Does extracorporeal membrane oxygenation attenuate hypoxic pulmonary vasoconstriction in a porcine model of global alveolar hypoxia?

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Background: During severe respiratory failure, hypoxic pulmonary vasoconstriction (HPV) is partly suppressed, but may still play a role in increasing pulmonary vascular resistance (PVR). Experimental studies suggest that the degree of HPV during severe respiratory failure is dependent on pulmonary oxygen tension (PvO2). Therefore, it has been suggested that increasing PvO2 by veno-venous extracorporeal membrane oxygenation (V-V ECMO) would adequately reduce PVR in V-V ECMO patients.

Objective: Whether increased PvO2 by V-V ECMO decreases PVR in global alveolar hypoxia.

Methods: Nine landrace pigs were ventilated with a mixture of oxygen and nitrogen. After 15 minutes of stable ventilation and hemodynamics, the animals were cannulated for V-V ECMO. Starting with alveolar normoxia, the fraction of inspiratory oxygen (FIO2) was stepwise reduced to establish different degrees of alveolar hypoxia. PvO2 was increased by V-V ECMO.

Results: V-V ECMO decreased PVR (from 5.5 [4.5-7.1] to 3.4 [2.6-3.9] mm Hg L⁻¹ min, P = .006) (median (interquartile range),) during ventilation with F I O2 of 0.15. At lower F I O2, PVR increased; at F I O2 0.10 to 4.9 [4.2-7.0], P = .036, at F I O2 0.05 to 6.0 [4.3-8.6], P = .002, and at F I O2 0 to 5.4 [3.5 - 7.0] mm Hg L⁻¹ min, P = .05.

Conclusions: The effect of increased PvO2 by V-V ECMO on PVR depended highly on the degree of alveolar hypoxia. Our results partly explain why V-V ECMO does not always reduce right ventricular afterload at severe alveolar hypoxia.

1 | INTRODUCTION

During hypoxic respiratory failure, for example, severe acute respiratory distress syndrome (ARDS), substantial parts of the lungs are collapsed or edematous, and therefore the oxygen tension in the alveoli (P A O2) in these regions is very low. Patients with severe ARDS could develop right heart failure due to the increased pulmonary vascular resistance (PVR), which may be partially caused by hypoxic pulmonary vasoconstriction (HPV).1-5 HPV redirects the blood flow from hypoxic areas of the lung to regions with intact ventilation maintaining systemic oxygenation. However, HPV may be damped in severe ARDS.6,7 HPV is influenced by P A O2, the oxygen tension in the pulmonary artery (PvO2) and the bronchial arteries with P A O2 being the strongest stimulus.8-11 Indeed, the oxygen tension, which acts as the stimulus for the HPV response (PsO2) at
the precapillary sensing site of the pulmonary circulation can be expressed as a function of $P_AO_2$ and $PvO_2$: 

$$P_{A}O_2 = P_{O_2}^{0.62} \times P_{vO_2}^{0.38}$$  \hspace{1cm} (1)$$

$P_AO_2$ cannot be measured directly and has therefore to be estimated by the alveolar gas Equation 12:

$$P_{A}O_2 = F_iO_2 \times (P_{atm} - P_{H_2O}) - PaCO_2 RQ^{-1}$$  \hspace{1cm} (2)$$

The specific pathways for hypoxia sensing are not fully understood. Nevertheless, low oxygen tensions activate pulmonary arterial smooth muscle contraction and thus increase PVR.

When conventional treatment in ARDS fails, extracorporeal membrane oxygenation (ECMO) is considered as a rescue procedure. ECMO is established either by the veno-venous (V-V ECMO) or the veno-arterial (V-A ECMO) approach. V-V ECMO is the method of choice for respiratory failure as long as cardiac function is preserved. In an experimental study of atelectatic lungs it has been found that $PvO_2 >100$ mm Hg $(>13.3$ kPa) effectively reduced PVR. Moreover, case series and reviews propose that V-V ECMO might adequately reduce PVR by increasing $PvO_2$ when HPV is present during severe respiratory failure, and thus, reduce the right ventricular afterload.

The rationale is that the increased $PvO_2$ would attenuate HPV and PVR by oxygen sensing and signal transduction mechanisms located in the precapillary region independently from $P_AO_2$.

In contrast to these reports, in our experience the increased $PvO_2$ by V-V ECMO does usually not counteract HPV in the most severe forms of ARDS. We hypothesized that this disagreement could be due to a difference in the degree of severity of the ARDS, that is, the amount shunting through lung units with low $P_AO_2$. Therefore, the aim of this study was to carefully dissect the specific influence of $PvO_2$ and $P_AO_2$ during V-V ECMO on HPV in a porcine model of global alveolar hypoxia.

2 | METHODS

2.1 | Ethics

The study was approved by the local ethics committee for animal research in Uppsala, Sweden, Dnr. 5.8.18-14077/2017. The animals were taken care off according to the ethical guidelines and the European Union’s directive. Twelve landrace pigs from a local breeder were used in the study, which was performed at the Hedenstierna Laboratory, Uppsala University, Sweden.

