed from a vascular catheter in the abdominal aorta. An 8 Fr balloon catheter was introduced into the inferior caval vein and positioned just below the right atrium for intermittent preload reduction. A 4 Fr catheter with side holes was placed in the coronary sinus for the collection of coronary venous blood. Transit time flow probes (Transonic Systems Inc, Ithaca, NY, USA) were placed around the pulmonary trunk and the three coronary arteries. Eight sonometric crystals (Sonometrics Corporation, Ontario, Canada) were implanted subendocardially, as described in Figure 1. At the end of the experiment, the heart was stopped by injection of 20 mmol KCl (1 mmol/ml). The heart was then excised, and through a balloon catheter in the left main coronary artery, Evans Blue (Sigma-Aldrich, Saint-Louis, Missouri, USA) was injected, and the stained and unstained heart muscle was weighed.

2.4 | Experimental protocol and drugs

The Riociguat dose was based on a dose-response study in four animals (Næsheim, How, & Myrmel, 2020), targeting...
a mean systemic blood pressure at 50 mmHg. In this study, the experiments were conducted using a repeated measures design. The ganglion blocker Hexamethonium chloride (Sigma-Aldrich Missouri, USA) 15 mg/kg was given to avoid autonomous reflexes during interventions and measurements (Douglas, 1952). Heparin, 2,500 IU, was given intravenously after instrumentation to prevent clotting of catheters. Following surgery, the pigs were allowed to rest for 30 min before baseline measurements.

Riociguat (100 μg/kg) was then given as an intravenous bolus, and the second recording was carried out after another 30 min. Finally, L-NAME (15 mg/kg) was given, also as a bolus. The dose of L-NAME was based on an earlier dose-response study in our lab (Nordhaug et al., 2004). The last recording was carried out after the hemodynamic parameters had stabilized after approximately 15 min. At each time point, a full set of cardiac function parameters and vascular measurements were recorded. Assessment of cardiometabolic efficiency was done according to an established protocol (Korvald et al., 2017) with some modification particularly related to measurements of cardiac volumes by sonometric crystals (Feneley et al., 1990). A downloading protocol was carried out after every drug intervention: By stepwise inflating the balloon catheter in the caval vein, stroke work, coronary flow, and arterial–to-coronary venous oxygen difference was recorded at 6–8 different preloads.

Riociguat was obtained from Chemoki Synthesi-TECH, Jiangsu, China, as a dry powder. pH neutral solutions were prepared with DMSO (dimethyl sulfoxide) and a 1:1 solution of Transcutol, Diethylene glycol ethyl ether (Sigma-Aldrich, Missouri, USA) and Cremophor, macrogolglycerol ricinolate (Sigma-Aldrich Missouri, USA). We used 5% Transcutol and Chremophore solutions, and the volume ratio between Transcutol, Diethylene glycol ethyl ether (Sigma-Aldrich, Missouri, USA) and Cremophor, macrogolglycerol ricinolate was 0.05:2.5:2.5. This solution was then further diluted with 0.9 mg·mL⁻¹ NaCl to a final concentration of test drug of 0.01 to 0.1 mg·mL⁻¹ depending on the dose to be given. The maximum DMSO concentration was 0.02%. L-NAME, N-omega-nitro-L-arginine methyl ester, 15 mg/kg, was used as a nitric oxide synthetase inhibitor (Rees, Palmer, Schulz, Hodson, & Moncada, 1990).

### 2.5 Registration of data and analysis

Data were sampled, digitized and analyzed (using ADI MPVS Ultra and Powerlab hardware and LabChart Pro software v 8.1.8, Dunedin, New Zealand). Cardiac dimension data, obtained by the sonometric crystals (Figure 1), were calibrated to cardiac volumes at baseline with the use of intrathoracic Echocardiography (Philips IE33, Philips Healthcare). Echocardiography was also used to confirm the sphericity of the left ventricle at all time points. For all the following recordings, cardiac volumes were calculated from the sonometric data using the following formulas:

The volume of the left ventricle was calculated by the biplane area-length formula as:

\[
V_{lt} = \frac{\pi}{6} D_{3-8}^2 \cdot D_{7-3}
\]

The volume of the right ventricle was estimated by a simplified shell subtraction model as:

\[
V_{re} = \frac{\pi}{6} D_{6-3} \left( D_{4-2}^2 - D_{3-8}^2 \right)
\]

\(D_{n-n}\) indicates the cardiac dimensions between selected crystals, as shown in Figure 1. This ignores the volume of the intraventricular septum, and will therefore probably overestimate right ventricular volumes. For assessment of end-systolic elastance (Ees, maximal ventricular tension as an index of contractility) and V₀ (unstressed ventricular volume), an abrupt reduction in preload was carried out by rapid inflation of the balloon catheter situated in the caval vein. Example loops from the left and right ventricle are presented in Figure 4. V₀ was calculated at baseline for each pig and used as a constant for the remainder of the experiment. Arterial elastance, Ea, in both ventricles was calculated as:

\[
Ea = ESP/SV
\]

ESP is the end-systolic pressure, and SV is the stroke volume (Morimont, Lambermont, & Ghuysen, 2008).

