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The effects of vasoconstriction and volume expansion on veno-arterial ECMO flow

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Running head: ECMO flow with vasoconstriction and volume expansion

Abstract

Background: Veno-arterial extracorporeal membrane oxygenation (VA-ECMO) is gaining widespread use in the treatment of severe cardiorespiratory failure. Blood volume expansion is commonly used to increase ECMO flow (Q_{ECMO}), with risk of positive fluid balance and worsening prognosis. We studied the effects of vasoconstriction on recruitment of blood volume as an alternative for increasing Q_{ECMO}, based on the concepts of venous return.

Methods: In a closed chest, centrally cannulated porcine preparation (n=9) in ventricular fibrillation and VA-ECMO with vented left atrium, mean systemic filling pressure (MSFP) and venous return driving pressure (VRdP) were determined in *Euvolemia*, during *Vasoconstriction* (norepinephrine 0.05, 0.125 and 0.2µg/kg/min) and following *Volume Expansion* (3 boluses of 10mL/kg Ringer´s lactate). Maximum achievable Q_{ECMO} was examined.

Results: *Vasoconstriction* and *Volume Expansion* both increased maximum achievable $Q_{ECMO,}$ delivery of oxygen (DO_2) and MSFP, but right atrial pressure increased in parallel. VRdP did not change. The vascular elastance curve was shifted to the left by *Vasoconstriction*, with recruitment of stressed volume. It was shifted to the right by *Volume Expansion* with direct expansion of stressed volume. *Volume Expansion* decreased resistance to venous return and pump afterload.

Conclusions: In a circulation completely dependent on ECMO support, maximum achievable flow directly depended on the vascular factors governing venous return - i.e. closing conditions, stressed vascular volume and the elastance and resistive properties of the vasculature. Both treatments increased maximum achievable ECMO flow at stable DO_2, via increases in stressed volume by different mechanisms. Vascular resistance and pump afterload decreased with *Volume Expansion*.

Key words: extracorporeal membrane oxygenation, norepinephrine, vasoconstrictor agents, crystalloid solutions, hemodynamics, mean systemic filling pressure, stressed volume, venous return

Introduction:

Extracorporeal membrane oxygenation (ECMO) has gained widespread use over the last decade for the treatment of severe cardiorespiratory failure and cardiac arrest. Despite remarkable technical improvements with improved system biocompatibility and centrifugal instead of roller pumps, the outcome of cardiogenic shock, extracorporeal resuscitation and post-cardiotomy shock remains modest at best (6-8). The physiology of ECMO is incompletely understood and data on hemodynamic support to optimize ECMO-flow with volume or vasopressors for patients on veno-arterial ECMO are scant (6, 8). Low blood flow and positive fluid balance on ECMO are strong predictors of mortality (28, 33). Volume expansion is the common choice for increasing ECMO flow (2), despite the risks of progressively positive fluid balance with worsening prognosis.

We and others have shown that the ECMO blood flow is directly dependent on venous return (VR) (13, 22), which is described as:

$$VR = (MSFP - RAP)/RVR \quad (1)$$

The mean systemic filling pressure (MSFP) is the elastic recoil pressure in the systemic vasculature (3), caused by the stressed blood volume and the vascular compliance. It can be measured in no-flow states (27). Conceptually, venous return driving pressure (VRdP), the gradient between MSFP and right atrial pressure (RAP) drives VR against the resistance to venous return (RVR) which reflects the lumped resistance of all vascular beds for blood returning to the heart (22). As around 70% of blood resides in the venous pool and the majority is unstressed, vasoconstriction can increase stressed volume and MSFP via recruitment of unstressed volume (19). This has been shown for epinephrine (5, 21), various α_1- and α_2-

agonists (1) and norepinephrine (11). An approach to limit volume expansion while achieving increased ECMO flow may clinically be beneficial. Since outcome data on optimal supportive hemodynamic treatment for VA-ECMO is currently lacking, a study on the underlying mechanisms of action for available treatments could provide the basis for clinical decision-making.

In this porcine model of ventricular fibrillation and veno-arterial (VA) ECMO support, we compared the effects of volume expansion with Ringer's lactate and vasoconstriction using norepinephrine on ECMO blood flow and delivery of oxygen (DO_2). Based on the concepts of venous return and recruitment or expansion of stressed vascular volume (19), we hypothesized that both volume expansion and vasoconstriction with norepinephrine could increase maximum achievable ECMO flow, although acting on stressed vascular volume via different mechanisms. We further hypothesized that the limits of ECMO flow are set by stressed volume and vascular circuit properties, rather than performance of the mechanical pump.

Material and Methods:

The study complied with the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences, 1996) and Swiss National Guidelines and was approved by the Commission of Animal Experimentation of Canton Bern, Switzerland (BE 16/17) Twelve domestic pigs (7 female, 5 male, mean body weight 40.0 ± 2.0 kg at twelve weeks of age) were fasted for 12 h with free access to water after a three day quarantine under veterinary observation at the animal hospital of the University of Bern. The first three animals were used in pilot studies

to establish the instrumentation and feasibility of procedures. As previously described (4, 22), after premedication with intramuscular ketamine (20 mg/kg) and xylazine (2 mg/kg), vascular access was established and anaesthesia was induced with midazolam (0.5 mg/kg) and atropine (0.02 mg/kg) followed by intubation and placement of a gastric tube.

Anaesthesia was maintained with propofol (4 mg/kg/h) and fentanyl (5 µg/kg/h) and the depth was controlled by repeatedly testing the response to nose pinch and targeting a bispectral index <60 (BIS Quatro, Covidien, Mansfield, MA, USA). During surgery, propofol and fentanyl infusions were increased to 6 mg and 20 µg/kg/h respectively. Additional injections of fentanyl (50 µg) or midazolam (5 mg) were given as needed. Cefuroxime (1.5 g) was given at skin incision and repeated after 4 h. Intermittent muscle relaxation was induced with rocuronium (0.5-1 mg/kg) for the study measurements. The pigs were mechanically ventilated in a volume-controlled mode (Servo-I, Maquet Critical Care, Solna, Sweden) using a PEEP of 5 cm H_2O, fraction of inspired oxygen of 0.30, I:E ratio 1:2 and tidal volume 7 mL/kg body weight. Respiratory rate was adjusted to maintain an end-tidal P_{CO_2} of 40 mmHg.