2.2 | Animal preparation

2.2.1 | Anesthesia and mechanical ventilation

Anesthesia was performed as previously reported. After arrival at the laboratory the pigs received an intramuscular injection of tiletamine/zolazepam (6 mg kg$^{-1}$; Zoletil$^6$; Virbac) and xylazine (2.2 mg kg$^{-1}$; Rompun$^6$, Bayer) into the neck or thigh for induction of anesthesia. After a sufficient degree of anesthesia was established, a peripheral intravascular catheter was placed in a left or right auricular vein. The animals were placed in supine position, and fentanyl (Leptanal$^6$), Janssen-Cilag AB, Sweden, 2-4.5 μg kg$^{-1}$ was administered before a tracheotomy was performed. Anesthesia was maintained by continuous infusion of midazolam (midazolam Actavis, Actavis Group, Hafnersjford, Iceland; 0.1-0.18 mg kg$^{-1}$ h$^{-1}$), fentanyl (Leptanal$^6$; Janssen-Cilag AB, Sweden; 3 - 6 μg kg$^{-1}$ h$^{-1}$) and ketamine (Ketaminol; Vetpharma, Zurich, Switzerland; 25-50 mg kg$^{-1}$ h$^{-1}$). After adequate levels of anesthesia and analgesia were ascertained by the absence of reaction to painful stimulation of the rear hooves, rocuronium bromide (2.5-5 mg kg$^{-1}$ h$^{-1}$; Esmeron, Organon, Oss, the Netherlands) was added for muscle relaxation. The animals received 30 mL kg$^{-1}$ hour$^{-1}$ of Ringer’s acetate solution during the first hour after induction of anesthesia and 20 mL kg$^{-1}$ hour$^{-1}$ afterward during the remaining experiment.

An endotracheal tube (ID 8.0, Mallinckrodt Pharmaceuticals, Athlone, Ireland) was placed into the trachea via a tracheostomy. Pressure-controlled ventilation (PCV) using a Servo-I (Maquet, Solna, Sweden) was started in all animals. Initial settings were a fraction of inspiratory oxygen $(F_iO_2)$ of 0.4, inspiratory: expiratory (I:E) 1:2, positive end-expiratory pressure (PEEP) 5-8 cm H$_2$O, and inspiratory pressure was set to achieve $PaCO_2$ 35-40 mm Hg. Ventilation remained unchanged during the study.

2.2.2 | Monitoring

A triple lumen 7.5 F pulmonary artery catheter (Swan-Ganz CCombo, Edwards Lifescience) and a 20G arterial catheter (Merit Medical) were inserted for hemodynamic (continuous cardiac output, pulmonary and peripheral arterial pressure) and oxygenation measurements. Hemodynamic monitoring was performed with a SC 9000 XL monitor (Siemens Medical Systems Inc). $F_iO_2$ was measured by the ventilator’s oxygen sensor during the experiments after its
accuracy was confirmed with a Deltatrac® (Datex-Ohmeda) in two pilot animals, and continuously recorded during the experiments by a custom-build data acquisition system. During the experiment, pre- and post-oxygenator blood samples were taken to evaluate membrane lung performance and oxygen transport by ECMO. Blood samples were immediately analyzed on an ABL 500 and an OSM3 blood gas analyzer (Radiometer).

2.2.3 | ECMO

After stabilization and unchanged circulatory and ventilatory parameters for 15 minutes, the animals were cannulated for V-V ECMO. ECMO cannulas were inserted into the right or left femoral vein and the right or left external jugular vein, by either an open surgical or a semi-percutaneous approach. Heparin was administered intravenously before cannulation (50 IE kg\(^{-1}\), LEO Pharma). We cannulated three animals for femoro-atrial V-V ECMO with a 21F/38cm cannula (Maquet) for drainage of desaturated blood and a 19F/23cm cannula for reinfusion and six animals for atrio-femoral V-V ECMO (21F/38cm or 25F/55cm for drainage and 17, 19, or 21F/23 cm for reinfusion) in a non-randomized manner. For the semi-percutaneous approach the diameter of the vessel was studied with ultrasound before cannulation, the open surgical approach did not require this examination. The largest possible cannula size was chosen to optimize the flow. We planned to treat hemodynamic instability during initiation of ECMO by continuous infusion of norepinephrine and/or dobutamine (mean arterial pressure below 60 mm Hg or cardiac output below 3.0 L min\(^{-1}\)).

Because alpha and beta agonists may change PVR and thereby bias the results, the protocol did not allow any changes of these drugs during the experiment regardless the hemodynamic situation. Cannula position was examined by X-ray. The position of the cannulas was assumed being correct when the tip of the atrial cannula was located at the beginning of the right atrium and the femoral cannula could not be detected in the same examination. Furthermore, during alveolar hypoxia with ECMO the cannulas could be repositioned by introducing or retracting to achieve the highest possible SvO\(_2\), thus decreasing the amount of recirculation. The ECMO systems used were (a) a Cardiohelp console with uncoated HLS set advanced (Maquet) (n = 2) or (b) a MDC console with a Deltastream, DP3 pump, 3/8 " tubing with an integrated Hilite 7000LT oxygenator (Medos Medizintechnik AG) or Novalung, X-lung set 3/8 " (Xenios AG) (n = 7).