Stroke work efficiency, the ratio of total ventricular mechanical work delivered to the circulation, was calculated by stroke work (SW)/ pressure–volume area (PVA) (Vanderpool et al., 2015). Stroke work was calculated as:

\[
SW = (P_{max} - EDP) \times SV
\]

\(P_{max}\) is the maximum pressure in the ventricle (mmHg), EDP is the end-diastolic pressure in the ventricle (mmHg), and SV is the stroke volume (ml). Pressure–volume area (PVA) was calculated as SW + potential energy (PE):

\[
PE = (ESP \times (ESV - V_0)) / 2 - (EDP \times (EDV - V_0)) / 4
\]

ESV is the end-systolic volume (ml), EDV is the end-diastolic volume (ml), and \(V_0\) is the x-axis intercept of the regression line of the end-systolic pressure–volume relation at baseline.

Left ventricular mechanoenergetic efficiency was assessed by the linear regression of PVA and myocardial oxygen consumption (MVO₂) at various workloads, where MVO₂ was calculated as:

\[\text{MVO}_2 = \text{MVO}_2 \times \text{PVA} \]
MVO₂ = (Hb × avdO₂ × LVCBF × 1.39) / (HR × 20.2)

Hb is hemoglobin concentration (g/dl), avdO₂ is the difference in arterial and great cardiac vein oxygen saturation (%), LVCBF is the left ventricular coronary blood flow (ml/min) or blood flow through the circumflex and left anterior descending arteries divided by left ventricular weight (LVW in grams, as described (Aghajani et al., 2004; Yao, Xue, Yu, Ni, & Chen, 2018)). HR is heart rate (beats per minute), 1.39 is a constant (ml O₂/g Hb), and 20.2 is a factor for J/mL O₂ to convert MVO₂ to mechanical energy equivalents. In the MVO₂/PVA framework the y-axis intercept represents myocardial oxygen cost for excitation–contraction coupling and basal metabolism, while 1/slope represents cardiac efficiency of contractile work (Myrmel & Korvald, 2000).

2.6 Statistical analysis

Calculations and statistical analyses were performed using a spreadsheet (Microsoft Excel 365, Microsoft, USA) and a statistical package (IBM SPSS Statistics for Windows, IBM Corp, Version 25.0, Armonk, NY). Values are presented as the mean ± standard deviation. Mixed model analysis with pig identity as random effect, with allowance for variation in both intercept and slopes between pigs, was performed on the graph in Figures 2 and 3. Pairwise comparisons with least significant difference were evaluated between time points. The autoregressive covariance structure was modeled to give the best fit of data. Mixed model linear regression with pig identity as random effect was used on cardiac energetics data. For these calculations, MVO₂ and the product of MVO₂ and the dummy variable for medication were used as fixed effects (linear regression Figure 7). p-values ***<.01 * <.05 were regarded as statistically significant.

**FIGURE 2** General hemodynamic measurements at baseline (Baseline), after a bolus of 100 µg/kg Riociguat (Rio) and finally after adding 15 mg/kg L-NAME. MAP is the mean systemic arterial pressure, MPAP is the mean pulmonary arterial pressure, CO is the cardiac output. SVR, PVR, and CVR are the systemic, pulmonary and coronary arterial resistances. dP/dt max is the maximum slope of left ventricular pressure development. Tau is the time constant of isovolumetric relaxation. EF is the left ventricular ejection fraction. N = 6, ** denotes p < .01 compared to the previous phase of the experiment.
RESULTS

3.1 Dose-response-study

Increasing doses of Riociguat up to 100 µg/kg induced progressive systemic vasodilatation and hypotension. No significant effects were seen in the pulmonary vasculature. At the maximum dose tested the mean systemic pressure reached the predefined threshold of 50 mmHg (manuscript in press). Heart rates in the control state during Riociguat infusion and after L-NAME bolus were 112 ± 9 min⁻¹, 111 ± 14 min⁻¹ and 111 ± 11 min⁻¹, respectively, with no significant difference (Table 1).