Installations:

The following catheters were surgically placed: a left carotid artery catheter, a right jugular three-lumen catheter and an introducer sheath in the right femoral vein for rapid volume exchange. Cystostomy was performed for urinary output monitoring. The thoracic cavity was entered via a median sternotomy and the pericardium was opened. After administration of 5000 U of heparin, the right atrium (RA), the ascending aorta and left atrium were cannulated (29 Fr 3-stage venous cannula MC2X, 18 Fr elongated-one-piece arterial cannula and 16 Fr DLP left atrial vent, Medtronic, Minnesota, USA) and connected to an ECMO circuit (centrifugal pump, non-pulsatile flow, Cardiohelp MECC set, Quadrox oxygenator, Maquet, Rastatt, Germany). The

VA-ECMO circuit had a shunt between the arterial and venous tubing. Clamping the inlet and outlet tubing while opening the shunt enabled rapid pressure and volume equilibration (22, 27). Flows in the pulmonary artery and the ECMO circuit were measured with appropriate transit time ultrasonic flow probes (PAU and ME9 PXL Tubing flow sensors respectively, Transonic, Ithaca, USA) and were monitored in real time to assist in volume and pump speed management (see below). Ventricular epicardial electrodes (MYO/Wire Temporary Atrial Cardiac Pacing Wires, A&E Medical Corporation, Farmingdale, New Jersey, USA), and passive pleural drains were placed. The pericardium, sternum and wound layers were closed. Intermittent heparin boluses were used to keep an activated clotting time > 180 s. During ECMO, tidal ventilation was continued with a respiratory rate fixed at 16/min and fraction of inspired oxygen of 0.21. The sweep gas flow (100% O_2) was adjusted to keep arterial P_{O_2} and P_{CO_2} in the normal range (ABL90Flex, Radiometer Medical ApS, Brønshøj, Denmark).

Pressure measurement and data acquisition:

Intravascular and airway pressures were measured using transducers (xtrans, Codan Medical, Lensahn, Germany) and a multi-modular monitor (S/5 Critical Care Monitor, Datex-Ohmeda, GE Healthcare, Helsinki, Finland) which also provided continuous electrocardiography and end-tidal P_{CO_2}. Output from the monitor and flow probes was recorded at 100 Hz in a data acquisition system (LabVIEW, National Instruments, Austin, TX, USA), and processed offline using customized analysis software (Soleasy, Alea Solutions, Zürich, Switzerland). The tip of the catheter used for right atrial pressure measurement, the venous drainage cannula, the inlet port of the ECMO pump, and all pressure transducers were fixed to the height of the mid-RA and verified by open chest palpation. Pressures were zeroed against the atmosphere and two-point

calibrated using a water manometer. Flow probes were zeroed and calibrated electronically. Baseline drift for pressure and flows was checked at the end of the experiment.

Fluid administration, volume state and ECMO pump speed:

During surgery, Ringer's lactate was infused at a rate of 10 mL/kg/h and thereafter reduced to 2 mL/kg/h. Hydroxyethyl starch (HES, 6% Voluven, Fresenius Kabi, Bad Homburg, Germany) was supplemented for measured blood loss during surgery (150 ± 109 mL). After closing the chest and allowing a stabilization period of 30 min ECMO flow (Q_{ECMO}) was adjusted to achieve a mixed venous O_2 saturation (S_VO_2, measured in the RA) of 50% (lower normal limit for pigs). During this period, if necessary, HES was added in 50 mL boluses to allow sufficient Q_{ECMO} to reach the S_VO_2 target, and to avoid RA collapse during tidal ventilation (total volume of HES, including that for blood loss replacement was 197 ± 199 mL). After this stage, defined as *Euvolemia*, no more HES was allowed.

Experimental protocol:

Ventricular fibrillation was induced by high rate pacing (1000 bpm, ventricular electrical output 18 mV, Pace 203, Osypka, Berlin, Germany). The protocol consisted of eight experimental conditions: *Euvolemia* was followed by three conditions of stepwise increasing rates of norepinephrine infusion [0.05, 0.125, and 0.2 µg/kg/min, each beginning with a bolus of 5 µg/kg (*Vasoconstriction 1-3* respectively)], with study measurements starting after five mins at each infusion rate. After completing measurements at *Vasoconstriction 3*, the norepinephrine rate was halved and three mins later discontinued completely, entering a state of *Post Vasoconstriction*. This was followed by three conditions of stepwise *Volume Expansion* (*VE1-3*) where 10 mL/kg of Ringer's lactate was infused over three mins at each step, with study measurements starting

after five mins (Figure 1). After completing the measurements, the animals were killed in deep anesthesia by withdrawing the ECMO support.

ECMO pump speed manoeuvers and venous return curves:

For each experimental condition, during tidal ventilation, *Maintenance* ECMO pump speed was adjusted to achieve a Q_{ECMO} resulting in SvO_2 of 50%. In order to find the *Maximum* Q_{ECMO} achievable without provoking clinically apparent RA collapse, the pump speed was increased during expiratory hold while observing the real-time flow, displayed on screen, and the ECMO tubing for signs of fluttering. The *Maintenance* and *Maximum* ECMO pump speeds and 80%, 60% and 50% thereof were applied during expiratory hold. Manoeuvers lasted for 30 s, after which pump speed was reset to *Maintenance* and tidal ventilation for at least 1 min until blood pressure returned to baseline. Data was extracted (as mean) for two seconds 9 s into hold after flow had reached its new steady state (4). In order to properly characterize the vascular return function, knowing from a previous experiment that unapparent closing conditions may occur (22), venous return curves of RAP-Q_{ECMO} data pairs were constructed after excluding all manoeuvers displaying vascular collapse in the offline analysis (independently assessed by authors PWM, AH, DB). Maximum achievable Q_{ECMO} however, was analysed with closing conditions included. To quantify the effect of the left atrial vent, in six animals in *Euvolemia*, *Vasoconstriction 3*, and *VE3*, manoeuvers with maximum and 50% pump speed were repeated with the vent closed. In order to quantify the shift of the venous return curves between *Vasoconstriction 3* and *VE3*, Q_{ECMO} was calculated for standard RAP, representing the mean of all conditions. Oxygen delivery (at *Maintenance* and *Maximum* Q_{ECMO}) and oxygen consumption (VO_2; at *Maintenance* Q_{ECMO}) were calculated using standard formulas for arterial and mixed venous blood oxygen content.

Determination of MSFP

MSFP was determined after the pump speed manoeuvers in each condition in a *Stop flow manoeuver* (22). The ECMO circuit was clamped with open shunt in expiratory hold (22). Flow was resumed after 30 s or if signs of a reflex-mediated increase in arterial blood pressure (ABP) were seen. MSFP was taken as the mean value of RAP during two seconds of equilibrium defined from ABP nadir (22). At least 3 min were allowed for blood pressure to return to baseline. Stability of MSFP was studied at *Vasoconstriction 3* and *VE3* by repeating the MSFP determinations three times over 40 minutes.

