Redistribution of intestinal microcirculatory oxygenation during acute hemodilution in pigs

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Schwarte, Lothar A., Artur Fournell, Jasper van Bommel, and Can Ince. Redistribution of intestinal microcirculatory oxygenation during acute hemodilution in pigs. J Appl Physiol 98: 1070–1075, 2005.—Acute normovolemic hemodilution (ANH) compromizes intestinal microcirculatory oxygenation; however, the underlying mechanisms are incompletely understood. We hypothesized that contributors herein include redistribution of oxygen away from the intestines and shunting of oxygen within the intestines. The latter may be due to the impaired ability of erythrocytes to off-load oxygen within the microcirculation, thus yielding low tissue/plasma PO2 but elevated microcirculatory hemoglobin oxygen (HB02) saturations. Alternatively, oxygen shunting may also be due to reduced erythrocyte deformability, hindering the ability of erythrocytes to enter capillaries. Anesthetized pigs underwent ANH (20, 40, 60, and 90 ml/kg hydroxyethyl starch; ANH group: n = 10; controls: n = 5). We measured systemic and mesenteric perfusion. Microvascular intestinal oxygenation was measured independently by remission spectrophotometry [microcirculatory HB02 saturation (μHB02)] and palladium-porphyrin phosphorescence quenching [microcirculatory oxygen pressure in plasma/tissue (μPO2)]. Microcirculatory oxygen shunting was assessed as the disparity between mucosal and mesenteric venous HB02 saturation (HB02-gap). Erythrocyte deformability was measured as shear stress-induced cell elongation (LORCA diffractometer). ANH reduced hemoglobin concentration from 8.1 to 2.2 g/dl. Relative mesenteric perfusion decreased (decreased mesenteric/systemic perfusion fraction). A paralleled reduction occurred in mucosal μHB02 (68 ± 2 to 41 ± 3%) and μPO2 (28 ± 1 to 17 ± 1 Torr). Thus the proposed constellation indicative for oxygen off-load deficits (sustained μHB02 at decreased μPO2) did not develop. A twofold increase in the HB02-gap indicated increasing intestinal microcirculatory oxygen shunting. Significant impairment in erythrocyte deformability developed during ANH. We conclude that reduced intestinal oxygenation during ANH is, in addition to redistribution of oxygen delivery away from the intestines, associated with oxygen shunting within the intestines. This shunting appears to be not primarily caused by oxygen off-load deficit but rather by oxygen/erythrocytes bypassing capillaries, wherein a potential contributor is impaired erythrocyte deformability.

mucosa; serosa; spectrophotometry; hemoglobin; splanchnic oxygenation

ACUTE NORMOVOLEMIC HEMODILUTION (ANH) reduces arterial hemoglobin (Hb) concentration, and thus arterial O2 content, triggering compensatory mechanisms at different levels of O2 transport to maintain tissue oxygenation. At the systemic level, ANH increases cardiac output (Qsys) and systemic O2 extrac-

tion ratio (ERO2) (22). Furthermore, it may redistribute blood flow to vital organs (3, 5, 29, 30). At the tissue level, ANH redistributes blood flow (11) and recruits underperfused capillaries (1). However, although these mechanisms initially preserve vital organ oxygenation, they may impair oxygenation of less-protected organs, such as the intestines. We found that, although a mild ANH maintains intestinal microvascular oxygenation (26), a more severe ANH impairs intestinal oxygenation (25) before failure of vital organs, such as the heart. This heterogeneous response was recently confirmed in a porcine model (27), showing that ANH causes significant desaturation of mesenteric venous blood at stages where cerebral tissue oxygenation and jugular venous oxygenation were still well maintained. Because the factors contributing to the ANH-induced impairment of intestinal oxygenation are unclear, the present study was undertaken to elucidate the underlying mechanisms.