FIGURE 3 Ventriculoarterial coupling (VA coupling) and stroke work efficiency (SWeff) in both the left (LV) and right (RV) ventricles after subsequent boluses of Riociguat and L-NAME (see Figure 2). Left column displays data from the systemic circulation and right column from the pulmonary vasculature. VA coupling was calculated as the ratio of ventricular end-systolic and arterial elastance (Ees/Ea). SWeff is the portion of total ventricular mechanical work (pressure–volume area, PVA) measured as external pressure and volume work (SW). N = 6, * p < .05, ** p < .01 compared to the previous measuring point in the experiment.
3.2 | Effects on vascular tone

A bolus of 100 µg/kg Riociguat gave modest systemic vasodilation, including the coronary circulation, as seen by a drop in coronary and systemic vascular resistance of 36% ± 21% (p = .01) and 26% ± 18% (p = .00), respectively (Figure 2). No significant effect was seen in the pulmonary vasculature.

A bolus of the NOS inhibitor L-NAME (15 mg/kg) after Riociguat resulted in both pulmonary and systemic vasoconstriction as seen by a 2.5 ± 1.6 (p = .01) and 1.5 ± 0.2 (p = .00) fold increase in PVR and SVR, respectively.

3.3 | Cardiac effects

The bolus of Riociguat did not alter cardiac output or ejection fraction (Figure 2). Left ventricular maximum pressure development (LV dP/dt) decreased by 24% ± 12% (p = .00), while no change in this index was seen for the right ventricle. Stroke work was reduced by 20% ± 8% (p = .00) for the left, but not the right ventricle. Left ventricular end-systolic pressure–volume relation, i.e., elastance (LV Ees), was reduced by 14% ± 14% (p = .01). Right ventricular elastance (RV Ees), remained stable. Preload recruitable stroke work (PRSW) decreased by 13% ± 7% (p = .01) for the left ventricle but did not change for the right ventricle. The diastolic relaxation index, Tau, was unaltered for the left ventricle. Ventricular dimensions remained unchanged.

L-NAME decreased cardiac output by 23% ± 7% (p = .00) and left ventricular ejection fraction (EF) decreased from 33% ± 5% to 25% ± 4% (p = .00). Heart rate was unchanged. Reduction in EF was accompanied by an increase in end-systolic volumes for both ventricles; 11% ± 8% (p = .010) for the left ventricle and 12% ± 10% (p = .04) for the right ventricle. Left ventricular elastance increased by 29% ± 23% (p = .01) and right ventricular elastance increased by 18% ± 10% (p = .02) (Figures 5 and 6). Left ventricular PRSW increased by 12% ± 8% (p = .00), while no effect was found on right ventricular PRSW.

3.4 | Effects on ventriculoarterial coupling

Riociguat did not impact the relationship between contractility and afterload demonstrated by an unchanged ventriculoarterial coupling and stroke work efficiency for both the systemic and pulmonary circulation. L-NAME, on the other hand, elevated afterload proportionately more than contractility, causing an offset in the Ees/Ea relationship for both ventricles. This resulted in impaired stroke work efficiency in both left and right ventricle by 19% ± 5% (p = .00) and 25% ± 10% (p = .00) (Figures 3, 5 and 6).

Figure 4 shows an example of pressure–volume relations during preload reductions in one pig at baseline, and Figures 5 and 6 the detailed effects of Riociguat and L-NAME on the time-varying interaction of the two ventricles with their afterloads (ventriculoarterial coupling). These compilations demonstrate that Riociguat mainly acts by reducing the afterload on the left ventricle giving reduced stroke work due to lesser pressure work but no altered ventricular volumes. L-NAME increases the arterial elastance, reduces the volume-related stroke work with an increased end-systolic volume (reduced ejection fraction) with a resulting reduced cardiac output.

3.5 | Effects on mechanoenergetic efficiency

The mechanoenergetic efficiency of the left ventricle was unaffected by Riociguat, displayed by unaltered MVO₂/PVA relationship with regard to both slope and intercept. Blocking NO by applying a subsequent L-NAME bolus did not impact the relative oxygen consumption in Riociguat-treated hearts (Figure 7).

4 | DISCUSSION

The main finding in this study was an observed neutral effect of Riociguat on myocardial oxygen consumption. There was no shift in the MVO₂/PVA relationship for Riociguat,

### Table 1: Dose-response study of Riociguat

| Riociguat, n = 4 | Vehicle | 10 µg/kg | 20 µg/kg | 50 µg/kg | 100 µg/kg |
|-----------------|---------|----------|----------|-----------|-----------|
| MAP, mmHg       | 88 ± 21 | 87 ± 22  | 66 ± 11<sup>+</sup> | 56 ± 13<sup>+</sup> | 50 ± 9<sup>+</sup> |
| MPAP, mmHg      | 25 ± 23 | 22 ± 2   | 23 ± 5   | 25 ± 7    | 25 ± 8    |
| SVR, dynes/s/cm<sup>–5</sup> | 1,066 ± 248 | 957 ± 204 | 760 ± 180<sup>+</sup> | 568 ± 124<sup>+</sup> | 497 ± 161<sup>+</sup> |
| PVR, dynes/s/cm<sup>–5</sup> | 166 ± 109 | 146 ± 87 | 162 ± 76 | 179 ± 76 | 167 ± 65 |

Note: Dose-response data for Riociguat submitted in other publication. MAP, Mean systemic arterial pressure; MPAP, Mean pulmonary arterial pressure; SVR, Systemic vascular resistance; PVR, Pulmonary vascular resistance. Values are mean ± standard deviation. Significance levels between doses of test drug against baseline.

