The relationship of CO2 metabolism to tissue perfusion, microcirculation, and treatment response in shock and sepsis
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Citation for published version (APA):

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ABSTRACT

**Objectives:** (1) To demonstrate that metabolic parameters are better indicators of tissue hypoxia than regional and whole oxygen consumption (VO$_2$). (2) To compare intramucosal pH (pHi) in different gastrointestinal segments.

**Design:** Prospective, interventional study.

**Setting:** Research laboratory at a university center.

**Subjects:** Fourteen anesthetized, mechanically ventilated dogs.

**Interventions:** Twenty milliliters per kilogram bleeding.

**Measurements and main results:** We placed pulmonary, aortic and mesenteric venous catheters, and an electromagnetic flow probe in the superior mesenteric artery, and gastric, jejunal and ileal tonometers to measure flows, arterial and venous blood gases and lactate, and intramucosal PCO$_2$. We calculated systemic and intestinal oxygen transport (DO$_2$) and consumption (VO$_2$), pHi and arterial minus intramucosal PCO$_2$ (ΔPCO$_2$). Then, we bled the dogs and repeated the measurements after 30 min. Systemic and intestinal DO$_2$ fell (26.0±7.3 versus 8.9±2.6 and 71.9±17.3 versus 24.6±9.6 ml/min per kg, respectively, p<0.0001). Systemic and intestinal VO$_2$ remained unchanged (5.5±1.3 versus 5.4±1.3 and 15.7±5.0 versus 14.9±5.3 ml/min per kg, respectively). Gastric, jejunal and ileal pH (7.13±0.11 versus 6.96±0.17, 7.18±0.06 versus 6.97±0.15, 7.12±0.11 versus 6.94±0.14, p<0.05) and ΔPCO$_2$ (21±13 versus 35±23, 15±5 versus 33±16, 23±17 versus 38±20, p<0.05) changed accordingly. Arterial and mesenteric venous lactate and their difference, rose significantly (1.7±0.9 versus 3.7±1.4 and 1.8±0.8 versus 4.3±1.5 mmol/l, 0.1±0.6 versus 0.6±0.7 mmol/l, p<0.05). **Conclusions:** During hemorrhage, systemic and intestinal VO$_2$ remained stable. However, hyperlactatemia and intramucosal acidosis evidenced anaerobic metabolism. pH changes paralleled in the three intestinal segments.

**Keywords.** Intramucosal pH - Tonometry - Shock - Oxygen consumption - Oxygen delivery - Lactate

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Adapted from Intensive Care Medicine 2001; 27:1931-1936
INTRODUCTION

Many studies have analyzed the effects of different types of injury on intestinal oxygenation. Nelson et al. showed that, during progressive bleeding, intestinal oxygen uptake (VO$_2$) is compromised early, even without changes in systemic VO$_2$ [1]. This limited ability for oxygen extraction makes the intestine suitable to track oxygen metabolism. Tissue oxygenation monitoring with tonometry, which measures gastrointestinal intramucosal pH (pHi), has been reasonably established in recent years [2]. Grum et al. showed that intestinal pHi reductions correlated with gut VO$_2$ decreases [3]. Other investigators have demonstrated the prognostic significance of gastric intramucosal acidosis. [2, 4]. However, there is no definite evidence that gastric intramucosal acidosis resembles the intramucosal acidosis of other gut sections. Some studies have analyzed pHi behavior in different segments of the gastrointestinal tract [5, 6], but their results have been inconclusive.

Our goal was to describe the effects of hemorrhage on intestinal oxygenation. We tried to answer the following questions: 1) Are there traces of tissue hypoxia, evidenced as intramucosal acidosis, before the beginning of VO$_2$ dependence on DO$_2$? 2) What is the relationship between pHi and other parameters of intestinal and systemic oxygenation? 3) Are pHi reductions in stomach, jejunum and ileum equivalent?