We hypothetized that, in addition to a redistribution of O2 away from the intestines, a shunting of O2 within the intestines occurs. Intestinal O2 shunting may herein be characterized by an intestinal microvascular oxygenation becoming progressively lower than oxygenation of corresponding effluent mesenteric venous blood. We observed such an impairment in intestinal microcirculatory oxygenation in various models of compromised systemic oxygenation, i.e., during hemorrhage, endotoxemia, anemia, and hypoxemia (16, 20, 27, 28). Thus we hypothetized that, during progressive ANH, intestinal microvascular HB02 becomes progressively lower than mesenteric venous HB02, indicative of intestinal O2 shunting.

O2 shunting, particularly during ANH, may be caused by an inability of erythrocytes to release Hb-bound O2 to the tissue rapidly enough, potentially due to shortened capillary transit times (12). In this case, a rather high microvascular HB02 saturation would be expected in the presence of low values of plasma/tissue PO2. To test this hypothesis, we simultaneously measured these two microcirculatory variables of tissue oxygenation, i.e., microcirculatory HB02 saturation by reflectance spectrophotometry (8) and microcirculatory PO2 (μPO2) by the phosphorescence quenching technique (14).

An alternative mechanism accounting for impaired microcirculatory oxygenation may be a reduced erythrocyte deformability (ED). ED is a major determinant of capillary perfusion and thus of tissue oxygenation. During ANH, erythrocytes are exposed to various stressors, e.g., rheological and metabolic factors that could impair their ability to deform (4, 13). Thus

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the erythrocytes may be forced to bypass the capillaries via larger (shunting) vessels or even become trapped in the microcirculation. Herein, especially splanchnic organs participate (17). To measure ED during ANH, we used the laser-assisted optical rotational cell analyzer technique (LORCA).

In summary, in this study, we hypothesized that during progressive ANH several mechanisms contribute to the reduction in O2 delivery to the intestinal microcirculation: redistribution of O2 delivery away from the intestines and shunting within the intestines, the latter potentially caused by O2 off-loading deficit of erythrocytes (due to reduced microcirculatory transit times associated with ANH) and/or impaired ED.

MATERIALS AND METHODS

Anesthesia and ventilation. The experiments were performed with the approval of the Animal Ethical Commission of the Academic Medical Centre, according to national laboratory animal care guidelines, as an extension of an established protocol (27).

Pigs (n = 15 Dutch land race-Yorkshire crossbred males, 25 ± 1 kg, mean ± SE) were fasted overnight, with unlimited access to water. The pigs were premedicated (0.02 mg/kg atropine, 1.0 mg/kg midazolam, and ketamine 20 mg/kg im), anesthetized (5 mg/kg thiopentone, 0.2 mg/kg midazolam, 0.02 mg/kg fentanyl, 0.1 mg/kg pancuronium iv), intubated, and mechanically ventilated. Anesthesia was maintained with fentanyl (0.01 mg·kg⁻¹·h⁻¹), midazolam (0.2 mg·kg⁻¹·h⁻¹), and pancuronium (0.1 mg·kg⁻¹·h⁻¹). Mechanical ventilation (AV-1, Drägerwerke, Lübeck, Germany; inspired O2 fraction /H11005 0.33, tidal volume /H11005 10 –15 ml/kg, mean 18 ± 3 ml/kg, respiratory rate /H11005 10 –15 breaths/min, positive end-expiratory pressure /H11005 15 ± 3 Torr). Ringer electrolyte solutions. For in vivo testing, we placed the spectrophotometry light guide on the ileum, allowing for steady-state conditions of microvascular oxygenation (μHbO2), and thereafter injected the Pd solution. Injection of Pd solution did not affect the spectrophotometry spectra or μHbO2 values obtained.

