Functional heterogeneity of oxygen supply with blood and hemoglobin-based oxygen carriers in porcine models of hemorrhagic shock
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Microcirculation follows macrocirculation in heart and gut in the acute phase of hemorrhagic shock and isovolemic autologous whole blood resuscitation in pigs

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Abstract
Disparity between the macro- and microcirculation is thought to occur as a result of (micro)vascular dysfunction in some types of shock. Whether this occurs during hemorrhagic shock, however, is unknown. We therefore investigated both macro- and microcirculatory parameters in the heart as a vital, and the gut as a non-vital organ. We hypothesized that the microcirculation in the gut would follow the macrocirculation in the acute phase of hemorrhagic shock and isovolemic autologous whole blood resuscitation, but that the microcirculation in the heart would be preserved even under conditions of macrocirculatory depression. Eleven pigs (23±4 kg) were anesthetized and subjected to a controlled hemorrhagic shock (30% and 45% reduction of total blood volume) and isovolemic resuscitation with autologous blood. Quantitative measurement of microvascular oxygen pressures (µPO2) was performed by phosphorimetry on the gut and heart simultaneously. Measurements of systemic hemodynamic and regional oxygen-derived parameters as well as µPO2 were performed at baseline, after the first and second phase of hemorrhage, and after resuscitation. Five pigs responded to resuscitation, while 6 pigs died spontaneously within 20-30 min after reinfusion of the withdrawn blood, without significant differences in macro- or microcirculatory parameters at baseline and after hemorrhage. Correlation analysis showed that microvascular PO2 in the heart and the gut were closely related to macrocirculatory parameters (cardiac index, mean arterial pressure, and oxygen delivery) during hemorrhage and resuscitation. The present study demonstrated that the microcirculation in the gut (being a non-vital organ) and heart (being a vital organ) follow the macrocirculation in the acute phase of hemorrhagic shock and isovolemic autologous whole blood resuscitation.

Introduction
In trauma patients, hemorrhage-induced hypotension is responsible for high mortality and morbidity due to early stage cardiac failure and/or intestinal ischemia,
possibly leading to multiple organ failure. Resuscitation of these patients is ultimately aimed at restoring oxygen delivery to organs by improving microcirculatory perfusion and oxygenation. In current clinical practice, guidelines for resuscitation are provided by monitoring macrocirculatory parameters such as arterial blood pressure, heart rate, and cardiac output. However, whether these resuscitation procedures are effective in restoring the microcirculation of and within different organ systems remains to be elucidated. Indeed, in other types of shock (e.g., septic shock) several studies have shown that correcting macrocirculatory parameters has little or no effect on microcirculatory perfusion and oxygenation and that monitoring the effects of resuscitation should be performed at the microcirculatory level. The possible disparity between the macro- and microcirculation is thought to occur as a result of (micro)vascular dysfunction induced by inflammatory activation associated with either infectious, inflammatory, or hypoxemic conditions and resulting in shunting of vulnerable microcirculatory beds. However, whether this occurs in hemorrhagic shock is as yet unknown and should be elucidated as it would have important consequences for monitoring the severity of hemorrhagic shock and the advantages of resuscitation in such scenarios.

In the present study we aimed to quantitatively investigate both macro- and microcirculatory parameters in two organ systems, i.e., the heart (being a vital organ) and the gut (being a non-vital organ), during hemorrhagic shock and autologous whole blood resuscitation in pigs. For this purpose, we measured coronary and mesenteric arterial flow, performed arterial and local venous blood gas analysis, and applied phosphorimetry on the left ventricle and the small intestinal serosa simultaneously to quantitatively monitor microvascular oxygen pressures. We hypothesized that the microcirculation in the gut would follow the macrocirculation in the acute phase of hemorrhagic shock and isovolemic autologous whole blood resuscitation, but that the microcirculation in the heart would be preserved even under conditions of macrocirculatory depression.