2.3 | Experimental design

The experiment was designed as a longitudinal study with repeated-measures within subjects where every animal was exposed to different experimental conditions. We studied the effect of increasing the PvO\(_2\) by V-V ECMO during different degrees of alveolar hypoxia and the effect of changing F\(_{\text{IO2}}\) from 0.15 and 0 to 0.21 on PVR. In total, nine comparisons were made in every animal. We assumed that changes in mean pulmonary arterial pressure (MPAP) reflect changes in PVR well and therefore MPAP was used when performing the experiments as the main parameter of interest. PVR was calculated after the experiments were finished.

The animals were ventilated with a mixture of oxygen and nitrogen. During the experiment F\(_{\text{IO2}}\) was reduced stepwise to achieve different degrees of alveolar hypoxia. PvO\(_2\) was increased by V-V ECMO. Oxygen was applied with a concentration of 100% and a flow rate of 1 liter into the membrane lung. With this setting the oxygenation of the blood passing the extracorporeal system is flow dependent. Therefore, during alveolar hypoxia the extracorporeal flow (ECC) was increased either until a decrease in MPAP occurred or the maximal possible flow leading to an increase in the saturation in the pulmonary artery (SvO\(_2\)) was reached. The maximal

![Flowchart of the experimental design](image)
| Animal | Weight (kg) | PIP (cmH₂O) | MAWP (cmH₂O) | PEEP (cmH₂O) | RR | Vt (mL) | Mv (L/min) | PaO₂ (mm Hg) | PaCO₂ (mm Hg) | SaO₂ (%) | PvO₂ (mm Hg) | SvO₂ (%) | pH | CI (L/min/m²) | MAP (mm Hg) | MPAP (mm Hg) | PAOP (mm Hg) |
|--------|-------------|--------------|--------------|-------------|----|--------|----------|-------------|--------------|----------|-------------|----------|----|----------------|-------------|-------------|--------------|
| 1      | 46          | 16           | 10           | 8           | 20 | 390    | 7.5      | 11.0        | 5.6          | 93.6     | 4.2         | 43.9     | 7.49 | 5.2            | 123         | 26          | 9            |
| 2      | 58.8        | 16           | 11           | 8           | 20 | 385    | 8        | 10.7        | 6.0          | 93       | 4.8         | 52.2     | 7.47 | 4.5            | 108         | 26          | 9            |
| 3      | 48.4        | 25           | 14           | 8           | 24 | 509    | 12.2     | 11.2        | 5.3          | 93.7     | 4.1         | 42       | 7.45 | 5.3            | 92          | 39          | 17           |
| 4      | 51.6        | 20           | 13           | 9           | 22 | 418    | 9.3      | 14.7        | 4.3          | 95.7     | 3.7         | 30.2     | 7.44 | 3.1            | 73          | 27          | 17           |
| 5      | 46.1        | 15           | 9            | 6           | 21 | 352    | 7.4      | 12.3        | 4.6          | 95.8     | 3.1         | 29.6     | 7.57 | 4.2            | 77          | 28          | 14           |
| 6      | 44.7        | 17           | 10           | 7           | 22 | 347    | 6.3      | 11.7        | 5.7          | 94.1     | 4.1         | 37       | 7.41 | 4.5            | 89          | 27          | 15           |
| 7      | 53.9        | 17           | 11           | 8           | 19 | 476    | 9.1      | 10.7        | 4.5          | 93.9     | 3.7         | 42.3     | 7.55 | 5.1            | 81          | 29          | 17           |
| 8      | 57          | 19           | 13           | 9           | 17 | 474    | 8.1      | 11.8        | 4.4          | 94       | 3.6         | 33.8     | 7.49 | 4              | 81          | 29          | 15           |
| 9      | 50          | 16           | 11           | 8           | 18 | 393    | 7.1      | 11.4        | 4.9          | 93.7     | 4.3         | 46.3     | 7.47 | 6.4            | 66          | 25          | 15           |