O2-derived variables. Under steady-state conditions, we simultaneously collected arterial, mixed venous, and mesenteric venous blood (2-ml preheparinized syringes). The samples were processed immediately (ABL-505 and OSM-3, Radiometer, Copenhagen, Denmark, calibrated for porcine blood) to measure O2-related (Hb, oxyhemoglobin saturation, PO2) and acid-/base-related (pH, base excess, Pco2) variables. O2-derived variables (arterial oxygen content, arterial-venous oxygen content difference, oxygen uptake, oxygen delivery, ERO2) were calculated according to standard formulas. To test for a capillary O2 off-load deficit during ANH, the relation between μPO2 and μHbO2 was plotted. If major O2 off-loading would occur, this should result in a flattening curve toward lower values, with a stable μHbO2 and progressively lowering μPO2 values.

Measurement of O2 delivery. O2 delivery was calculated at baseline, at 40 ml/kg HD, and at the final HD step (90 ml/kg). ED was measured at both points. The experiments were performed in duplicate and averaged.

Experimental protocol. After completion of instrumentation, we allowed a stabilization period of ~45 min. Thereafter, the animals were randomly assigned to either the HD (n = 10) group or the sham control (SC; n = 5) group. Initial baseline measurements of hemodynamics (heart rate, MAP, Qs, Qmes) and microcirculatory oxygenation (μHbO2 and μPO2, both mucosal and serosal) were obtained as detailed above. After a second baseline measurement (30 min after initial baseline), which ensured
Table 1. Systemic hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>BL-1</th>
<th>BL-2</th>
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<th>HD-40</th>
<th>HD-60</th>
<th>HD-90</th>
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<tbody>
<tr>
<td>Hb, g/dl</td>
<td></td>
<td></td>
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<tr>
<td>HD</td>
<td>8.1±0.3</td>
<td>8.1±0.4</td>
<td>5.4±0.2†</td>
<td>3.9±0.2†</td>
<td>3.0±0.1†</td>
<td>2.2±0.1†</td>
</tr>
<tr>
<td>SC</td>
<td>8.2±0.4</td>
<td>8.3±0.4</td>
<td>8.1±0.4</td>
<td>8.1±0.2</td>
<td>8.0±0.1</td>
<td>8.8±0.2</td>
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<tr>
<td>VRIsys</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HD</td>
<td>32±4</td>
<td>37±4</td>
<td>32±2†</td>
<td>28±2†</td>
<td>24±2†</td>
<td>20±2†</td>
</tr>
<tr>
<td>SC</td>
<td>36±2</td>
<td>41±2</td>
<td>41±3</td>
<td>40±3</td>
<td>42±3</td>
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<td></td>
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</tr>
<tr>
<td>HD</td>
<td>210±11</td>
<td>242±16</td>
<td>236±17*</td>
<td>213±13*</td>
<td>191±14*</td>
<td>167±9†</td>
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<td>304±25</td>
<td>309±16</td>
<td>324±30</td>
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</tr>
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Values are means ± SE. HD, hemodilution (n = 10); SC, sham controls (n = 5); BL, baseline; VRIsys, systemic vascular resistance index; VRImes, mesenteric vascular resistance index. For the HD steps, the exchange volume is given in ml/kg; *P < 0.05 vs. SC. †P < 0.05 vs. baseline. ‡P < 0.05 vs. previous measurement.

stability of the porcine preparation, stepwise isovolemic HD was performed in the HD group. Blood was withdrawn via the femoral artery catheter and simultaneously replaced by isovolemic intravenous infusion of warmed (~38°C) hydroxyethyl starch (6% hydroxyethyl starch 200/0.5, Fresenius, Germany). The following blood volumes were exchanged: 20, 40, 60, and 90 ml/kg. In pilot experiments (n = 3), HD was advanced to 120 ml/kg, resulting in a Hb of 159±4 ± 3.1 mmHg, whereas stroke volume index remained almost constant (1.8 ± 0.1 to 1.9 ± 0.1 ml/kg), resulting in an ~30% increase in Qsys. Systemic vascular resistance indexes decreased significantly, and mean arterial pressure also tended to decrease from 103 ± 2 to 94 ± 3 mmHg.