<sup>+</sup>=p < 0.05 (mixed model statistics with pig identity as random effect).
indicating that stimulation of sGC did not influence myocardial metabolism in healthy pigs in the dose given in this study. The dose chosen was the highest dose possible to administer while avoiding excessive hypotension. Of notice, the subsequent administration of the NO-blocker L-NAME did not increase the relative oxygen consumption in the myocardium, and this suggests that the expected surplus MVO₂ after using L-NAME (Nordhaug et al., 2004) was attenuated by this dose of Riociguat.

The indication for using Riociguat is idiopathic pulmonary artery hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH), two out of five categories of pulmonary hypertension (PH), according to the World Health Organization (Farber, Miller, & Poms, 2015). Inadequate NO-mediated vasodilation is believed to have an essential role in the pathogenesis of PAH. Thus, stimulating the NO-sGC-cGMP-PKG pathway is a seemingly attractive treatment target (Ghofrani, Humbert, & Langleben, 2017). Indeed, inhibition of the cGMP degradation by the PDE5 inhibitor Sildenafil has improved outcomes for patients with PAH (Galié et al., 2005). However, PAH is associated with reduced NO availability and reduced sensitivity of the sGC as caused by oxidative stress that may restrict the effectiveness of PDE5 inhibitors (Hoepner, Simonneau, & Corris, 2017; Lang, Kojonazarov, & Tian, 2012). Riociguat works through the same pathway; by stimulating sGC partly independent of available NO and with higher potency than NO when sGC has undergone oxidation in pathological tissues (Thoonen et al., 2015). While efforts have been taken to investigate the vascular effects of Riociguat in the diseased state with particular emphasis on PAH, little attention has been on the physiological effects of the drug on the heart in general and the right ventricle in particular. This is relevant since PAH is the leading cause of right heart failure, and heart failure determines the prognosis in PAH (Kylhammar, Kjellström, & Hjalmarsson, 2018; Vonk-Noordegraaf et al., 2013).

The impact of NO on contractility and cardiometabolic efficiency has been an area of particular attention. NO and cGMP seem to influence contractile function in a concentration-dependent bidirectional fashion. In an in vitro model, the effect of NO on cardiomyocytes was found to be dependent on intact endothelium. In this study, cyclic GMP affected the contractile response of the myocytes via a biphasic inhibition and then stimulation of cGMP-dependent cAMP phosphodiesterase inhibitor through modulation of intracellular stress that may restrict the effectiveness of PDE5 inhibitors (Hoeper, Simonneau, & Corris, 2017; Lang, Kojonazarov, & Tian, 2012). Riociguat works through the same pathway; by stimulating sGC partly independent of available NO and with higher potency than NO when sGC has undergone oxidation in pathological tissues (Thoonen et al., 2015). While efforts have been taken to investigate the vascular effects of Riociguat in the diseased state with particular emphasis on PAH, little attention has been on the physiological effects of the drug on the heart in general and the right ventricle in particular. This is relevant since PAH is the leading cause of right heart failure, and heart failure determines the prognosis in PAH (Kylhammar, Kjellström, & Hjalmarsson, 2018; Vonk-Noordegraaf et al., 2013).

The impact of NO on contractility and cardiometabolic efficiency has been an area of particular attention. NO and cGMP seem to influence contractile function in a concentration-dependent bidirectional fashion. In an in vitro model, the effect of NO on cardiomyocytes was found to be dependent on intact endothelium. In this study, cyclic GMP affected the contractile response of the myocytes via a biphasic inhibition and then stimulation of cGMP-dependent cAMP phosphodiesterase inhibitor through modulation of intracellular stress that may restrict the effectiveness of PDE5 inhibitors (Hoeper, Simonneau, & Corris, 2017; Lang, Kojonazarov, & Tian, 2012). Riociguat works through the same pathway; by stimulating sGC partly independent of available NO and with higher potency than NO when sGC has undergone oxidation in pathological tissues (Thoonen et al., 2015). While efforts have been taken to investigate the vascular effects of Riociguat in the diseased state with particular emphasis on PAH, little attention has been on the physiological effects of the drug on the heart in general and the right ventricle in particular. This is relevant since PAH is the leading cause of right heart failure, and heart failure determines the prognosis in PAH (Kylhammar, Kjellström, & Hjalmarsson, 2018; Vonk-Noordegraaf et al., 2013).