Median (IQR)
| Animal | Weight (kg) | PIP (cmH₂O) | MAWP (cmH₂O) | PEEP (cmH₂O) | RR | Vt (mL) | Mv (L/min) | PaO₂ (mm Hg) | PaCO₂ (mm Hg) | SaO₂ (%) | PvO₂ (mm Hg) | SvO₂ (%) | pH | CI (L/min/m²) | MAP (mm Hg) | MPAP (mm Hg) | PAOP (mm Hg) |
|--------|-------------|--------------|--------------|-------------|----|--------|----------|-------------|--------------|----------|-------------|----------|----|----------------|-------------|-------------|--------------|
| 1      | 46          | 16           | 10           | 8           | 20 | 390    | 7.5      | 11.0        | 5.6          | 93.6     | 4.2         | 43.9     | 7.49 | 5.2            | 123         | 26          | 9            |
| 2      | 58.8        | 16           | 11           | 8           | 20 | 385    | 8        | 10.7        | 6.0          | 93       | 4.8         | 52.2     | 7.47 | 4.5            | 108         | 26          | 9            |
| 3      | 48.4        | 25           | 14           | 8           | 24 | 509    | 12.2     | 11.2        | 5.3          | 93.7     | 4.1         | 42       | 7.45 | 5.3            | 92          | 39          | 17           |
| 4      | 51.6        | 20           | 13           | 9           | 22 | 418    | 9.3      | 14.7        | 4.3          | 95.7     | 3.7         | 30.2     | 7.44 | 3.1            | 73          | 27          | 17           |
| 5      | 46.1        | 15           | 9            | 6           | 21 | 352    | 7.4      | 12.3        | 4.6          | 95.8     | 3.1         | 29.6     | 7.57 | 4.2            | 77          | 28          | 14           |
| 6      | 44.7        | 17           | 10           | 7           | 22 | 347    | 6.3      | 11.7        | 5.7          | 94.1     | 4.1         | 37       | 7.41 | 4.5            | 89          | 27          | 15           |
| 7      | 53.9        | 17           | 11           | 8           | 19 | 476    | 9.1      | 10.7        | 4.5          | 93.9     | 3.7         | 42.3     | 7.55 | 5.1            | 81          | 29          | 17           |
| 8      | 57          | 19           | 13           | 9           | 17 | 474    | 8.1      | 11.8        | 4.4          | 94       | 3.6         | 33.8     | 7.49 | 4              | 81          | 29          | 15           |
| 9      | 50          | 16           | 11           | 8           | 18 | 393    | 7.1      | 11.4        | 4.9          | 93.7     | 4.3         | 46.3     | 7.47 | 6.4            | 66          | 25          | 15           |

Note: Weight in kg; PIP: peak inspiratory pressure (cmH₂O); MAWP: mean airway pressure (cmH₂O); PEEP: positive endexpiratory pressure (cmH₂O); RR: respiratory rate; Vt: tidal volume (mL); Mv: minute volume (L/min); PaO₂: arterial oxygen tension (mm Hg); PaCO₂: arterial carbon dioxide tension (mm Hg); SaO₂: arterial hemoglobin oxygen saturation (%); PvO₂: oxygen tension in the pulmonary artery (mm Hg); SvO₂: hemoglobin oxygen saturation in the pulmonary artery; CI: cardiac index (L/min/m²); MAP: mean arterial pressure (mm Hg); MPAP: mean pulmonary artery pressure (mm Hg); PAOP: pulmonary artery occlusion pressure (mm Hg).

Individual data, median values (interquartile range) are shown.
possible extracorporeal flow is named “maximal V-V ECMO support” in the text. Figure 1 shows a flowchart of the experimental design. Hemodynamic and oxygenation parameters were obtained before changing an experimental condition when no further change in MPAP could be detected or before if severe hemodynamic instability occurred. Maximal pulmonary vasoconstriction was expected to occur approximately 10 minutes after a change in $F_O_2$. Because of the interindividual variability in the vascular response to the hypoxic challenge the duration of study periods were somewhat different.

After the last measurements, the animals, still in deep anesthesia, were killed by an intravenous injection of 20 mL (100 mmol) KCl.

2.4 | Calculations

$P_S{O_2}$ (mm Hg) and $P_A{O_2}$ (mm Hg) were calculated according to Equations 1 and 2, respectively. $P_V$ (mm Hg $L^{-1} min^{-1}$) = (MPAP - PAOP) $CO^{-1}$ where PAOP (mm Hg) is the pulmonary artery occlusion pressure and $CO$ ($L min^{-1}$) the cardiac output. The systemic vascular resistance (SVR) was calculated as SVR (mm Hg $L^{-1} min^{-1}$) = (MAP - CVP) $CO^{-1}$. MAP = mean arterial pressure, CVP = central venous pressure.

2.5 | Sample size and power calculation

We calculated the sample size before study start based on the results of published case series and one experimental study. We expected a change of approximately 20%-25% of MPAP between two different conditions during the experiment. To reach a power of 80% ($1 - \beta = 0.8$) with a moderate effect size and a type I error of 5% the number of animals to be studied was calculated as $n = 32$. After analysis of data of 9 of 12 consecutive included animals the study had reached a power of 99% for MPAP and PVR. Therefore, the study was stopped.