Systematic hemodynamics and O2-derived variables. HD decreased Hb concentration from baseline values of 8.1 to 2.2 g/dl at end-stage HD (Table 1). Similar baseline values were obtained and maintained without significant changes in the SC group. In HD animals, heart rate increased by ~20% (130 ± 4 to 159 ± 3 beats/min), whereas stroke volume index remained almost constant (1.8 ± 0.1 to 1.9 ± 0.1 ml/kg), resulting in an ~30% increase in Qsys. Systemic vascular resistance indexes decreased significantly, and mean arterial pressure also tended to decrease from 103 ± 2 to 94 ± 3 mmHg.

Mechanical ventilation resulted in stable arterial PO2 and Pco2 in both SC and HD animals (Table 2). HD decreased arterial O2 concentration from ~11 to one-third of that value, i.e., to ~3.5 ml/dl. Although Qsys increased (Fig. 1), systemic O2 delivery decreased from ~26 to ~11 ml/kg·min−1. This decrease in systemic O2 delivery was paralleled by an increase in systemic O2 extraction (ERO2 from ~0.3 to ~0.5), as reflected in decreases of venous PO2 and O2 saturation (Table 2).
A significant drop in systemic O2 uptake was only observed after the last step of HD (90 ml/kg). HD pigs did not reveal histological alterations, such as mucosal villus tip lesions, that exceeded those in SC animals.

Regional intestinal hemodynamics and O2-derived variables. In the SC group, regional hemodynamics and oxygenation variables remained unchanged throughout the time-matched experiments. Although during HD Qmes tended to increase (Fig. 1), mesenteric O2 delivery decreased from ~4 to 1.35 ml·kg⁻¹·min⁻¹. In turn, mesenteric ER02 increased from 0.2 to ~0.5 at end-stage HD, as reflected in decreased mesenteric venous O2 return (mesenteric P02 and O2 saturation).

We observed a progressive redistribution of perfusion away from the intestines during the course of HD. Flow was redistributed away from the mesentery (Fig. 1), as expressed by a significantly decreased Qmes-to-Qsys ratio (minus ~20% at end-stage HD). The VRImes decreased progressively during HD, an effect that we interpret as being caused by a reduction in Hb (e.g., viscosity) associated with HD.

Intestinal μHbO2 and μP02. Progressive ANH induces a two-phase response of intestinal microcirculatory oxygenation. Initially, from baseline conditions to mild HD, microvascular oxygenation was preserved but progressively impaired during severe ANH. At the intestinal mucosa, both μHbO2 and μP02 followed a similar profile during HD (Fig. 2). Mucosal μHbO2 was well preserved during baseline measurements and the initial HD (~65, ~68, and ~65%, respectively), but thereafter μHbO2 progressively decreased to ~41% at end-stage HD (equal to ~60% of baseline values). In parallel, mucosal μP02 was also maintained during the two baseline measurements and the initial HD step (~25, 28, and 26 Torr, respectively) and declined thereafter progressively to ~17 Torr (also equal to ~60% of baseline values).

Both measurements presented a markedly higher baseline oxygenation at the intestinal serosa (μHbO2 of ~87% and μP02 of ~60 Torr, respectively), compared with the intestinal mucosa (Fig. 3). The serosal μHbO2 was ~85% at baseline measurements and declined thereafter to ~74%. The μP02 decreased already at the initial steps of HD from ~60 to ~50 Torr, with a further progressive decrease to ~20 Torr at end-stage HD.

The evolution of a divergence between μHbO2 and mesenteric Hb saturation [i.e., μHbO2 - mesenteric HbO2 (HbO2-gap)] in the course of progressive HD is presented in Fig. 4. The gradient between mucosal μHbO2 and the mesenteric venous HbO2 remained stable up to an exchange volume of 40 ml/kg but then progressively increased to twice the baseline value at end-stage HD.