The impact of NO on contractility and cardiometabolic efficiency has been an area of particular attention. NO and cGMP seem to influence contractile function in a concentration-dependent bidirectional fashion. In an in vitro model, the effect of NO on cardiomyocytes was found to be dependent on intact endothelium. In this study, cyclic GMP affected the contractile response of the myocytes via a biphasic inhibition and then stimulation of cGMP-dependent cAMP phosphodiesterase inhibitor through modulation of intracellular stress that may restrict the effectiveness of PDE5 inhibitors (Hoeper, Simonneau, & Corris, 2017; Lang, Kojonazarov, & Tian, 2012). Riociguat works through the same pathway; by stimulating sGC partly independent of available NO and with higher potency than NO when sGC has undergone oxidation in pathological tissues (Thoonen et al., 2015). While efforts have been taken to investigate the vascular effects of Riociguat in the diseased state with particular emphasis on PAH, little attention has been on the physiological effects of the drug on the heart in general and the right ventricle in particular. This is relevant since PAH is the leading cause of right heart failure, and heart failure determines the prognosis in PAH (Kylhammar, Kjellström, & Hjalmarsson, 2018; Vonk-Noordegraaf et al., 2013).

The impact of NO on contractility and cardiometabolic efficiency has been an area of particular attention. NO and cGMP seem to influence contractile function in a concentration-dependent bidirectional fashion. In an in vitro model, the effect of NO on cardiomyocytes was found to be dependent on intact endothelium. In this study, cyclic GMP affected the contractile response of the myocytes via a biphasic inhibition and then stimulation of cGMP-dependent cAMP phosphodiesterase inhibitor through modulation of intracellular stress that may restrict the effectiveness of PDE5 inhibitors (Hoeper, Simonneau, & Corris, 2017; Lang, Kojonazarov, & Tian, 2012). Riociguat works through the same pathway; by stimulating sGC partly independent of available NO and with higher potency than NO when sGC has undergone oxidation in pathological tissues (Thoonen et al., 2015). While efforts have been taken to investigate the vascular effects of Riociguat in the diseased state with particular emphasis on PAH, little attention has been on the physiological effects of the drug on the heart in general and the right ventricle in particular. This is relevant since PAH is the leading cause of right heart failure, and heart failure determines the prognosis in PAH (Kylhammar, Kjellström, & Hjalmarsson, 2018; Vonk-Noordegraaf et al., 2013).

The impact of NO on contractility and cardiometabolic efficiency has been an area of particular attention. NO and cGMP seem to influence contractile function in a concentration-dependent bidirectional fashion. In an in vitro model, the effect of NO on cardiomyocytes was found to be dependent on intact endothelium. In this study, cyclic GMP affected the contractile response of the myocytes via a biphasic inhibition and then stimulation of cGMP-dependent cAMP phosphodiesterase inhibitor through modulation of intracellular...
Ca\(^{2+}\) levels and myofilament Ca\(^{2+}\) sensitivity. Increasing NO and cGMP levels first induced an increase in contractility followed by a fall at high concentrations (Mohan, Brutsaert, Paulus, & Sys, 1996). In a human study on cardiac failure, on the other hand, blockade of NO synthetase increased inotropic response to β-adrenergic stimulus (Hare, Givertz, Creager, & Colucci, 1998). The complex interaction between the NO-sGC-cGMP-pathway and contractility makes it challenging to predict the exact optimum dose of NO-donors or sGC activators or stimulators when aiming for therapeutically altered contractility.

In our study using sympathetically blocked healthy animals, Riociguat had a predominantly systemic vasodilatory effect. The concomitantly observed hemodynamic effects of the drug can, for a large part, be a consequence of this unloading effect. The effects on contractile function (elastance and PRSW), ventriculoarterial matching (Ees/Ea ratio) and left ventricular pump efficiency (SW efficiency) were overall minor. The model is therefore well designed to pick up relatively minor direct metabolic or oxygen-consuming effects of test-drugs. Using the same experimental setup, in earlier studies, we have shown that NO-blockade, inotropic drugs and systemic infections have clear metabolic effects in the myocardium evaluated in the frame of an MVO\(_2\)/PVA analysis (Aghajani, Korvald, Nordhaug, Revhaug, & Myrmel, 2004; Aghajani, Nordhaug, et al., 2004; Elvenes et al., 2000; How et al., 2005; Korvald, Elvenes, Ytrebø, Sørlie, & Myrmel, 1999; Nordhaug, Steensrud, Korvald, Aghajani, & Myrmel, 2002).