Materials and Methods
Animal anesthesia
All experiments were performed in conformity with the regulations of the animal ethics committee of the Academic Medical Center at the University of Amsterdam. After an overnight fast, 11 female crossbred Landrace x Yorkshire pigs (mean±SD weight = 23±4 kg) were sedated with ketamine (Nimatek, AUV, Cuijk, The Netherlands; 10 mg·kg⁻¹ i.m.). Anesthesia was induced by mask ventilation with nitrous oxide in oxygen (ratio of 7:3). Anesthesia was maintained by continuous infusion of fentanyl citrate (Fentanyl, Janssen Pharmaceutical, Tilburg, The Netherlands; 12.5 µg·kg⁻¹ i.v.) and midazolam (Dormicum,
Hoffmann-La Roche, Mijdrecht, The Netherlands; 0.5 mg·kg⁻¹ bolus, followed by 0.5 mg·kg⁻¹·h⁻¹ i.v.). Muscle relaxation was maintained with pancuronium bromide (Pavulon, Organon Teknika, Boxtel, The Netherlands; 0.1 mg·kg⁻¹ bolus, followed by 0.3 mg·kg⁻¹·h⁻¹ i.v.). During induction, the depth of anesthesia was adapted according to the heart rate and blood pressure. After this had been established the infusion rates of fentanyl citrate and midazolam were kept constant.

Subsequently, the animals were intubated and ventilation (Servo 900B, Siemens, Munich, Germany) was performed by intermittent positive pressure ventilation with oxygen in air (FiO₂: 0.36). During preparation, artificial ventilation was instituted to maintain an end tidal PCO₂ of 35-40 mmHg. Positive end expiratory pressure of 5 mmHg was used to prevent atelectases. Lactated Ringer’s solution (10 ml·kg⁻¹·h⁻¹) was administered via the ear vein as maintenance fluid throughout the experiment. The central temperature was maintained by a heating pad.

**Surgical preparation**

Fluid-filled catheters were placed in both the right brachial artery (4 Fr.) for measurement of blood pressure and collection of arterial blood samples and the left femoral vein (6 Fr.) for blood withdrawal to create hemorrhagic shock and for reinfusion of the withdrawn blood. A pulmonary artery catheter (7.5 Fr., Baxter Healthcare, Deerfield, IL) was positioned in the pulmonary artery via the right jugular vein for the measurement of cardiac output and central temperature and for the collection of mixed venous blood samples. A supra-pubic bladder catheter was positioned for prevention of vagal stimulation associated with bladder distension.

Following a median laparotomy, a flow probe (2.5 mm, Transonic Systems, Ithaca, NY) was placed around the superior mesenteric artery for the measurement of blood flow to the splanchnic region. A mesenteric vein draining the ileum was cannulated (4 Fr.) for collection of mesenteric venous blood samples. A 10-cm segment of the terminal ileum was exteriorized outside the abdomen. Desiccation was prevented by continuous irrigation with preheated 0.9% NaCl solution. The median abdominal incision was closed loosely by three staples to prevent rises in abdominal pressure.

The pericardium was opened following a midsternal thoracotomy. The proximal part of the left anterior descending coronary artery was dissected free and a flow probe (1.5 mm, Transonic Systems) was placed around it for measurement of blood flow to the left ventricle. The vein accompanying the left anterior descending coronary artery was cannulated (venflon 18G) for collection of coronary venous blood.
Experimental protocol
One hour prior to completion of the surgical preparation, 12 mg·kg⁻¹ of Pd-porphyrin was administered via the cannulated ear vein at a rate of 2 ml·min⁻¹. Before controlled hemorrhage, the animals were allowed to stabilize for 30 min, during which two sets of baseline measurements were performed. The blood collection duration for blood gas analysis was approximately 5 min. Controlled hemorrhagic shock was induced in two phases: 1) 20 ml·kg⁻¹ blood withdrawal over a period of 20 min, corresponding to 30% blood loss and 2) 10 ml·kg⁻¹ blood withdrawal over a subsequent period of 20 min, corresponding to 45% blood loss cumulatively. The two phases were separated for 20 min to allow hemodynamic stabilization (~10 min) and measurements (~10 min). The withdrawn blood was collected in CPDA (citrate-phosphate-dextrose-adenine)-containing blood bags and reinfused over 20 min for resuscitation. Measurements (~10 min) were performed at baseline (t<0 min), after the first phase of blood withdrawal (t=30-40 min), after the second phase of blood withdrawal (t=70-80 min), and 10-20 min after resuscitation (t=110-120 min). The experiments were terminated by i.v. administration of 10 mmol of KCL.