2.6 | Statistical calculation

Differences in sum of ranks of MPAP and PVR of the entire study population between different experimental conditions were tested with non-parametric one-way analysis of variance (the Friedman test) and Dunn’s post-test for multiple comparisons. The Kendall’s $W$ coefficient was calculated as the effect size for the Friedman test. The following correlations were calculated by Spearman’s rank correlation: between $P_V$, $P_A{O_2}$, $P_V{O_2}$, venous pH, arterial pH, venous $P_C{O_2}$, arterial $P_C{O_2}$, MPAP, and $CO$; correlations between $CO$ and MPAP and SVR and MAP.
| F1O2 | %O2 ML | ECC | RPM | CO   | PVR | MPAP | PAO2 | PVCO2 | PSO2 | PAO2 | MAP | SaO2 | SvO2 |
|------|--------|-----|-----|------|-----|------|------|-------|------|------|-----|------|------|
| 0.21 | 0.6    | 1800 (650-2950) | 4.5 (3.9-5.0) | 3.3 (2.7-3.7) | 27 (26-29) | 13.5 (12.9-14.5) | 4.1 (3.6-4.3) | 8.5 (8.2-8.7) | 11.4 (10.9-12.1) | 81 (75-100) | 94 (94-95) | 42 (32-45) |
| 0.15 | 0.6    | 2000 (800-2950) | 5.1 (3.8-5.3) | 5.5 (4.5-7.1) | 42 (38-45) | 8.3 (7.4-8.7) | 2.8 (2.5-3.4) | 5.3 (5.2-5.6) | 6.1 (5.5-6.9) | 65 (52-75) | 77 (70-81) | 20 (17-30) |
| 0.15 | 100    | 7500 (5287-8350) | 5.0 (4.0-5.7) | 3.4 (2.6-3.9) | 29 (28-34) | 8.8 (8.5-9.3) | 8.2 (6.5-9.7) | 8.5 (7.5-9.3) | 11.1 (9.1-11.6) | 92 (73-113) | 94 (90-95) | 86 (75-92) |
| 0.15 | 100    | 7600 (5288-8750) | 4.6 (4.3-5.8) | 3.5 (2.3-4.6) | 28 (27-35) | 8.8 (8.5-9.3) | 7.8 (6.5-10) | 8.4 (7.7-9.4) | 10.4 (8.9-11.5) | 86 (61-110) | 94 (91-95) | 87 (76-92) |
| 0.15 | 100    | 7500 (5288-8550) | 4.7 (4.2-5.6) | 3.2 (2.2-4.3) | 29 (25-32) | 8.9 (8.5-9.4) | 7.5 (6.3-10.1) | 8.3 (7.5-9.5) | 11 (9.4-11.5) | 79 (71-101) | 94 (92-95) | 85 (76-92) |
| 0.15 | 0.4    | 7500 (4083-8550) | 4.7 (3.9-5.6) | 6.7 (4.1-7.3) | 42 (39-46) | 7.7 (7.3-8.9) | 2.6 (2.4-3.0) | 5.2 (5.0-5.5) | 5.6 (5.1-7.1) | 64 (42-88) | 75 (63-81) | 18 (14-23) |
| 0.21 | 0.4    | 7500 (4083-8550) | 4.3 (3.9-5.6) | 3.2 (2.4-3.6) | 28 (27-31) | 13.2 (12.8-14.4) | 3.7 (3.4-4.2) | 8.3 (8.1-8.5) | 10.4 (9.8-13.5) | 88 (71-114) | 94 (92-96) | 37 (31-44) |
| 0.15 | 100    | 7500 (5288-9100) | 4.2 (4.0-6.4) | 3.4 (2.1-4.3) | 29 (26-33) | 8.8 (8.4-9.1) | 7.4 (7.0-9.7) | 8.4 (7.8-9.2) | 11.1 (9.3-11.8) | 80 (67-104) | 93 (92-95) | 84 (78-91) |
| 0.1  | 100    | 9250 (8450-9575) | 4.9 (3.9-6.0) | 4.9 (4.2-7.0) | 38 (37-46) | 4.1 (3.7-4.4) | 8.3 (6.7-10.5) | 5.4 (4.6-6.2) | 7.7 (6.5-8.2) | 79 (54-94) | 86 (81-89) | 88 (75-94) |
| 0.05 | 100    | 9500 (6910-9800) | 4.9 (4.1-6.0) | 6.0 (4.3-8.6) | 45 (39-51) | 0 | 8.0 (6.0-10.3) | 0 | 5.8 (5.1-6.3) | 70 (53-83) | 73 (62-78) | 85 (68-92) |
| 0   | 100    | 9500 (7050-10000) | 5.5 (4.2-6.0) | 5.4 (3.5-7.0) | 45 (36-47) | 0 | 6.7 (6.3-12.5) | 0 | 4.6 (4.0-5.6) | 49 (33-71) | 58 (47-66) | 75 (70-96) |
| 0.21 | 100    | 9500 (7050-10000) | 5.0 (4.2-6.4) | 3.6 (2.2-4.4) | 31 (28-34) | 14.2 (13.6-15.3) | 9.3 (8.0-12.5) | 11.9 (11.3-13.5) | 15.3 (13.3-16.3) | 99 (71-119) | 95 (93-96) | 88 (81-93) |