There is consistent evidence that blocking enzymes in the NO pathway causes reduced cardiac efficiency by surplus myocardial oxygen consumption (Chen, Traverse, Du, Hou, & Bache, 2002; Nordhaug et al., 2004; Suto et al., 1998). This is at least partly caused by vasoconstriction and
subsequent disruption of the ventriculoarterial matching by elevated afterload without a concomitant increase in contractility (Nordhaug et al., 2004). The maximum stroke work at a given end-diastolic volume will occur when Ees equals Eea (Figure 6). Stroke work efficiency (the fraction of total cardiacoxygen consumption, PVA, delivered to the circulation as stroke work), on the other hand, increases as the Ees/Ea ratio increases (Burkhoff & Sagawa, 1986). In these efficient states, a lower proportion of oxygen utilization is metabolized as potential energy (PE), where potential energy is the metabolism necessary to preserve the wall tension of the heart at end-systole. This means that a higher proportion of metabolic oxygen consumption is used for energy transfer to the aorta, and the oxygen cost of stroke work is, therefore, lower (De Tombe, Jones, Burkhoff, Hunter, & Kass, 2017). The correlation between oxygen consumption and ventriculoarterial matching is well established (Hayashida et al., 1992; Nozawa, Yasmura, & Futaki, 1987), and also holds true in our study with a linear correlation between measured oxygen consumption and PVA.

In addition, NO has a distinct metabolic effect independent of loading conditions (Recchia et al., 1999). Various metabolic studies, as reviewed by Chang, Diers, and Hogg (2015), have demonstrated that the normal NO-tone in tissues reduces the mitochondrial oxygen consumption by reducing the oxygen interaction with cytochrome c oxidase/complex I. However, there is only one study demonstrating a decrease in non-contractile oxygen utilization combined with an unchanged contractile efficiency (MVO₂/PVA) after infusion of the NO-precursor l-arginine. In that study the authors also observed the predicted opposite effect by infusion of a NO synthetase inhibitor (Suto et al., 1998). By employing a large animal model (pigs) in our laboratory, we have not been able to reproduce a

**FIGURE 6** Ventriculoarterial coupling, right ventricle. Schematic pressure–volume curves for the right ventricle based on the average values of the end-diastolic pressure–volume relationship (EDPVR), the maximum systolic pressure–volume relationship, and the pressure–volume relationship at the start of diastole. Curves are fitted through these three points. Also shown is the average volume at the x-axis intercept of the end systolic pressure–volume line (ESPVR line) at baseline, V₀. The slope of the ESPVR line is the right ventricular elastance (Ees) and the slope from the maximum systolic pressure–volume point to the x-axis intercept of the end diastolic volume is the pulmonary arterial elastance (Ea). Error bars are standard deviation for pressures and volumes. N = 6
decrease in basal oxygen requirements in the post ischemic, stunned heart after infusion of L-arginine, even though biochemical assessments demonstrated a substantial turnover of L-arginine to L-citrulline and thus a concomitant production of NO (Andersen, Igumnova, Kildal, & Myrmel, 2012). When studying experimental cardiogenic shock study in our lab, however, L-NAME was shown to increase the basal oxygen requirements in the heart (Nordhaug et al., 2004). The impaired stroke work efficiency induced by L-NAME was not mirrored in our present study, since there was no surplus MVO₂ when L-NAME was given after Riociguat. This is in contrast to the effect of L-NAME given alone in other studies, as this compound has been shown to induce increased unloaded oxygen consumption (Nordhaug et al., 2004; Suto et al., 1998). Of notice, in this study we have not included a separate group of NO-blockade alone (L-NAME) due to the consistent energetic effect observed with this intervention.

Our study shows that Riociguat given to healthy animals has only a minor effect in the pulmonary circulation with no impact on the right ventriculo-pulmonary arterial coupling.

Riociguat (Adempas®) has been approved for treatment in pulmonary hypertension based on pulmonary vasodilatory effects in animal disease models and human studies. The effect of this sGC stimulator is therefore different in healthy and diseased lung vasculature.

5 | CONCLUSION

Riociguat alone has no effect on myocardial oxygen consumption in the healthy heart. Both ventriculoarterial coupling and basal myocardial oxygen metabolism are unaffected by infusion of 100 µg/kg Riociguat. However, the subsequent administration of L-NAME did not increase the relative oxygen consumption in the myocardium, and this suggests that the expected surplus basal MVO₂ in the heart after blocking the endogenous NO-tone by L-NAME was attenuated by this dose of Riociguat. This oxygen-conserving effect during L-NAME stimulation indicates that Riociguat can induce “metabolic protection” in stressed myocardium and potentially also in pathological states. Such a potential should be explored in experimental disease models and during various pharmacological combinations.

6 | DATA SHARING NOTICE

The data used for the study can be shared upon personal contact with the authors.

ACKNOWLEDGMENTS

Thanks to the laboratory technicians in the cardiovascular research laboratory at the Arctic University in Tromsø.

CONFLICT OF INTEREST

None of the authors has any conflicts of interest.