Measurement of hemodynamic and oxygenation parameters
Systolic and diastolic arterial pressures (SAP and DAP, mmHg) and heart rate (HR, beats·min⁻¹) were determined from brachial artery pulse waves, which were recorded continuously. Mean arterial blood pressure (MAP, mmHg) was calculated as MAP = DAP + (SAP - DAP) / 3. Cardiac output (CO, l·min⁻¹) was measured by the thermodilution technique and indexed to body weight as cardiac index [l·min⁻¹·kg⁻¹]. The mean of three measurements, using 5 ml of room temperature-equilibrated saline, was calculated and recorded. Coronary artery flow (ml·min⁻¹) and superior mesenteric artery flow (ml·min⁻¹) were recorded continuously. A value of flow at each measurement point was calculated as the average over a sampling period of 5 min (~30 measurements).
Arterial and local venous blood samples were collected in heparinized 1-ml plastic tubes. Samples for blood gas analysis were directly placed on ice and analyzed within 5 min for hemoglobin concentration (Hb, g·dl⁻¹) and its oxygen saturation (SO₂) (Spectrophotometer OSM3, Radiometer, Copenhagen, Denmark, calibrated for porcine blood), PO₂ (mmHg), PCO₂ (mmHg), pH (ABL 505, Radiometer) and calculation of base excess (BE, mmol·dl⁻¹) as BE=0.93×([HCO₃⁻]-24.4+14.8×[pH 7.4]). Left ventricular and gut oxygen delivery and oxygen consumption were calculated by Fick’s principle according to standard formulas. Oxygen delivery was calculated as: DO₂ (ml·min⁻¹) = flow (ml·min⁻¹) × CₐO₂, where CₐO₂ is arterial oxygen content, calculated as 1.39 × Hb × SₐO₂ + 0.0031 × PₐO₂. Oxygen consumption was calculated as VO₂ (ml·min⁻¹) = C(a-v)O₂ × flow, where c(a-v)O₂ is the difference between arterial and venous oxygen content. Values were indexed to body weight.
Measurement of microvascular oxygen pressures

Microvascular partial oxygen pressures were assessed by measuring oxygen-quenched phosphorescence lifetimes of Pd-porphyrin, using two time-domain phosphorimeters. The principles of phosphorimetry and the applied phosphorimeters in large animal studies have been described in detail in chapter 1. Temperature readings on the serosal site of the ileum and on the outer surface of the left ventricle were used for correction of the oxygenation calculations.

The optical fiber of the first phosphorimeter was placed just above the surface of the apical site of the left ventricle and the optical fiber of the second phosphorimeter was placed just above the outer surface (serosal site) of the ileum. Care had been taken to place the fibers on a region without visible vasculature. One hundred phosphorescent decay curves were acquired at 50 Hz and averaged. Measurements were performed and recorded every 20 s.

Data analysis

Statistical analysis was performed in GraphPad Prism (GraphPad Software, San Diego, CA). All data are presented as mean±SD. Comparative analysis of values obtained at different time points was performed using the non-parametric Friedman test for repeated measures with the Dunns post-hoc test. Differences were considered statistically significant at p<0.05. Correlation analysis between macrohemodynamic parameters and microvascular PO2 was performed using Spearman’s correlation test.

Results

All animals responded similarly to 30% and 45% blood loss, which resulted in a significant decrease in cardiac index and MAP (Figure 1) and a significant increase in heart rate. Arterial PO2 remained unchanged during the entire experiment.