To test whether microvascular O2 release from Hb was impaired (O2 off-load deficit) the relation between μP02 and μHbO2 was plotted. If major O2 off-loading would have occurred, this should have resulted in a flattening curve toward lower values, i.e, a stable μHbO2 and progressively declining μP02 values. This, however, was not observed; in contrast, the data correlated linearly (r = 0.94).

ED. ED was determined for both tested shear stresses, 30 Pa and the more sensitive 3.0-Pa shear stress. ED under high shear stress conditions (30 Pa) remained unaltered (0.562 ± 0.02, 0.568 ± 0.01, and 0.571 ± 0.01 for baseline, 40 ml/kg HD, and 90 ml/kg HD, respectively). However, ED at the lower shear...
stress (3.0 Pa) decreased significantly from baseline (0.407 ± 0.01) already at an exchange volume of 40 ml/kg (0.369 ± 0.03) and decreased further at 90 ml/kg (0.358 ± 0.02), indicating that, during ANH, a progressive stiffening of erythrocytes occurred.

DISCUSSION

In this study, we investigated the mechanisms underlying the changes in intestinal microcirculatory oxygenation induced by acute normovolemic HD. Our results show that severe HD is associated with redistribution of O2 delivery away from the intestines and also shunting of the residual O2 within the intestines itself. The correlation between mucosal μHbO2 and μPO2 during reduction of intestinal microvascular oxygenation indicates that impaired erythrocyte O2 off-loading is not the key mechanism responsible for the decreased microcirculatory mucosal oxygenation. Thus O2 shunting bypassing the microcirculation to the venous compartment appears to be the main factor. Herein, rheological alterations may contribute, and a potential candidate is the observed erythrocyte rigidification during ANH.

In HD pigs, mucosal μHbO2 was well preserved during mild ANH but finally progressively declined. This finding of a two-phase response of intestinal microvascular oxygenation (maintained oxygenation during mild and progressive impairment of oxygenation during severe HD) to ANH confirms earlier studies in rats (25) and pigs (9). The initial phase suggests the presence of compensatory mechanisms on the systemic and microvascular level, e.g., by capillary recruitment (1), counterbalancing the initial reduction in Hb (31).

The observed baseline differences in microvascular oxygenation between intestinal serosa and mucosa are in accordance with other studies (9, 10), independent of the measurement method applied. Although a slight reduction of mucosal oxygenation was observed at 40 ml/kg exchange, the mucosal μHbO2 still approximated baseline values. Serosal μHbO2 tended to decrease already with the first step of HD, although the level of statistical significance was achieved only at 60 and 90 ml/kg, probably due to the larger heterogeneity of serosal μHbO2. In agreement with this pattern, the μPO2 measurements indicated a maintained mucosal oxygenation during mild HD and a decrease during progressive HD (Fig. 2). The drop of mucosal μPO2 occurred at a later stage than the drop of serosal μPO2, agreeing with a redistribution of O2 from the serosal to the mucosal layer. This is in accordance with other models of impaired splanchnic perfusion/oxygenation (2, 21, 23). However, although intestinal perfusion may be redistributed between serosa and mucosa (15), this buffer mechanism finally exhausts and does not compensate for the mucosal metabolic demand anymore, leading to a decreased mucosal μHbO2 and μPO2, respectively, both to ~60% of the baseline values.

In the present study, we observed a marked increase in the HbO2-gap between mesenteric venous HbO2 and mucosal μHbO2 during severe HD, indicating that O2 transport to this microcirculatory compartment was being increasingly shunted to the venous pool (12). Microvascular oxygenation was maintained during mild HD (20 ml/kg), accompanied by only a small HbO2-gap and a parallel course of both saturations from baseline to 40 ml/kg HD. However, thereafter, we observed a significant broadening of the HbO2-gap to about twofold of the baseline value. Whatever mechanisms are responsible for the increased shunting during HD, our study shows that a relatively high regional O2 return (i.e., mesenteric venous HbO2) does not necessarily reflect adequate tissue oxygenation during HD.