AUTHOR CONTRIBUTIONS

Torvind Næsheim: Drafting protocol, sourcing medications, setting up the lab, instrumentation, data collection, analysis, and authoring; Ole-Jakob How: Idea, analysis, authoring; Truls Myrmel: Idea, drafting protocol, instrumentation, data collection, analysis, and authoring.

ETHICAL STATEMENT

The research complies with the ethical guidelines of animal research as given by the the Norwegian Animal Research Authority. The protocol was approved in advance with the reference number FDF 2012/55972.

ORCID

Torvind Næsheim https://orcid.org/0000-0002-6294-3667
Ole-Jakob How https://orcid.org/0000-0003-1495-0413
Truls Myrmel https://orcid.org/0000-0002-4148-9223
REFERENCES

Aghajani, E., Korvald, C., Nordhaug, D., Revhaug, A., & Myrmel, T. (2004). Increased oxygen cost of contractility in the endotoxemic porcine left ventricle. *Scandinavian Cardiovascular Journal, 38*(3), 187–192. https://doi.org/10.1080/14017430410031164

Aghajani, E., Nordhaug, D., Korvald, C., Steensrud, T., Husnes, K., & Ingebritsen, O. ... Myrmel, T. (2004). Mechanoenergetic inefficiency in the septic left ventricle is due to enhanced oxygen requirements for excitation-contraction coupling. *Cardiovascular Research, 63*(2), 256–263. https://doi.org/10.1016/j.cardiores.2004.04.019

Andersen, I. A., Igunnova, E., Kildal, A. B., & Myrmel, T. (2012). Increased oxygen cost of contractility in the endotoxemic porcine left ventricle. *Scandinavian Cardiovascular Journal, 38*(3), 187–192. https://doi.org/10.1080/14017430410031164

Aghajani, E., Korvald, C., Ytrebø L. M., Sørlie D. G., & Myrmel T. (1999). Oxygen-wasting effect inotropy in the “virtual work model”. *American Journal of Physiology-Heart and Circulatory Physiology, 276*(4), H1339–H1345. http://dx.doi.org/10.1152/ajpheart.1999.276.4.h1345

Korvald C., Elvenes O. P., & Myrmel T. (2000). Myocardial substrate metabolism influences left ventricular energetic in vivo. *American Journal of Physiology-Heart and Circulatory Physiology, 278*, (4), H1345–H1351. http://dx.doi.org/10.1152/ajpheart.2000.278.4.h1345

Farber, H. W., Miller, D. P., Poms, A. D., Badesch, D. B., Frost, A. E., Rouzie, E. M. -L., ... Benza, R. L. (2015). Five-Year outcomes of patients enrolled in the REVEAL Registry. *Chest, 148*(4), 1043–1054. https://doi.org/10.1378/chest.15-0300

Feneley M. P., Elbeery J. R., Gaynor J. W., Gall S. A., Davis J. W., Rankin J. S. (1990). Ellipsoidal shell subtraction model of right ventricular volume. Comparison with regional free wall dimensions as indexes of right ventricular function. *Circulation Research, 67*(6), 1427–1436. http://dx.doi.org/10.1161/01.res.67.14.1427

Galié, N., Ghofrani, H. A., Tobricki, A., Barst, R. J., Rubin, L. J., Badesch, D., ... Simonneau, G. (2005). Sildenafil citrate therapy for pulmonary arterial hypertension. *New England Journal of Medicine, 353*(20), 2148–2157. https://doi.org/10.1056/NEJMoa050010

Ghofrani, H.-A., Humbert, M., Langleben, D., Schermuly, R., Stasch, J.-P., Wilkins, M. R., & Klinger, J. R. (2017). Riociguat: Mode of action and clinical development in pulmonary hypertension. *Chest, 151*(2), 468–480. https://doi.org/10.1016/j.chest.2016.05.024

Hare, J. M., Givertz, M. M., Creager, M. A., & Colucci, W. S. (1998). Increased sensitivity to nitric oxide synthase inhibition in patients with heart failure: Potentiation of β-adrenergic inotropic responsiveness. *Circulation, 97*(2), 161–166. https://doi.org/10.1161/10.1.97.2.161

Hayashi, K., Sunagawa, K., Noma, M., Sugimachi, M., Ando, H., & Nakamura, M. (1992). Mechanical matching of the left ventricle with the arterial system in exercising dogs. *Circulation Research, 71*(3), 481–489. https://doi.org/10.1161/106.RES.71.3.481

Hooper, M. M., Simonneau, G., Corris, P. A., Ghofrani, H.-A., Klinger, J.R., Langleben, D., ... Benza, R.L. (2017). RESPITE: Switching to riociguat in pulmonary arterial hypertension patients with inadequate response to phosphodiesterase-5 inhibitors. *European Respiratory Journal, 50*(3). https://doi.org/10.1183/13993003.02425-2016