Blood volume determination:

Plasma volume was measured using indocyanine green dye dilution at *Euvolemia, Vasoconstriction 3*, and *VE3*, as previously described (4). Changes in plasma volume were calculated based on hematocrit and hemoglobin concentrations using Beaumont's method (16) when no direct plasma volume measurements were available.

Determination of vascular elastance, stressed and unstressed volumes:

Vascular elastance (E_{vasc}) was determined at *Vasoconstriction 3* and *VE3* using the difference between MSFP obtained before and immediately after rapid bleeding of 9 mL/kg from the arterial ECMO tubing into a transfusion bag. The bled volume was re-transfused. Systemic vascular elastance was calculated as $E_{vasc} = \Delta MSFP/\Delta BV$. Stressed and unstressed volumes were determined from the x-intercept of the E_{vasc} function (4, 23).

Statistics:

Based on previous data, a sample size of eight animals was needed to detect a clinically relevant difference in MSFP of 1 mmHg (4). Data were analysed using SPSS software (Version 21; SPSS Inc., Chicago Illinois, USA). Two-way repeated measurements analysis of variance (ANOVA), within-subject factors treatment (vasoconstriction *vs*. volume expansion) and level of treatment intensity (0-3), was used to assess the effects of interventions. In case of significant interaction (treatment × intensity), each treatment was tested separately with one-way repeated measurements ANOVA to assess where changes occurred. Bonferroni correction was applied as appropriate. The vent effect was assessed with two-way repeated measurements ANOVA (within-subject factors condition [*Euvolemia*, *Vasoconstriction 3* and *VE 3*] and pump speed [maximum *vs*. 50%]). Blood volumes, elastances and hemoglobin concentrations (*Vasoconstriction 3 vs*. *VE3*), and urine output during *Vasoconstriction 1-3 vs*. *VE 1-3* were compared with paired t-test. Generalized estimating equations [(GEE) first order auto-regressive working correlation matrix] was used to characterize the linear relations between flow *vs.* pump speed, pressure head *vs.* flow, MSFP *vs.* time, and the venous return function flow *vs.* RAP. Proportion of variance for these variables in individual animals was assessed as Pearson correlation coefficient squared (r^2). Assumptions of equal variance and normality were assessed as studentized residuals < ±3, visually by Q-Q plots and histograms, and by Kolmogorov-Smirnov testing.

Results

Ventricular fibrillation could be achieved in all nine animals with complete cessation of pulmonary artery blood flow (7 ± 12 mL/min over all conditions). Opening or closing the vent

did not affect Q_{ECMO} (range of relative changes, open to closed, 98.4 ± 3.3% to 100.1 ± 0.7%), with no difference seen between conditions or pump speeds (p=0.502 and 0.598 respectively). Pump function (mL/revolution) was highly linear over the experimental conditions and pump speeds used [r^2 (median, range) for individual animals over *Euvolemia*, *Vasoconstriction 3* and *VE 3* 0.999 (0.811 – 1.0), Supplemental Digital Content, Table e1 and Figure e1, http://links.lww.com/SHK/A762].

Both treatments progressively increased MSFP. For the doses used, the effect was more pronounced for *Volume Expansion*. Mean arterial pressure was higher with *Vasoconstriction*. Hemoconcentration and hemodilution were seen with *Vasoconstriction* and *Volume Expansion* respectively. The blood lactate increased together with VO_2 despite maintenance of the target S_vO_2 (Table 1). Factors defining venous return were not different between *Euvolemia* and *Post Vasoconstriction* (Supplemental Digital Content, Table e2, http://links.lww.com/SHK/A762). Urine output was 2.6 ± 1.1 *vs*. 2.7 ± 1.4 mL/kg/h during *Vasoconstriction 1-3* and *Volume Expansion 1-3* respectively (n=6, p=0.832).

Maximum ECMO flow

Both treatments increased maximum achievable Q_{ECMO}. For the doses used, the effect was more pronounced for *Volume Expansion*, but this did not translate into higher DO_2 compared to *Vasoconstriction*, due to concomitant hemodilution (Table 2).

Venous return function

Signs of vascular collapse were observed in 17 % of pump speed manoeuvers with equal distribution between treatments. When pump speeds were varied, there was a linear negative

correlation between Q_{ECMO} and RAP [median for individual Q_{ECMO}/RAP responses r^2 0.975 (0.626-1.000); Table 3, Figure 2]. MSFP and flow increased significantly with both treatments, and the respective VR curves were shifted to the right (Tables 1-2, Figure 2; for VR plots with all data pairs included, see Supplemental Digital Content, Table e3, Figure e2, http://links.lww.com/SHK/A762). Increase in flow was less pronounced in *Vasoconstriction* compared to *Volume Expansion* (Table 2). VRdP was not different between conditions and at maintenance speed (Table 1 and 4). The response of resistance to venous return from *Vasoconstriction* was highly variable, with equal distribution of increasing, decreasing or unchanged RVR in individual animals (p=.445). *Volume Expansion* consistently and progressively reduced RVR (Tables 3-4, Figure 2). The flow corresponding to the standard RAP of 2.8 mmHg was 2978 ± 1046 mL/min in *Euvolemia* and increased to 3529 ± 648 mL/min during *Vasoconstriction 3* and to 6195 ± 1787 mL/min during *VE3* (p <0.0005, for details see Supplemental Digital Content, table e4, http://links.lww.com/SHK/A762).

Pressure head vs. Q_{ECMO}

The relationship between pressure head (mean arterial pressure MAP minus RAP) and Q_{ECMO}, was highly linear in all conditions. (r^2 for individual animals (median, range) in *Euvolemia*: 0.983 (0.936-0.999); *Vasoconstriction 3*: 0.990 (0.969-1.000); and *VE3*: 0.965 (0.643-0.995). The resistance needed to be overcome by the pump was lower in *VE3* as compared to *Euvolemia* and/or *Vasoconstriction 3* (GEE, Table 5).

Vascular elastance, stressed and unstressed blood volumes

Compared to *Euvolemia*, *Vasoconstriction* increased MSFP and decreased total blood volume due to loss of plasma (Table 1 and 6, Figure 3). *Volume Expansion* restored and increased the

blood volume slightly above the base level at *Euvolemia* due to plasma expansion. *Vasoconstriction* resulted in higher vascular elastance than *Volume Expansion*. *Vasoconstriction* led to a leftward shift of the elastance curve, and unstressed volume was recruited into stressed volume. *Volume Expansion* shifted the elastance curve back to the right, through increases in both stressed and unstressed volumes (Table 6, Figure 3).