MATERIALS AND METHODS

Animal preparation

This study was approved by the local animal care committee. Fourteen mongrel dogs, weighing 22.1±3.1 kg (mean ± SD), were anesthetized with 30 mg/kg of sodium pentobarbital, with supplementary doses of 1 mg/kg given each hour or as necessary. They were intubated and ventilated with a volumetric respirator (Harvard Apparatus Dual Phase Control Respirator Pump Ventilator, model 613 A, Harvard Apparatus, Southnatick, Mass., USA), with a tidal volume of 15 ml/kg, FIO$_2$ of 0.21 and respiratory rate of 15/min. Neuromuscular blockade was provided by intravenous 0.06 mg/kg of pancuronium bromide. Ranitidine, 50 mg, was infused intravenously.

We advanced a Swan-Ganz catheter (flow-directed thermodilution fiberoptic pulmonary artery catheter model P 7110, Abbott Critical Care Systems, Mountain View, Calif., USA) into the pulmonary artery through the right external jugular vein to measure pressures and to sample blood. Catheters were placed in the left femoral artery and vein to measure mean arterial pressure (MAP), to administer drugs and fluids, and to perform bleeding. A gastric tonometer was placed in the stomach to measure pH and its position was checked manually through palpation.

After a midline laparotomy had been performed, a splenectomy was carried out to prevent autotransfusion. The superior mesenteric artery was dissected and
an electromagnetic flow probe was placed around it. A catheter was advanced through a small mesenteric proximal vein to the intestine into the superior mesenteric vein to sample blood. Two small enterotomies were performed to place jejunal and ileal tonometers. After careful hemostasis, the abdominal contents were returned to the cavity and the abdomen was closed.

**Measurements and calculations**

Mean arterial pressure (MAP) was measured (Statham P23 AA, Statham, Hato Rey, Puerto Rico) and registered (Gould RS 3400, Gould, Cleveland, Ohio, USA) through the whole experiment. Cardiac output (CO) was measured in triplicate by thermodilution using 5 ml of saline at 0°C (Oximetrix SO2/CO Computer, Abbott Laboratories, North Chicago, Ill. USA). CO was indexed to body weight. Superior mesenteric artery blood flow (SMAF) was measured with an electromagnetic probe (Spectramed Blood Flowmeter model SP 2202 B, Spectramed, Oxnard, Calif., USA) and is related to gut weight.

Arterial, mixed venous and mesenteric venous PO2, PCO2 and pH were measured with a blood gas analyzer (ABL 30, Radiometer, Copenhagen, Denmark). Hemoglobin and oxygen blood saturations (%HbO2) were measured by using a co-oximeter calibrated for canine blood (OSM 3, Radiometer, Copenhagen, Denmark). Arterial, mixed venous and mesenteric venous contents (CaO2, CvO2 and CmvO2) were calculated as Hb = 1.34 x %HbO2 + PaO2 x 0.0031. Systemic and intestinal oxygen transport and uptake (DO2, VO2, DO2i and VO2i, respectively) were estimated as DO2 = CO x CaO2, VO2 = CO x (CaO2-CvO2), DO2i = SMAF x CaO2 and VO2i = SMAF x (CaO2-CmvO2). Systemic and intestinal oxygen extraction ratios (O2ER and O2ERi) were calculated as (CaO2-CvO2)/CaO2 and (CaO2-CmvO2)/CaO2, respectively.

Intramucosal PCO2 and pHi were measured with tonometers (TRIP NGS II Catheter, Tonometrics Division, Instrumentarium, Helsinki, Finland) [7]. We calculated differences between intramucosal PCO2 (gastric, jejunal and ileal) and arterial PCO2 (gastric ΔPCO2, jejunal ΔPCO2 and ileal ΔPCO2, respectively). To determine whether mucosal anaerobic metabolism developed after hemorrhage, we used the Dill nomogram to predict mesenteric venous %HbO2 (%HbO2vDill) from intramucosal PCO2 [8].