Note: F1O2: fraction of inspiratory oxygen; %O2 ML: percent of oxygen over membrane lung, oxygen was applied with a flow rate of 1 L min⁻¹; ECC: veno-venous extracorporeal circuit flow (L min⁻¹); RPM: round per minute; CO: cardiac output (L min⁻¹); PVR: pulmonary vascular resistance (mm Hg L⁻¹ min⁻¹); MPAP: mean pulmonary arterial pressure (mm Hg); PAO2: alveolar oxygen tension (kPa); PVCO2: pulmonary artery oxygen tension (kPa); PSO2: hypoxic stimulus (kPa); PAO2: partial pressure of oxygen in arterial blood (kPa); MAP: mean arterial blood pressure (mm Hg); SaO2: arterial hemoglobin oxygen saturation (%); SvO2: hemoglobin oxygen saturation in the pulmonary artery (%). Data are expressed as median values (interquartile range).
TABLE 3  Blood carbon dioxide, pH, and ventilatory parameters during the experiment

| $F_{O_2}$ | %O$_2$ ML | ECC | $PaCO_2$ | Arterial pH | $PvCO_2$ | Venous pH | $V_t$ | $Mv$ |
|---------|----------|-----|----------|-------------|----------|-----------|------|------|
| 0.21    | 0        | 0.6 (0.1-1.2) | 4.9 (4.4-5.7) | 7.47 (7.45-7.52) | 6.6 (5.6-7.1) | 7.42 (7.36-7.47) | 393 (369-475) | 8.0 (7.3-9.2) |
| 0.15    | 0        | 0.6 (0.3-1.2) | 4.6 (4.4-5.6) | 7.50 (7.45-7.56) | 6.1 (5.5-7.1) | 7.41 (7.37-7.48) | 406 (367-471) | 7.9 (7.5-9.1) |
| 0.15    | 100      | 4.3 (3.7-5.2) | 4.3 (3.9-4.7)* | 7.51 (7.47-7.58) | 5.6 (5.2-6.1)* | 7.44 (7.38-7.48) | 387 (356-470) | 7.2 (6.8-7.8) |
| 0.15    | 100      | 4.8 (3.9-5.2) | 4.2 (3.9-4.7) | 7.54 (7.48-7.59) | 5.5 (5.1-6.2) | 7.45 (7.37-7.49) | 386 (347-477) | 7.0 (6.8-7.9) |
| 0.15    | 100      | 4.5 (3.9-4.9) | 4.1 (3.9-4.6) | 7.54 (7.46-7.59) | 5.4 (5.1-6.4) | 7.45 (7.37-7.50) | 390 (345-480) | 7.0 (6.8-7.9) |
| 0.15    | 0        | 4.2 (3.2-5.0) | 5.0 (4.2-5.6)* | 7.47 (7.44-7.54)* | 6.2 (5.6-6.9)* | 7.40 (7.29-7.46) | 370 (323-437) | 6.9 (6.3-7.7) |
| 0.21    | 0        | 4.2 (3.2-5.0) | 5.2 (4.5-5.7) | 7.44 (7.40-7.51) | 6.5 (5.8-7.3) | 7.39 (7.31-7.46) | 428 (380-472) | 7.4 (6.7-9.4) |
| 0.15    | 100      | 4.6 (4.1-5.0) | 4.3 (4.2-4.7)* | 7.54 (7.47-7.58)* | 5.7 (5.0-6.3)* | 7.44 (7.35-7.48)* | 385 (342-471) | 7.4 (6.7-8.1) |
| 0.1    | 100      | 5.4 (5.1-6.0) | 4.4 (4.0-4.7) | 7.55 (7.46-7.56) | 5.5 (5.2-6.5) | 7.43 (7.35-7.47) | 379 (331-456) | 7.4 (6.7-7.6) |
| 0.05   | 100      | 5.8 (5.1-6.1) | 4.5 (4.1-5.0) | 7.50 (7.44-7.54) | 5.6 (5.3-5.7) | 7.37 (7.32-7.45) | 365 (329-455) | 7.4 (5.9-7.7) |
| 0     | 100      | 5.6 (5.1-6.1) | 4.4 (3.8-5.3) | 7.50 (7.39-7.52) | 5.5 (5.1-6.1) | 7.38 (7.34-7.46) | 369 (335-455) | 7.4 (5.9-7.6) |
| 0.21   | 100      | 5.5 (5.0-6.1) | 4.8 (3.9-5.4) | 7.46 (7.43-7.53) | 5.7 (5.2-6.9) | 7.37 (7.32-7.35) | 378 (326-412) | 6.7 (5.9-7.5) |

Note: $F_{O_2}$: fraction of inspiratory oxygen; %O$_2$ ML: percent of oxygen over membrane lung, oxygen was applied with a flow rate of 1L min$^{-1}$; ECC: veno-venous extracorporeal circuit flow (L min$^{-1}$); $PaCO_2$: arterial carbon dioxide tension (kPa); $PvCO_2$: pulmonary artery carbon dioxide tension (kPa); $V_t$: tidal volume (ml); $Mv$: minute volume (L min$^{-1}$). Data are expressed as median values (interquartile range)

*Indicates a significant difference ($P < .05$) between two experimental conditions. Values from one animal are missing.

A P-value of <.05 (two sided tests) was considered significant. We report the multiplicity-adjusted P-value of the rank sum differences for each comparison.

Statistical analysis was performed with SPSS (IBM SPSS Statistics, version 21 for Windows) and Graph Pad Prism (GraphPad Software, version 8.2.1 for Macintosh). Power was calculated with G*Power (version 3.1.9.4 for Macintosh).