ANH may recruit previously unperfused capillaries (1), allowing more homogenous tissue perfusion by minimizing intercapillary distances and thus distances from capillaries to O2-consuming cells. Thus capillary recruitment could redirect blood toward the O2-consuming cells, enhance O2 delivery efficiency, and minimize O2 shunting. Therefore, induction of a more homogenous flow pattern through the capillaries into the draining veins should minimize the HbO2-gap. Although we observed a stable HbO2-gap during mild HD, we observed the opposite response during severe HD, i.e., increased shunting during progressive HD, as indicated by the marked widening of the intestinal HbO2-gap, indicating exhaustion of the before-mentioned compensatory mechanisms.

One mechanism that potentially contributes to the shunting of O2, and thus accounts for impaired intestinal oxygenation in our study, is altered erythrocyte rheology, i.e., our finding of reduced ED during ANH. This alteration may contribute to redistribution of blood flow away from splanchnic organs (17) or redirect perfusion to larger, nonnutrient conducting and shunting vessels within the splanchnic region. Although we found alteration in red blood cell deformability ex vivo by use of the LORCA technique, the question remains whether red blood cell deformability also occurs in vivo in the microcirculation. New techniques developed to observe red blood cell kinetics in vivo, such as Orthogonal Polarization Spectral Imaging (16), may provide answers in future research.

In contrast to these regional changes, systemic markers of oxygenation remained more stable. Supporting the concept that systemic markers of oxygenation are less-sensitive indicators of tissue distress (16), these markers of oxygenation (arterial PO2 and O2 saturation; Table 2) remained preserved during HD, and systemic O2 consumption was maintained up to an exchange volume of 60 ml/kg and decreased significantly only with the final HD step. The prolonged maintenance of systemic O2 consumption was achieved by an increased Qsys and
systemic ERO2, both of which already reached significance with the first step of HD.

Although we are aware that experimental data may not be translated directly to the clinical setting, our results indicate that progressive HD by several mechanisms decreases intestinal tissue oxygenation before systemic measures of oxygenation. Thus monitoring of intestinal oxygenation in the clinical setting may allow individualization of HD and detection of impaired intestinal oxygenation in advance of systemic impairment. Herein, reflectance spectrophotometry, as applied in the present study, appears an attractive option and has already been demonstrated to be applicable in patients (7).

In summary, there are several major findings of our study. Although systemic oxygenation (arterial O2 saturation and PO2) was maintained throughout the entire experiment and systemic O2 consumption was maintained until the last step of HD, the intestinal oxygenation, in contrast, was impaired at earlier stages in the course of HD. Herein, we observed by two independent measures of microcirculatory oxygenation, i.e., µHbO2 and µPO2, impairment of intestinal microcirculatory oxygenation. As mechanisms for this impairment, we found, other than redistribution of perfusion away from the mesentery, redistribution within the intestinal wall and an increased shunting within the intestines, with the potential contribution of altered ED. Thus, regarding the marked intestinal shunting as indicated by the increasing gap in microvascular and venous HbO2 evolving during ANH, a major mucosal O2 off-load failure was not detected, as mucosa µHbO2 and µPO2 both decreased by the same fraction, i.e., to ~60% of the respective baseline values, and thus the proposed constellation of elevated ED. Thus, regarding the marked intestinal shunting as indicated by the increasing gap in microvascular and venous HbO2 evolving during ANH, a major mucosal O2 off-load failure was not detected, as mucosa µHbO2 and µPO2 both decreased by the same fraction, i.e., to ~60% of the respective baseline values, and thus the proposed constellation of elevated µHbO2 in conjunction with decreased µPO2 was not observed.

REFERENCES