An enzymatic electrode (Stat profile 9 plus, Nova Biomedical, Waltham, Mass., USA) was used to measure arterial and venous lactate. Intestinal efflux of lactate was calculated as SMAF times arterial minus mesenteric venous lactate difference.

**Experimental procedure**

Basal measurements were performed after a stabilization period, which was defined by steady CO, SMAF and mixed venous %HbO2. Then, 20 ml/kg of blood was extracted through the femoral artery at a rate of 5 ml/kg per min. The
measurements were repeated after 30 min (ischemia). Dogs’ temperatures were maintained at 37°C, with a heating lamp, throughout the experiment.

**Statistical analysis**

Measurements are reported as means ± SD. The data of the basal period were compared with ischemia with paired t-test. Multiple comparisons were analyzed with ANOVA and Student-Newman-Keuls test. Differences with a $p$ value of less than 0.05 were considered significant.

**RESULTS**

Hemorrhage caused marked effects on hemodynamic and oxygen transport variables (Fig. 1). MAP, CO, SMAF, DO$_2$, and DO$_2$i fell significantly, but both systemic and intestinal VO$_2$ remained stable due to increases in systemic and intestinal oxygen extractions (Table 1). Notwithstanding this, signs of tissue hypoxia were quite evident, like decreases of systemic pH, bicarbonate and arterial, mixed venous and mesenteric venous base excesses (Table 2) and arterial and mesenteric venous hyperlactatemia. Gastric, jejunal and ileal ∆PCO$_2$ and pHi changed accordingly (Fig. 2). pHi variation coefficients were 1.6, 0.8 and 1.6%, respectively, in the basal period, and 2.4, 2.2 and 2.1% during ischemia. However, the decreases of gastric, jejunal and ileal pHi were greater than that of arterial pH (0.16 ± 0.09, 0.21 ± 0.14 and 0.18 ± 0.13 versus 0.07 ± 0.03, respectively; $p$<0.05). Arterial and mesenteric venous lactate levels rose significantly (1.7 ± 0.9 versus 3.7 ± 1.4, $p$<0.001, and 1.8 ± 0.8 versus 4.3 ± 1.5 mmol/l, $p$<0.0001) as did the lactate veno-arterial difference (0.1 ± 0.6 to 0.6 ± 0.7 mmol/l, $p$<0.05). Intestinal efflux of lactate increased more than 100% (0.037 ± 0.064 versus 0.082 ± 0.114 mmol/min per kg), but it did not reach statistical significance due to a large SD.

![Fig. 1. Systemic and intestinal oxygen transport and consumption (DO$_2$, DO$_2$i, VO$_2$ and VO$_2$i, respectively) in basal and ischemic conditions.](image)
Basal and ischemic %HbO$_2$ vDill values, derived from gastric, jejunal and ileal tonometer PCO$_2$ values, were 19 ± 62% and -17 ± 66% (p<0.02). Ischemic values were not only different from basal ones but became negative (Fig. 3).

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**Table 1. Systemic and intestinal hemodynamic and oxygen transport parameters in basal conditions and after hemorrhage.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>Ischemia</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>145±21</td>
<td>88±24</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Cardiac output (ml/min per kg)</td>
<td>121±29</td>
<td>48±14</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Superior mesenteric artery blood flow (ml/min per kg)</td>
<td>338±91</td>
<td>135±55</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Systemic oxygen transport (ml/min per kg)</td>
<td>26.0±7.3</td>
<td>8.9±2.6</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Systemic oxygen consumption (ml/min per kg)</td>
<td>5.5±1.3</td>
<td>5.4±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Intestinal oxygen transport (ml/min per kg)</td>
<td>71.9±17.3</td>
<td>24.6±9.6</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Intestinal oxygen consumption (ml/min per kg)</td>
<td>15.7±5.0</td>
<td>14.9±5.3</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic oxygen extraction ratio</td>
<td>0.22±0.06</td>
<td>0.62±0.11</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Intestinal oxygen extraction ratio</td>
<td>0.23±0.07</td>
<td>0.60±0.10</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

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Fig. 2. Gastric, jejunal and ileal intramucosal arterial PCO$_2$ gradients and intramucosal pH (pHi) behaviors in basal and ischemic conditions. Black dots are each individual experiment. Open squares are mean values ± SD.