How, O., Aasum, E., Kunnathu, S., Severson, D. L., Myhr, E. S. P., & Larsen, T. S. (2005). Influence of substrate supply on cardiac efficiency, as measured by pressure-volume analysis in ex vivo mouse hearts. *American Journal of Physiology-Heart and Circulatory Physiology, 288*(6), H2979–H2985. https://doi.org/10.1152/ajpheart.00084.2005
Myrmel, T., & Korvald, C. (2000). New aspects of myocardial oxygen consumption. Invited review. Scandinavian Cardiovascular Journal, 34(3), 233–241. https://doi.org/10.1080/713783125

Næsheim, T., O. J. How, and T. Myrmel. 2020. Hemodynamic effects of a soluble guanylate cyclase stimulator, riociguat, and an activator, cinaciguat, during NO-modulation in healthy pigs. Journal of Cardiovascular Pharmacology. https://doi.org/10.1177/10742450204842094897.

Nordhaug D., Steensrud T., Aghajani E., Korvald C., & Myrmel T. (2004). Nitric oxide synthase inhibition impairs myocardial efficiency and ventriculo-arterial matching in acute ischemic heart failure. European Journal of Heart Failure, 6, (6), 705–713. http://dx.doi.org/10.1016/j.ejheart.2003.11.010.

Nordhaug, D., Steensrud, T., Korvald, C., Aghajani, E., & Myrmel, T. (2002). Preserved myocardial energetics in acute ischemic left ventricular failure – studies in an experimental pig model. European Journal of Cardio-Thoracic Surgery, 22(1), 135–142. https://doi.org/10.1016/S1010-7940(02)00201-4.

Nozawa, T., Yasumura, Y., Futaki, S., Tanaka, N., Igarashi, Y., Goto, Y., & Suga, H. (1987). Relation between oxygen consumption and pressure-volume area of in situ dog heart. American Journal of Physiology-Heart and Circulatory Physiology, 253(1), H31–H40. https://doi.org/10.1152/ajpheart.1987.253.1.H31

Recchia F (1999). Nitric oxide controls cardiac substrate utilization in the conscious dog. Cardiovascular Research, 44, (2), 325–332. http://dx.doi.org/10.1016/s0008-6363(99)00245-x.

Rees, D. D., Palmer, R. M. J., Schulz, R., Hodson, H. F., & Moncada, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. British Journal of Pharmacology, 101(3), 746–752. https://doi.org/10.1111/j.1476-5381.1990.tb14151.x

Suto N., Mikuniya A., Okubo T., Hanada H, Shinozaki N, & Okumura K. (1998). Nitric oxide modulates cardiac contractility and oxygen consumption without changing contractile efficiency. American Journal of Physiology-Heart and Circulatory Physiology, 275, (1), H41–H49. http://dx.doi.org/10.1152/ajpheart.1998.275.1.h41.

Thoonen, R., Cauwels, A., Decaluwe, K., Geschka, S., Tainsh, R. E., Delanghe, J., …, Brouckaert, P. (2015). Cardiovascular and pharmacological implications of haem-deficient NO-unresponsive soluble guanylate cyclase knock-in mice. Nature Communications, 6, 8482. https://doi.org/10.1038/ncomms9482.

Vanderpool, R. R., Pinsky, M. R., Naeije, R., Deible, C., Kosaraju, V., Bunner, C., …, Simon, M. A. (2015). RV-pulmonary arterial coupling predicts outcome in patients referred for pulmonary hypertension. Heart, 101(1), 37–43. https://doi.org/10.1136/heartjnl-2014-306142.

Vasu M. A., O’Keefe D. D., Kapellakis G. Z., Vezeridis M. P., Jacobs M. L., Daggett W. M., & Powell W. J. (1978). Myocardial oxygen consumption: effects of epinephrine, isoproterenol, dopamine, norepinephrine, and dobutamine. American Journal of Physiology-Heart and Circulatory Physiology, 235, (2), H237–H241. http://dx.doi.org/10.1152/ajpheart.1978.235.2.h237.

Vonk-Noordegraaf A., Haddad F., Chin K. M., Forfia P. R., Kawut S. M., Lumens J., …, Hassoun P. M. (2013). Right Heart Adaptation to Pulmonary Arterial Hypertension. Journal of the American College of Cardiology, 62, (25), D22–D33. http://dx.doi.org/10.1016/j.jacc.2013.10.027.

Yao, L., Xue, X., Yu, P., Ni, Y., & Chen, F. (2018). Evans blue dye: A revisit of its applications in biomedicine. Contrast Media & Molecular Imaging, 2018, 18–24. https://doi.org/10.1155/2018/7628037.

How to cite this article: Næsheim T, How O-J, Myrmel T. The effect of Riociguat on cardiovascular function and efficiency in healthy, juvenile pigs. Physiol Rep. 2020;8:e14562. https://doi.org/10.14814/phy2.14562.