Five animals (45%) responded to isovolemic autonomic blood transfusion resuscitation by surviving more than one hour after resuscitation and six animals (55%) did not respond to resuscitation and died within 20-30 min after reinfusion of the withdrawn blood as a result of ventricular fibrillation. Post-resuscitation measurements (i.e., 10-20 min after reinfusion of the withdrawn blood) could be performed in all animals. In the responders, resuscitation resulted in a significant increase in cardiac index and MAP, while resuscitation had no effect on these parameters in the non-responders. No significant differences existed between the responders versus non-responders at baseline, 30%, and 45% blood loss with respect to cardiac index, MAP, heart rate, and arterial PO2. Hence, prior to resuscitation, no identifiable factor predicting outcome following autologous whole blood resuscitation was identified.
Oxygen delivery and consumption

Measurement of arterial flow and oxygenation and venous oxygenation allowed calculation of oxygen delivery and oxygen consumption. In the heart (ns) and gut (p<0.05), oxygen delivery decreased during the first (30%) and second (45%) phase of hemorrhage (Figure 2). In the responders, resuscitation restored oxygen delivery to baseline level, whereas resuscitation was not effective in increasing oxygen delivery to either organ in the non-responders. Oxygen consumption in the gut decreased during the second phase of hemorrhage and was restored to baseline in the responders, but decreased significantly further in the non-responders.

**Figure 1.** Cardiac index (upper left), mean arterial pressure (MAP, upper right), heart rate (lower left), and arterial PO$_2$ (lower right) during controlled hemorrhage (30% and 45% blood loss) and resuscitation. * p<0.05 versus baseline and † p<0.05 versus previous time point (t.p.).
Venous base excess
After 45% blood loss, venous BE decreased significantly in the heart and gut in both the responders and the non-responders (Figure 3). In the responding group, resuscitation stabilized the venous BE, but in the non-responding group, venous BE continued to significantly decline during resuscitation.

Figure 2. Oxygen delivery (upper graphs) and consumption (lower graphs) of the heart and gut in responders (left) and non-responders (right). * p<0.05 versus baseline and † p<0.05 versus previous time point (t.p.).

Figure 3. Venous base excess (BE) of the heart and gut in the responders (left) and non-responders (right). * p<0.05 versus baseline and † p<0.05 versus previous time point (t.p.).
**Microvascular oxygenation**

Thirty percent blood loss caused a significant decrease in microvascular PO$_2$ (measured quantitatively by Pd-porphyrin phosphorimetry) in both the heart and the gut (Figure 4). However, this decrease was more profound in the gut than in the heart. Forty-five percent blood loss significantly decreased microvascular PO$_2$ even further in the heart, but not in the gut. Resuscitation led to a significant increase in microvascular PO$_2$ in the responders, but a significant decrease in the non-responders. In the responders, the heart microvascular PO$_2$ restored to approximately 80% of baseline, while the gut microvascular PO$_2$ returned to only 50%, indicating that the gut is less responsive to resuscitation than the heart and that isovolemic autologous whole blood resuscitation is not associated with complete resuscitation of the gut, leaving it hypoxic.

**Micro- versus macrocirculation**

Correlation analysis showed that microvascular PO$_2$ in the heart and the gut were closely related to macrocirculatory parameters (CI, MAP, and oxygen delivery) during hemorrhage and resuscitation (Figure 5). Heart microvascular PO$_2$ correlated to cardiac index (Spearman’s $r = 0.79$), MAP (Spearman’s $r = 0.83$), and heart oxygen delivery (Spearman’s $r = 0.80$). Similarly, gut microvascular pO$_2$ correlated to cardiac index (Spearman’s $r = 0.76$), MAP (Spearman’s $r = 0.80$), and gut oxygen delivery (Spearman’s $r = 0.71$). This suggests that the microcirculation in both the heart (vital organ) and the gut (non-vital organ) follows the macrocirculation, during hemorrhage and resuscitation.

![Figure 4. Microvascular PO$_2$ in the heart and gut in responders (left) and non-responders (right). * $p<0.05$ versus baseline and † $p<0.05$ versus previous time point (t.p.).](image-url)
Discussion and conclusions

In the present study we investigated the effects of hemorrhagic shock and isovolemic autologous whole blood resuscitation on macro- and microcirculatory parameters in the heart and gut. All animals responded similarly to hemorrhagic shock both at the macro- and microcirculatory level. However, only 45% of the animals responded to the whole blood resuscitation protocol by surviving more than one hour after resuscitation and 55% of the animals did not respond to the resuscitation protocol by dying due to ventricular fibrillation within 20-30 min after resuscitation. The primary results of this study were that: 1) the gut was more vulnerable to hemorrhage than the heart and autologous whole blood resuscitation restored the microcirculation more effectively in the heart than in the gut and 2) macro- and microcirculatory parameters are profoundly affected and closely related to each other during hemorrhagic shock and resuscitation in vital organs such as the heart as in non-vital organs such as the gut.