Basal and ischemic %HbO$_2$ vDill values, derived from gastric, jejunal and ileal tonometer PCO$_2$ values, were 19 ± 62% and -17 ± 66% (p<0.02). Ischemic values were not only different from basal ones but became negative (Fig. 3).
Table 2. Arterial, mixed venous and mesenteric venous blood gases in basal conditions and after hemorrhage.

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Ischemia</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>16.3±2.1</td>
<td>14.6±2.2</td>
<td>0.04400</td>
</tr>
<tr>
<td>Arterial oxygen saturation</td>
<td>0.99±0.02</td>
<td>0.96±0.03</td>
<td>0.00270</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.33±0.04</td>
<td>7.26±0.06</td>
<td>0.00200</td>
</tr>
<tr>
<td>Arterial PO₂ (torr)</td>
<td>101±16</td>
<td>84±13</td>
<td>0.00510</td>
</tr>
<tr>
<td>Arterial PCO₂ (torr)</td>
<td>34±4</td>
<td>33±3</td>
<td>NS</td>
</tr>
<tr>
<td>Arterial HCO₃ (mmol/l)</td>
<td>17±1</td>
<td>14±2</td>
<td>0.00010</td>
</tr>
<tr>
<td>Arterial BE (mmol/l)</td>
<td>-7±2</td>
<td>-12±3</td>
<td>0.00025</td>
</tr>
<tr>
<td>Mixed venous oxygen saturation</td>
<td>0.70±0.06</td>
<td>0.36±0.10</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Mixed venous pH</td>
<td>7.31±0.04</td>
<td>7.22±0.07</td>
<td>0.00048</td>
</tr>
<tr>
<td>Mixed venous PO₂ (torr)</td>
<td>52±6</td>
<td>33±5</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Mixed venous PCO₂ (torr)</td>
<td>38±8</td>
<td>46±6</td>
<td>0.00710</td>
</tr>
<tr>
<td>Mixed venous HCO₃ (mmol/l)</td>
<td>18±3</td>
<td>18±2</td>
<td>NS</td>
</tr>
<tr>
<td>Mixed venous BE (mmol/l)</td>
<td>-7±3</td>
<td>-10±3</td>
<td>0.02000</td>
</tr>
<tr>
<td>Mesenteric venous oxygen saturation</td>
<td>0.76±0.07</td>
<td>0.38±0.09</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Mesenteric venous pH</td>
<td>7.30±0.04</td>
<td>7.17±0.07</td>
<td>0.00003</td>
</tr>
<tr>
<td>Mesenteric venous PO₂ (torr)</td>
<td>51±7</td>
<td>35±5</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Mesenteric venous PCO₂ (torr)</td>
<td>41±4</td>
<td>50±7</td>
<td>0.00035</td>
</tr>
<tr>
<td>Mesenteric venous HCO₃ (mmol/l)</td>
<td>20±1</td>
<td>18±3</td>
<td>0.03700</td>
</tr>
<tr>
<td>Mesenteric venous BE (mmol/l)</td>
<td>-6.3±1.8</td>
<td>-11.3±4.4</td>
<td>0.00076</td>
</tr>
</tbody>
</table>

To convert torr to kPa, multiply the value by 0.1333.
DISCUSSION

In recent years, tonometry has emerged as a useful tool with which to evaluate gut mucosal oxygen metabolism, as blood flow to the stomach and intestine is early and disproportionately reduced in shock and low-flow states. Intramucosal acidosis can rise from several mechanisms. First, it might appear because of tissue hypoxia. At the onset of anaerobic metabolism, CO\textsubscript{2} rises because of the bicarbonate buffering of protons generated by strong acids and ATP hydrolysis [9]. Tonometry might certainly identify this CO\textsubscript{2} excess. Besides, in hypoperfusion states, aerobically generated CO\textsubscript{2} could also accumulate as a result of lack of removal [10]. Moreover, in experimental endotoxemia, intramucosal acidosis could develop as a signal of real, deep metabolic disorders of sepsis, in the absence of tissue hypoxia and hypoperfusion [11].