Stability of effects

At *Vasoconstriction 3* and *VE3*, repeated measurements of MSFP over 40 minutes showed a decline over time (mean 1.7 mmHg), with no difference between treatments (GEE, Supplemental Digital Content, Table e5, http://links.lww.com/SHK/A762). Changes in plasma volume under these conditions were small (0.3 ± 6.5% for *Vasoconstriction 3*, -2.5 ± 7.7% for *VE3*, p=0.24).

Discussion

We have applied the principles of venous return (13-15), verified in a series of experiments with (22), and without mechanical circulatory assist (4, 27, 31), to modern VA-ECMO treatment. We found that both vasoconstriction with norepinephrine and volume expansion increased MSFP and the maximum achievable Q_{ECMO} with similar oxygen delivery. The effect of volume expansion on blood flow was larger than that of vasoconstriction. In our model, the ECMO pump replaces the cardiac function. The pump function was constant, as indicated by the linear relationship between pump flow and revolutions per minute (rpm). Accordingly, our results can be interpreted solely as changes in the circuit properties, as we have previously demonstrated (22). The evaluation of effects and mechanisms of vasoconstriction and volume expansion on ECMO flow is highly relevant for the clinical application of modern ECMO treatment.

The maximum ECMO flows in each condition were associated with imminent vascular collapse, which could not be observed clinically. The vascular collapse, when present, dissociated the Q_{ECMO}-RAP relationship, since RAP no more served as the backpressure for VR (22, 34). Closing conditions via vascular waterfalls were recognized as the main limitation to further flow increase in the early seminal studies by Guyton (14, 15). The venous return plots showed a strictly linear RAP-Q_{ECMO} relationship (Figure 2), as predicted by Guyton's model. As we had hypothesized, both *Vasoconstriction* and *Volume Expansion* allowed for increasing maximum ECMO flow and increased MSFP. In this sequential treatment study design, we observed larger effects regarding blood flow from volume expansion than from vasoconstriction. Both treatments thereby shifted the VR curve to the right. In addition, *Volume Expansion* also decreased resistance to venous return. As compared to *Euvolemia*, the increase in flow under *Vasoconstriction* was accompanied by decreased total blood volume (a leftward shift of the vascular elastance curve) and increased MSFP by recruitment of stressed volume from unstressed vascular volume (Figure 3). Recruitment of stressed volume was modified by two phenomena. Firstly, vasoconstriction with norepinephrine increased the vascular elastance (29) and thereby increased MSFP for the given stressed volume. We did not measure elastance in *Euvolemia* and therefore cannot quantify the elastance increase under *Vasoconstriction*. The value of elastance reported here under norepinephrine is larger than we found in a similar experiment under euvolemic conditions (4), and increasing elastances have been shown for different vasoconstrictors (1, 11). Secondly, roughly a fifth of the plasma volume and therefore part of the recruited volume may have been lost by plasma leakage. Plasma leakage may be related to inflammation induced by use of extracorporeal circulation (20). *Volume Expansion* shifted the elastance curve further to the right. Stressed and unstressed volumes were both

expanded, but to a lesser extent than expected from the large amount of volume infused (30 mL/kg). As urinary output remained stable between conditions, this rightward shift may have been limited by ongoing plasma leakage and/or increases in vessel bed diameter, as described below.

For both *Vasoconstriction* and *Volume Expansion,* the maximum flow increased. Maximum flows were often associated with vascular collapse. Under closing conditions, the MSFP-RAP pressure gradient does not reflect the driving pressure for venous return. When venous return was evaluated in conditions without signs of collapse, a decrease in RVR following volume expansion was evident, and explains the further increase in VR despite unchanged VRdP. Recruitment of stressed from unstressed volume via vasoconstriction of veins and venules can occur without changes in the resistance to venous return (9, 10), which is here reproduced with an unchanged slope on average (representing RVR) of the *Vasoconstriction* VR curves (Tables 3-4, Figure 2). Notably, individual responses to norepinephrine varied, exhibiting unchanged, rising of falling resistances. Such variable reactions of venous return to vasoconstriction have been reported earlier (18) and are clinically important, when VA-ECMO is used as support for severe heart failure, as increases in resistance and afterload may have detrimental effects (25). Maximum flow may have been influenced by cannula tip-vessel wall interaction (34) via centralisation of blood volume from vasoconstriction. We have therefore estimated the increases in Q_{ECMO} at a standardized RAP, in order to exclude artefacts from dissociated Q_{ECMO} and RAP, which confirmed the flow increases and curve shifts. *Volume Expansion* showed progressively and uniformly lower RVR and pressure heads, allowing higher flows at stable VRdP. Despite higher flow from volume expansion, oxygen delivery was limited due to concomitant hemodilution, and the resulting DO_2 was similar in the two treatments. Besides resistance

changes, an additional mechanism may be at play with volume expansion. The linear pump function illustrates that the flow generated per rpm will depend on the variables of the Hagen-Poiseuille equation (17), i. e. flow is directly proportional to the pressure gradient (or head) and the fourth power of vessel radius, but inversely proportional to viscosity and tubing length. The pressure heads and RVR at *Volume Expansion* may have been influenced by viscosity changes due to hemodilution or -concentration. Whether the decreasing resistances are a direct vasodilatory effect of *Volume Expansion* after vasopressor weaning, as is clinically often seen (30), or if ongoing SIRS and instability of the experimental preparation were the cause of vasodilation, cannot be determined with certainty.

What are the clinical consequences of our findings? Operating close to the maximum possible flow brings a high risk of clinically unapparent closing conditions. Volume depletion and high airway pressure (4, 32) may increase the likelihood of vessel closure or directly reduce venous return via elevated right atrial pressure (22, 27, 31). Preferential drainage from the inferior caval vein (which in pigs has an intrathoracic part exposed to pleural pressure) with a three-stage cannula may have promoted vessel collapse and dissociated flow from right atrial pressure (34) as compared to a right angle cannula in a previous experiment (22). In the absence of clear evidence for optimal hemodynamic supportive measures, the clinician's choice between volume expansion and vasoconstriction should be guided by the disease process and the expected physiological limits and effects of a treatment.