Figure 5. Correlation analysis of microvascular PO$_2$ in the heart and gut versus cardiac index (upper left), mean arterial pressure (MAP) (upper right), heart and gut oxygen delivery (lower left and lower right, respectively) during hemorrhagic shock and resuscitation. p<0.001 for all correlations.
Several studies have been conducted looking at tissue and/or microcirculatory oxygenation of different organ systems simultaneously in models of hemorrhage and resuscitation. None of these studies, however, incorporated isovolemic autologous whole blood resuscitation in their protocol. Hence, this is the first study quantitatively investigating the relation between the micro- and macrocirculation in both a vital (the heart) and a non-vital (the gut) organ. We found that at 45% hemorrhage, no significant differences in macro- and microcirculatory parameters between the responders and non-responders were present. Ten-to-twenty min after autologous whole blood transfusion, however, micro- and macrocirculatory parameters improved in the responders but worsened in the non-responders. Since the animals initially comprised a homogenous group, bred and kept under identical conditions, undergoing the same procedure, the finding that only 45% of the animals survived the resuscitation protocol is, in itself, of interest inasmuch as it implies that subtle differences between animals govern the response to autologous whole blood resuscitation. Furthermore, autologous whole blood may not constitute the optimal medium for resuscitation and whole blood resuscitation may require additional pharmacological intervention to improve macro- and microcirculatory parameters during resuscitation.

In both responders and non-responders the gut was shown to be more vulnerable to hemorrhagic shock than the heart and, additionally, the heart responded better to resuscitation than the gut. At 30% blood loss the decrease in microvascular oxygenation in the gut was much more severe than in the heart. Furthermore, whole blood resuscitation restored heart microvascular oxygenation to 80% of baseline and in the gut, microvascular oxygenation was restored to only 50% of baseline. In addition, measurement of tissue and/or microcirculatory oxygenation was shown to be of great relevance inasmuch as restoration of these parameters should be regarded as the ultimate end point of resuscitation. The present study clearly showed that, even though macrocirculatory parameters and heart microvascular oxygenation were significantly improved by whole blood resuscitation, gut microvascular oxygenation in responders remained hypoxic. This renders the gut more susceptible to ischemic insult and shock and complies with the designation of this organ as “the canary of the body” even when oxygen delivery to the gut ultimately restores to baseline. Consequently, although currently largely abandoned in clinical practice, these findings underscore the importance of monitoring the effects of shock and resuscitation at the microcirculatory level in vulnerable organs such as the gut for guidance and optimization of resuscitation protocols.

Nevertheless, during the entire protocol, micro- and macrocirculatory parameters were closely related and no distributive effects of hemorrhagic shock and autologous
whole blood resuscitation were observed in the present study. This is in contrast to septic shock, where increasing MAP does not result in an increase in microcirculatory density and perfusion as was shown by Dubin et al. in septic patients requiring norepinephrine in addition to fluid resuscitation to maintain an adequate MAP.\textsuperscript{124} The authors studied the sublingual microcirculatory density and perfusion using intravital microscopy and investigated the effects of norepinephrine on MAP and the microcirculation. It was concluded that even though the MAP increased by norepinephrine administration, the microcirculation remained suboptimal. However, unlike septic shock, hemorrhagic shock does not appear to be of a (re)distributive nature where a depressed microcirculation coexists with a normalized macrocirculation. Realizing this is of particular importance as it might have profound consequences for the treatment and monitoring of hemorrhagic shock.

In conclusion, the present study has provided new insights into the response of the cardiovascular system to hemorrhagic shock and whole blood resuscitation by examining the response at both at the macrocirculatory level and at the microcirculatory level in a vital (i.e., the heart) and a non-vital (i.e., the gut) organ. We have demonstrated that the microcirculation in the gut and heart follow the macrocirculation in the acute phase of hemorrhagic shock and isovolemic autologous whole blood resuscitation.