3 | RESULTS

The data from nine animals (six females and three males; weight 44.7-58.8 kg) of the 12 animals were analyzed. Interim effect size analysis (Kendall’s W for PVR = 0.81 and for MPAP = 0.976) showed a power of 99%. The remaining three animals died during the instrumentation, thus, before the actual experiment; one animal developed refractory ventricular fibrillation during insertion of the pulmonary artery catheter, one circulatory failure during start of V-V ECMO, and one technical complication during cannulation for V-V ECMO. Baseline median values (interquartile range) of hemodynamic, blood gases and ventilatory parameters for all analyzed animals are presented in Table 1. Detailed data of the atrio-femoral and femoro-atrial groups are presented in a digital online supplement.

4 | ENTIRE STUDY POPULATION

Rank sums differed significantly in seven of nine comparisons for PVR and in eight for MPAP (Figure 2A,B). MPAP and PVR increased when alveolar hypoxia was established ($P = .0005$ and 0.0023, respectively). When V-V ECMO was started with 100% $O_2$ into the membrane lung, MPAP and PVR decreased ($P = .0097$ and 0.0061, respectively), the calculated $PaO_2$ increased slightly, and $PvO_2$ increased markedly ($P = .0005$), (Figure 2A,B and Figure 3A-D, Table 2, Figure S2A-D of the digital online supplement shows individual data).

All parameters were stable during V-V ECMO and ventilation with $F_{O_2} = 0.15$. At $F_{O_2} 0.10, 0.05$, and 0, MPAP and PVR increased despite V-V ECMO generated normal or supranormal $PvO_2$ values (MPAP: $P = .0068$, .0003 and .012, respectively; PVR: $P < .021$, <.001 and =.036, respectively, (Figure 2B,D and Figure 3A-D, Table 2)).

PVR was inversely correlated with $PvO_2$ ($r_s = -0.46$ [95% CI: -0.60 to -0.29], $P < .0001$), $PvO_2$ ($r_s = -0.51$ [95% CI: -0.64 to 0.35], $P < .0001$) and CO ($r_s = -0.37$ [95% CI: -0.53 to 0.19], $P < .0001$) but not to $PvO_2$ alone ($r_s = -0.16$ [95% CI: -0.35 to 0.04], $P = .10$). Despite significant changes in pH and $pCO_2$ at some stages during the experiment (Table 3), there were no correlations between PVR and arterial pH ($r_s = -0.06$, [95% CI: -0.26 to 0.13], $P = .51$), pulmonary arterial pH ($r_s = -0.09$, [95% CI: -0.09 to 0.34], arterial $PCO_2$ ($r_s = -0.10$ [95% CI: -0.10 to 0.28], $P = .34$), or pulmonary arterial $PCO_2$ ($r_s = 0.12$, [95% CI: -0.08 to 0.31], $P = .23$) $PCO_2$ and pH values were also in the normal range during the study.

There was a strong relationship between MPAP and PVR ($r_s = 0.84$ [95% CI: 0.78 to 0.89], $P < .0001$) but not between MPAP and CO ($r_s = -0.05$ [95% CI: -0.25 to 0.14], $P = .59$) MAP and systemic vascular resistance (SVR) decreased with increasing degree of alveolar hypoxia (Figure 3 digital online supplement). One animal developed circulatory instability during the initiation of ECMO (MAP < 60 mm Hg). Blood pressure was stabilized by continuous infusion of norepinephrine (0.08 µg kg$^{-1}$ min$^{-1}$) before the start of the experiment and not changed during the experiment. Five animals
developed severe circulatory instability (MAP < 50 mm Hg) during ventilation with F\textsubscript{O\textsubscript{2}} < 0.15. In all but one of these animals MAP returned back to baseline when F\textsubscript{O\textsubscript{2}} was increased from 0 to 0.21. MAP and SVR were closely correlated \((r_5 = 0.82 \ [95\% \ CI: 0.74 \text{ to} \ 0.84], P < .0001)\).

5 | DISCUSSION

This is, to the best of our knowledge, the first controlled experimental study that has investigated the effect of increasing PvO\textsubscript{2} by peripheral V-V ECMO on MPAP, PVR, and the systemic circulation during different degrees of global alveolar hypoxia. We found in this model that it was not possible to attenuate hypoxic pulmonary vasoconstriction and to stabilize systemic circulation with V-V ECMO in severe alveolar hypoxia. On the other hand, alveolar normoxia regulated PVR despite low PvO\textsubscript{2}.

We used a longitudinal model of repeated measures to reduce the number of animals and to avoid possible fatal events during severe alveolar hypoxia without ECMO. We chose a closed chest model of global pulmonary hypoxia instead of an ARDS model to control the different degrees of alveolar hypoxia and SvO\textsubscript{2} on MPAP/PVR and the systemic circulation.