Vasoconstriction may allow increase in flow by recruitment of stressed volume and thereby decreasing the need to infuse volume, where the amount is associated with worsening outcome for patients on VA-ECMO (28). Such volume sparing effects may be of special value in cases of severe respiratory failure. The physiological reaction to vasoconstriction is much more variable

and therefore less predictable than that of volume expansion. Especially the increases in resistance may have negative effects in patients with failing hearts supported with temporary mechanical assist. Here, prudent volume expansion to facilitate vasodilation may be appropriate. As the components of venous return are not easily measured, monitoring the true effects of vasoconstriction is demanding. As a compromise, ECMO blood flow may be kept as low as clinically reasonable and adverse effects of repeated vascular collapse need to be considered when high flows are necessary. Vasoconstriction and volume expansion, as used in this study, are equal regarding oxygen delivery. Both show a similar decline in MSFP over time, probably due to ongoing plasma leakage. Plasma leakage under vasoconstriction has been described (12). The upper limit for recruitment of unstressed volume into stressed volume using vasoconstriction is reported as 10 to 18 mL/kg (19). Up to 3/4 of volume expansion with crystalloids will be lost into the interstitial space over time and eventually impair tissue perfusion in case of severe oedema. In pilot animals for this study, we used higher doses of norepinephrine, which led to more pronounced leakage and unstable preparation with inability to sustain the SvO_2 target. The recruitable reserve and the ongoing leakage may be further influenced by inflammation associated with ECMO (20), and must be taken into clinical consideration.

Limitations

Lack of randomization between *Volume Expansion* and *Vasoconstriction* is a limitation. We chose the sequential use of norepinephrine followed by volume expansion in order to minimize shifts in blood volume before norepinephrine. In clinical use of ECMO vasoconstriction and volume expansion are often used simultaneously or consecutively, and their effects are modified by deterioration of the underlying disease, ongoing plasma leakage and inflammation. Alternatively, volume could have been expanded and then removed to facilitate randomization.

Transfusion and bleeding was not possible due to lack of pig blood for volume expansion. The ECMO system used did not have a volume reservoir or allow for ultrafiltration. Adding a CRRT device would have further increased the technical complexity of an already challenging setup. In addition, the effects of ultrafiltration on equilibration between interstitial and intravascular space and on vasoregulation would have interfered with restoration of baseline intravascular volume state after fluid removal. Our sequential approach was a pragmatic compromise. The main determinants of venous return did not differ between the two baseline conditions *Euvolemia* and *Post Vasoconstriction*. This suggests that the conclusion regarding the basic mechanisms of vasoconstriction and volume expansion still hold true at least during an early clinical course on VA-ECMO.

We did not encounter clinical instability during this experiment and similar previous experiments (4, 22). Urinary output was stable during both *Vasoconstriction* and *Volume Expansion*. We attribute the rising lactate to an ongoing inflammatory reaction - a known phenomenon on ECMO (20). This is supported by the increasing oxygen consumption in the course of the experiment. We cannot exclude gut ischemia or liver dysfunction due to venous outflow obstruction but consider them unlikely due to the clinical stability. The continuously fibrillating heart may also have contributed, but not to a quantitatively relevant extent.

We tested whether vasoconstriction and volume expansion could increase maximum achievable ECMO flow and assed the underlying mechanisms. Our study was not designed to evaluate a treatment benefit of one approach over the other. We show that both volume expansion and vasoconstriction, when used in moderate doses, increase maximum achievable ECMO flow, with similar effects on DO_2. We conclude that ECMO flow is primarily dependent on the factors governing venous return - in our view a central finding for anyone in clinical care of ECMO

patients. In order to find an optimal treatment regarding outcome, the basic mechanisms should be understood. To what degree our findings can be translated to diseases other than cardiac arrest, like septic shock or severe pulmonary failure, is still to be explored. Particularly, our results cannot be extrapolated to treatment of respiratory failure using veno-venous ECMO.

We omitted an elastance measurement at *Euvolemia*, which could have provided interesting information as we found changing elastances between *Vasoconstriction* and *Volume Expansion*. As there is little doubt about the linear behaviour of the elastance curve in the physiological range (4, 22) (1, 10, 23), a one-step change of blood volume seems warranted. Values presented here are in agreement with previous experiments performed by us and others (4, 24) and increasing elastance with vasoconstrictors is well described (1, 11).

We used low doses of norepinephrine as titration of higher doses in the pilot series led to progressive instability. Still, higher doses may have shown clearer results. Similar results to ours were found with much higher doses in endotoxemic pig models (9).

Statistical approach

Linear regression has been the standard method of describing venous return (27). We could reproduce our earlier findings using standardized RAP and Generalized Estimating Equations (22), which allowed statistical interference from repeated measurements.

Validity of RAP and VRdP

Increasing pump speed shifts volume away from the RA, progressively lowering RAP and increasing VRdP (22) – which is the difference of intravascular pressures over a vascular segment. The limit of maximum flow however, is determined by transmural pressure (26, 34): at

closing conditions, the vascular wall interacts with the cannula tip causing flow to drop with subsequent build-up of pressure until wall and cannula separate anew and flow is restored (staccato flow) (34). This phenomenon is associated with a net increase in resistance. A RAP valid for calculation of VRdP can only be measured at the orifice of unobstructed flow in a multistage cannula (14, 34). We therefore verified our main results by estimation of increasing flows at standard RAP, independently of VRdP.

Independently of these limitations, the corollary of our experiment is that in a circulation completely dependent on a mechanical pump, maximum pump output is determined by vascular factors. This should be taken into consideration in the clinical management.

Conclusions

In a circulation completely dependent on ECMO support, blood flow is directly dependent on the vascular factors that govern venous return - i.e. closing conditions, stressed vascular volume and the elastance and resistive properties of the vasculature.

Abbreviations

ABP	arterial blood pressure
ANOVA	analysis of variance
DO_2	delivery of oxygen
ECMO	extracorporeal membrane oxygenation
GEE	general estimating equation
HES	hydroxyethyl starch

MAP	mean arterial pressure
MSFP	mean systemic filling pressure
PEEP	positive end-expiratory pressure
RAP	right atrial pressure
rpm	revolutions per minute
S_VO_2	mixed venous oxygen saturation
VE	volume expansion
VO_2	oxygen consumption
VR	venous return
VRdP	Venous return driving pressure (=MSFP – RAP)

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Figure Legends

Figure 1

Experimental protocol. After stabilization, Euvolemia was reached by adding HES as necessary to allow an ECMO flow resulting in SvO_2 of 50% without RA collapse during tidal ventilation. Ventricular fibrillation was induced and the study period consisting of eight conditions began. Pump speed and Stop flow manoeuvers were performed during expiratory hold (for a detailed description, please refer to reference (22)).

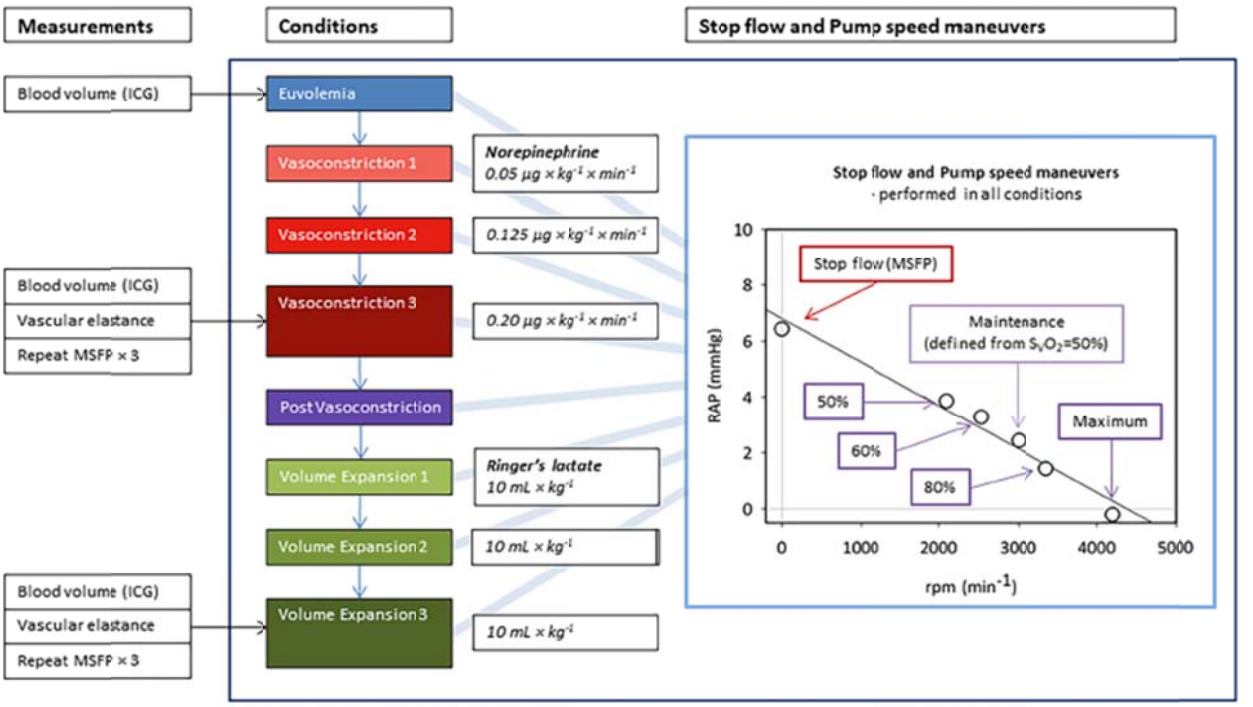

Figure 2

Venous return curves after exclusion of closing conditions. The left panel shows *Euvolemia* and *Vasoconstriction 1-3*. The right panel *Post Vasoconstriction* and *Volume Expansion 1-3*. The lines indicate the mean slopes of GEE (for equations, see Table 3).

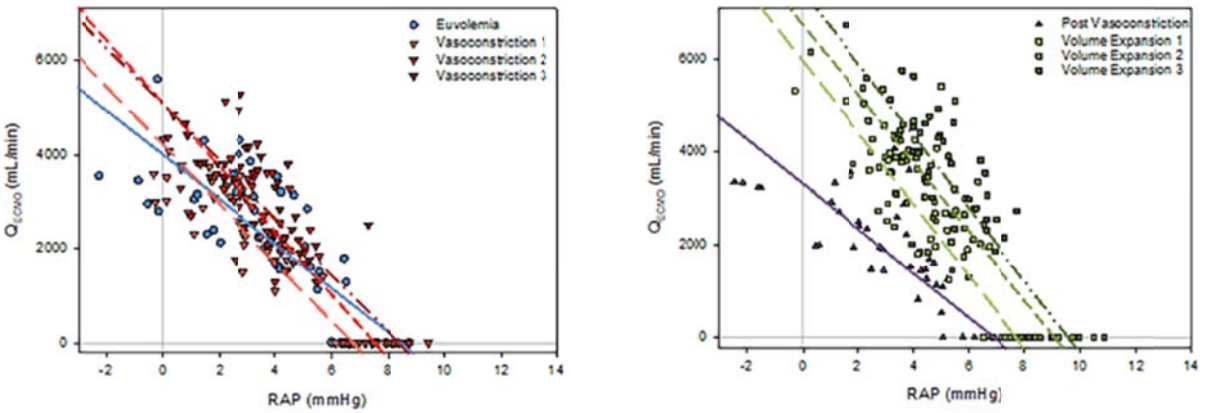

Figure 3

Vascular elastance derived from bleeding manoeuvers at *Vasoconstriction 3* and *VE3* (Table 6).