In a small case series, Reis Miranda and co-workers found that initiating V-V ECMO in patients with severe ARDS resulted in a statistical significant, but minor decrease in MPAP.\textsuperscript{19} However, these patients had respiratory acidosis before initiation of ECMO, which may have contributed to the pulmonary hypertension. In a case report by Mongodi and colleagues, initiation of V-V ECMO was associated with improved right ventricular function and stabilized circulation.\textsuperscript{20} However, first, the results in case and case series reports are prone to chance, and second, it is not possible to know whether the reduction in right ventricular afterload in the reports were due to an increase in PvO\textsubscript{2}, a combination of improved oxygenation in PvO\textsubscript{2} and increased pH by decarboxylation, by the corrected respiratory acidosis alone, or by change in ventilator settings.\textsuperscript{28,29} In our study mechanical ventilation and pH were kept constant throughout the entire experiment and did therefore not influence the results. Bishop and Cheney found in oleic acid lung injury that the intrapulmonary shunt increased but PVR was not affected by increased PvO\textsubscript{2}.\textsuperscript{30} In contrast, changes in intrapulmonary shunt could not be detected when varying PvO\textsubscript{2} and F\textsubscript{O\textsubscript{2}} in patients with severe respiratory failure treated with V-V ECMO.\textsuperscript{31} Importantly, two influential reviews advocate that V-V ECMO attenuates HPV and improves right ventricular failure in severe ARDS.\textsuperscript{21,22} However, based on the existing literature and our results, we argue that at severe respiratory failure with a substantially reduced mean P\textsubscript{A}\textsubscript{O\textsubscript{2}}, V-V ECMO, despite maximal possible increase in PvO\textsubscript{2}, cannot increase PsO\textsubscript{2} \((PsO_2 = P_{A\textsubscript{O\textsubscript{2}}}^{0.62} \times \text{PvO}_2^{0.38})\) to a level (>6.5 kPa) that sufficiently attenuates HPV. Although PvO\textsubscript{2} could be increased by a large extent by V-V ECMO, the increase is hampered by i) the admixture of venous blood from the native circulation to the oxygenated blood from the extracorporeal device, and ii) the recirculation of the reinfused blood due to draining through the venous cannula. Indeed, in this experiment, maximal V-V ECMO support was unable to reduce MPAP, and PVR when P\textsubscript{A}\textsubscript{O\textsubscript{2}} was below 5 kPa (\(F\textsubscript{O\textsubscript{2}} < 0.15\)). (Figure 3A–C).

There is no consensus about ventilator settings during V-V ECMO, but usually F\textsubscript{O\textsubscript{2}} will be reduced significantly to avoid or reduce toxic effects of high oxygen concentrations.\textsuperscript{32} This and reducing inspiratory pressure and PEEP may lead to decreased alveolar oxygen concentrations in the ventilated areas of the lung. As a consequence the increase in PVR will result in increased right ventricular afterload. Therefore, in our clinical experience, the progression of right ventricular failure during V-V ECMO is usually depended on lung function and not an “ominous sign, pointing to lack of recovery” as described by Krishnan and Schmidt.\textsuperscript{33} Attempts to increase PvO\textsubscript{2} by increasing the extracorporeal flow usually fail to reduce PVR. Hence, the results from this study support our clinical experience, and we are convinced that the circuit should preferentially be changed to V-A ECMO in these situations to stabilize the circulation.\textsuperscript{17,23}

6 | LIMITATIONS

Our study has several limitations: (1) this is an experimental study in an animal model with all its inherent limitations, and although the HPV response to hypoxia is similar in most mammals, the results can only cautiously be transferred to human conditions. (2) Three animals could not be studied due to complications during pre-experimental preparations. However, the set-up of this study was elaborate, and all complications were procedural. According to the 3-R principle, we did not include the a priori calculated 32 animals because the results were clear-cut with a calculated power of 99% after nine animals. (3) We studied a model of global alveolar hypoxia in normal lungs and not of ARDS. This was done to estimate P\textsubscript{A}\textsubscript{O\textsubscript{2}}, with the assumption that the results from an ARDS model would give similar results when HPV is present. However, this notion has not been examined in this study. (4) The estimated P\textsubscript{A}\textsubscript{O\textsubscript{2}} when V-V ECMO was started after inducing pulmonary hypertension with an \(F\textsubscript{O\textsubscript{2}} = 0.15\) could be somewhat higher than the real value, due to CO\textsubscript{2} removal by ECMO, reducing the predicted RQ of 0.8.\textsuperscript{24} (5) ECMO may theoretically interfere with thermodilution and continuous cardiac output measurements with a pulmonary artery catheter, but, to the best of our knowledge, this has not been studied yet. However, due to these reasons, our findings regarding cardiac output should be interpreted cautiously. Moreover, the long updating time during continuous cardiac output measurement could make sudden changes in cardiac output undetectable.\textsuperscript{35} Since there was no relationship between MPAP and CO, the increase in MPAP could not be due to increased flow. Moreover, MPAP and PVR were strongly correlated indicating that changes in MPAP adequately represented the changes in PVR.
7 | CONCLUSION

In this porcine model of alveolar hypoxia, V-V ECMO, despite it increased \( P_{vO_2} \), did not decrease MPAP, and PVR, or attenuated systemic hypoxemia at \( F_1O_2 \) below 0.15. Our results could contribute to explain the fact that V-V ECMO sometimes in our experience fails to stabilize hemodynamics in patients with the most severe forms of ARDS. In these patients conversion from V-V to V-A ECMO is usually needed until the lungs have recovered.

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CONFLICT OF INTEREST

BH has received lecture fees from Xenios. HK, SE, and AL do not report any conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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