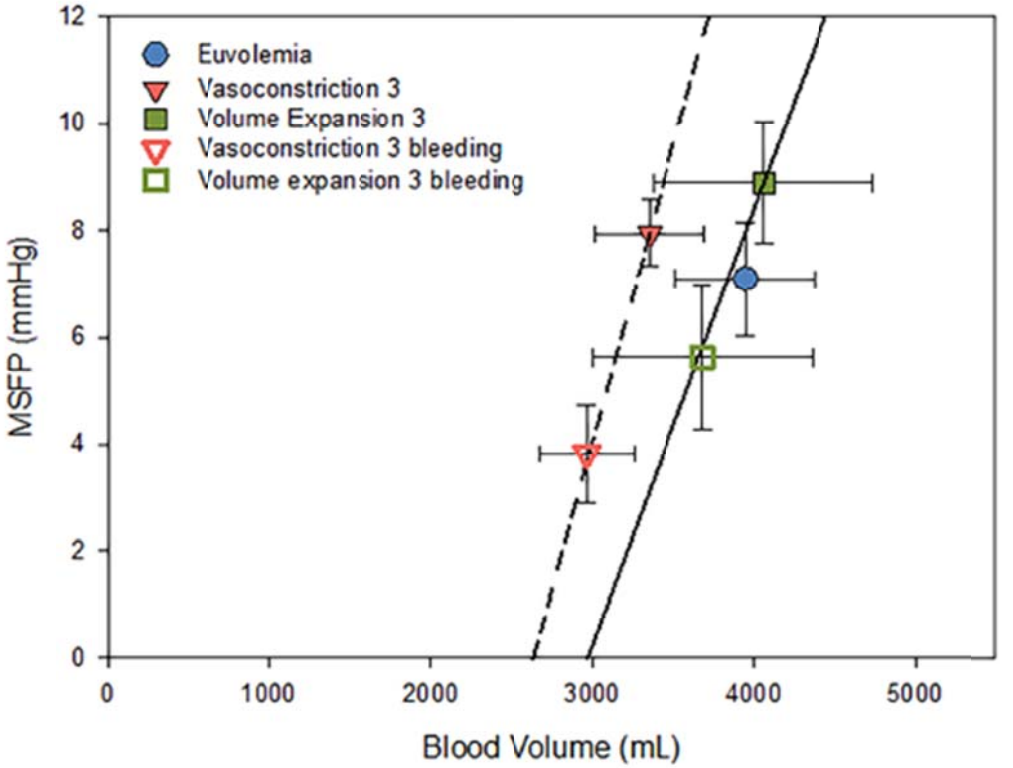

Table 1: Hemodynamics at maintenance pump speed

	Euvolemia	Vasoconstriction			Post Vasoconstr.	Volume Expansion			*p* values		
		Step 1	Step 2	Step 3		Step 1	Step 2	Step 3	treatment	intensity	interaction
MSFP: mmHg	7.1 ± 1.1	6.7 ± 0.6	7.4 ± 1.0	7.9 ± 0.6	6.5 ± 0.7	7.8 ± 0.7	8.6 ± 1.1	8.9 ± 1.1	0.023	<0.0005	<0.0005
									Vasoconstriction 0.021* Volume Expansion <0.0005*		
Q_{ECMO}: mL × min^{-1}	3382 ± 199	3454 ± 262	3515 ± 163	3606 ± 207	3144 ± 682	3887 ± 308	4237 ± 492	4317 ± 386	0.002	<0.0005	<0.0005
									Vasoconstriction 0.20* Volume Expansion 0.015*		
rpm: min^{-1}	2978 ± 164	3047 ± 296	3133 ± 218	3144 ± 213	2944 ± 403	3206 ± 248	3344 ± 292	3422 ± 327	0.017	0.004	0.079
SvO_2: %	54 ± 7	52 ± 3	54 ± 4	56 ± 5	46 ± 9	49 ± 5	52 ± 5	54 ± 5	0.027	0.047	0.376
VRdP: mmHg	5.1 ± 2	5.2 ± 1.4	4.8 ± 1.0	5.1 ± 1.1	4.7 ± 2.8	4.7 ± 0.8	4.5 ± 1.3	4.0 ± 1.5	0.209	0.524	0.663
MAP: mmHg	70 ± 15	82 ± 12	90 ± 22	76 ± 14	54 ± 10	60 ± 8	60 ± 11	59 ± 11	0.001	0.001	0.015
Hemoglobin: g × L^{-1}	94 ± 6	100 ± 7	106 ± 7	108 ± 7	108 ± 6	101 ± 5	97 ± 6	93 ± 7	0.079	0.818	<0.001
DO_2: mL× min^{-1}	435 ± 43	471 ± 75	505 ± 42	532 ± 52	463 ± 109	535 ± 48	552 ± 78	551 ± 82	0.018	<0.0005	0.299
VO_2: mL× min^{-1}	201 ± 36	225 ± 16	230 ± 26	232 ± 35	240 ± 39	276 ± 37	271 ± 55	254 ± 56	0.016	0.040	0.298
Lactate: mmol × L^{-1}	1.2 ± 0.8	1.6 ± 1.1	2.1 ± 1.3	3.2 ± 2.0	5.7 ± 3.3	6.2 ± 2.5	6.0 ± 4.5	6.0 ± 4.5	0.003	0.036	0.003

Data is mean ± SD. n=9. 2-way repeated measurements ANOVA, within-subject factors treatment modality and treatment intensity. *In case of significant interaction, simple main effect for intensity was assessed with repeated measurements ANOVA, within-subject factor treatment intensity. MSFP= mean systemic filling pressure; Q_{ECMO}= ECMO flow; rpm: revolutions per minute; VRdP= MSFP-RAP= venous return driving pressure; MAP= mean arterial pressure; DO_2 = delivery of oxygen; VO_2= oxygen consumption.

Table 2: Maximum flow

	Euvolemia	Vasoconstriction			Post Vasoconstr.	Volume Expansion			*p* values		
		Step 1	Step 2	Step 3		Step 1	Step 2	Step 3	treatment	intensity	interaction
Q_{ECMO} max: mL × min^{-1}	4376 ± 887	4369 ± 802	4670 ± 746	4967 ± 977	3470 ± 902	4792 ± 677	5469 ± 382	5649±1040	0.127	<0.0005	0.003
									Vasoconstriction 0.012* Volume Expansion <0.0005*		
rpm max: min^{-1}	3756 ± 644	3744 ± 570	3572 ±603	4000 ± 634	3104 ± 585	3756 ± 413	4167 ± 508	4322 ± 626	0.663	<0.0005	<0.0005
									Vasoconstriction 0.064* Volume Expansion <0.0005*		
Pump efficiency: mL × revolution^{-1}	1.17 ± 0.15	1.16 ± 0.10	1.18 ± 0.12	1.23 ± 0.07	1.11 ± 0.12	1.27 ± 0.07	1.31 ± 0.10	1.30 ± 0.12	0.042	<0.0005	0.008
									Vasoconstriction 0.203* Volume Expansion <0.0005*		
DO_2 max: mL × min^{-1}	561 ± 112	597 ± 124	673 ± 133	734 ± 160	510 ± 140	661 ± 105	729 ± 161	726 ± 177	0.489	<0.0005	0.051

Data is mean ± SD at maximum flow, including closing conditions, n=9. Two-way repeated measurements ANOVA, within-subject factors treatment modality and treatment intensity. *Simple main effect for intensity: repeated measurements ANOVA, within-subject factor treatment intensity. Q_{ECMO} max= maximum ECMO flow; rpm max= pump speed giving Q_{ECMO} max; Pump efficicency= mL × revolution^{-1} at rpm max; DO_2 max = delivery of oxygen at maximum ECMO flow.

Table 3: Venous return function

		Covariate		Dependent variable		r^{2*}	Equation parameters		p
		RAP; mmHg		Q_{ECMO}; mL × min^{-1}			Slope (95% CI) mL × min^{-1} × mmHg^{-1}	Intercept (95% CI) mL × min^{-1}	
	n	mean ± SD	range	mean ± SD	range	median (range)			
Euvolemia	47	3.7 ± 2.5	-2.3 – 8.8	2284 ±1412	0 – 5598	0.98 (0.89 – 1.00)	-471 (-618 – -324)	3996 (3096 – 4895)	≤0.0005
Vasoconstriction 1	48	3.2 ± 2.2	-0.3 – 8.1	2288 ± 1385	0 – 4824	0.99 (0.76 – 1.00)	-621 (-713 – -529)	4203 (3635 – 4772)	
Vasoconstriction 2	48	3.9 ± 2.1	0.0 – 9.5	2461 ± 1467	0 – 5123	0.98 (0.81 – 0.99)	-674 (-811 – -537)	5084 (4266 – 5903)	
Vasoconstriction 3	44	4.4 ± 2.2	0.8 – 8.8	2423 ± 1499	0 – 5281	0.97 (0.63 – 1.00)	-602 (-687 – -517)	5068 (4545 – 5591)	
Post Vasoconstriction	44	3.2 ± 2.5	-2.5 – 7.3	1747 ± 1195	0 – 4065	0.95 (0.76 – 0.99)	-479 (-629 – -328)	3316 (2424 – 4208)	
Volume Expansion 1	48	4.5 ± 2.1	-0.3 – 9.0	2625 ± 1590	0 – 5403	0.97 (0.78 – 1.00)	-767 (-885 – -649)†	5950 (5196 – 6704)†	
Volume Expansion 2	47	5.2 ± 2.2	0.3 – 10.5	2895 ± 1750	0 – 6156	0.98 (0.81 – 1.00)	-735 (-816 – -654)†	6718 (6322 – 7115)†	
Volume Expansion 3	46	5.8 ± 2.1	1.5 – 10.9	2985 ± 1843	0 – 6727	0.97 (0.74 – 1.00)	-775 (-900 – -649)†	7448 (6480 – 8415)†	

Generalized Estimating Equations for Q_{ECMO} vs. RAP in the *per protocol* data set including MSFP. n=number of data pairs per condition. Slope of the line = (resistance to venous return)$^{-1}$ = (RVR)$^{-1}$; *p*-value for equation parameters valid for both slopes and intercepts. *Median (range) of proportion of variance (Pearson correlation coefficient squared; r^2) for individual animals. † The slopes and intercepts of Volume Expansion 1, 2, and 3 were significantly different from those of Euvolemia and Post Vasoconstriction.

Table 4: Venous return function, closing conditions excluded

	Euvolemia	Vasoconstriction			Post Vasoconstr.	Volume Expansion			*p* values		
		Step 1	Step 2	Step 3		Step 1	Step 2	Step 3	treatment	intensity	interaction
Q_{ECMO}: $mL \times min^{-1}$	3828 ± 756	3858 ± 564	4139 ± 625	4103 ± 594	3093 ± 723	4415 ± 713	4891 ± 761	5078 ± 934	0.057	<0.0005	0.005
									Vasoconstriction 0.2* Volume Expansion <0.0005*		
VRdP : mmHg	5.76 ± 1.56	5.40 ± 1.27	5.54 ± 1.07	5.47 ± 1.12	5.35 ± 2.29	5.07 ± 1.68	5.34 ± 1.83	4.96 ± 2.04	0.558	0.583	0.960
RVR: $mmHg/(mL \times min^{-1})$	1.59 ± 0.57	1.54 ± 0.40	1.44 ± 0.36	1.48 ± 0.25	2.00 ± 0.84	1.30 ± 0.25	1.19 ± 0.30	1.06 ± 0.31	0.348	0.007	0.041
									Vasoconstriction 0.445* Volume Expansion 0.014*		

Data is mean ± SD. n=9. Two-way repeated measurements ANOVA, within-subject factor treatment modality and treatment intensity. *Simple main effect for intensity: repeated measurements ANOVA, within-subject factor treatment intensity. Q_{ECMO} highest= maximum ECMO flow in the *per protocol* data set; rpm highest= revolutions per min associated with highest Q_{ECMO}; VRdP highest= venous return driving pressure at highest Q_{ECMO}, calculated as MSFP-RAP; RVR= resistance to venous return calculated as inverse slope of individual animal RAP-Q_{ECMO} pairs.

Table 5: Pressure head *vs.* Q_{ECMO} per condition

		Covariate		Dependent variable		Equation parameters		Difference of slopes
		Q_{ECMO}; mL×min^{-1}		Pressure head; mmHg		Slope m (95% CI) mmHg × min ×mL^{-1}	*p*	
	n	mean ± SD	range	mean ± SD	range			
Euvolemia	45	3069 ± 1083	1135 - 5598	62.3 ±27.4	18.7 – 138.4	0.023 (0.019 – 0.260)	≤0.0005	n.s.
Vasoconstriction 3		3381 ± 1179	1292 - 6379	67.3 ±31.6	21.7 – 158.5	0.024 (0.020 – 0.029)	≤0.0005	
Volume Expansion 3		3971 ± 1328	1462 - 7042	52.0 ±17.3	24.8 – 117.7	0.002 (0.008 – 0.015)	≤0.0005	<0.05*

Generalized Estimating Equations for Pressure head vs. Q_{ECMO}. n=number of data pairs per condition (9 animals × 5 pump speeds). *ΔPressure head / ΔQ_{ECMO} (=slope) in Volume Expansion 3 is significantly different from slopes of Euvolemia and Vasoconstriction 3 respectively. Proportion of variance (Pearson correlation coefficient squared; r^2) for individual animals (median, range) in Euvolemia: 0.983 (0.936-0.999); Vasoconstriction 3: 0.990 (0.969-1.000); and Volume Expansion 3: 0.965 (0.643-0.995).

Table 6: Blood volumes and voume shifts

	Euvolemia	Vasoconstriction	Volume Expansion	*p*
Blood volume: mL	3947 ± 424	3354 ± 335[a]	4061 ± 679[b]	0.011†
Plasma volume; mL	2804 ± 280	2230 ± 234	2849 ±460	0.001
Total vascular elastance: mmHg × L^{-1}		11.44 ± 0.42	8.67 ± 2.26	0.06*
Stressed volume: mL		721 ± 166	1089 ± 304	0.002*
Unstressed volume; mL		2605 ± 320	2972 ±775	0.189

Data is mean ± SD. n=9. † One-way repeated measurements ANOVA. Pairwise comparisons with Bonferroni correction: [a] Vasoconstriction *vs.* Euvolemia p=0.026, [b] Volume Expansion *vs.* Euvolemia p=0.013. * Paired t-test.