In the present study, though DO\textsubscript{2} fell, systemic and intestinal VO\textsubscript{2} were maintained through increases in systemic and intestinal O\textsubscript{2}ER. This was to be expected, as we did not reach the critical DO\textsubscript{2} level needed to jeopardize VO\textsubscript{2}, stated by Nelson et al. as 7.9 ± 1.9 and 9.7 ± 2.7 ml/min per kg for systemic and intestinal VO\textsubscript{2}, respectively [1]. However, and as a key finding of this study, there was multiple evidence of anaerobic metabolism, such as metabolic acidosis, hyperlactatemia and intramucosal acidosis. We reason that, in this model, tissue hypoxia might have been the main mechanism responsible for intramucosal acidosis. During the experiment, intestinal VO\textsubscript{2} remained stable but the veno-arterial lactate difference rose significantly, which can be ascribed to the onset of intestinal anaerobic metabolism.

Schlichtig and Bowles, in a low-flow experimental model and with the aid of the nomogram of Dill, concluded that, below critical DO\textsubscript{2}, the appearance of CO\textsubscript{2} in the gut lumen was due to anaerobic production and not to the accumulation of aerobic source [8]. Briefly, Dill’s nomogram rests upon the almost perfect linear relationship between venous PCO\textsubscript{2} and venous %HbO\textsubscript{2} in aerobic conditions, with stable CO\textsubscript{2} to O\textsubscript{2} exchange ratio. It follows that, if the venous PCO\textsubscript{2} is known, venous %HbO\textsubscript{2} is predictable (%HbO\textsubscript{2,vDill}) and, if it agrees with the measured venous %HbO\textsubscript{2} value, the appearance of dissolved CO\textsubscript{2} absolutely indicates aerobic metabolism. On the other hand, %HbO\textsubscript{2,vDill} less than measured %HbO\textsubscript{2} or less than zero, would indicate the production of metabolic acids like lactate, and then conversion of CO\textsubscript{3}H\textsuperscript{−} to CO\textsubscript{2} in anaerobiosis. During the ischemic period, our %HbO\textsubscript{2,vDill} values obtained from gastric, jejunal and ileal mucosal PCO\textsubscript{2} became remarkably negative, which would further imply the presence of anaerobic processes.

The gut is especially sensitive to systemic hypoperfusion. As was pointed out before, the beginning of oxygen supply dependence occurs earlier in the gut than in the whole body [1]. Besides, measurements of regional oxygen transport and uptake parameters have been claimed as a way to assess oxygen metabolism. However, in our study, intestinal VO\textsubscript{2} remained stable in the face
of altered metabolic parameters. Similarly, Fink et al. found ileal intramucosal acidosis in the presence of normal mesenteric VO$_2$ [12] and Gutierrez et al. have demonstrated that increases in DO$_2$ with dobutamine in septic patients can reverse hyperlactatemia and gastric intramucosal acidosis, in spite of unmodified VO$_2$ [13]. Other investigators have reported different results. Grum et al. described a consistent correlation between pHi and intestinal VO$_2$ during DO$_2$ decreases induced by ischemia and hypoxia. Initially, both pHi and intestinal VO$_2$ remained stable and fell when critical DO$_2$ was achieved [3]. Rozenfeld et al. found that metabolic markers of intestinal hypoxia appeared with the onset of critical DO$_2$ [14]. These contrary results could be ascribed to different experimental models of hypoperfusion. In the aforementioned studies there was selective reduction in superior mesenteric blood flow, while our model consisted in hemorrhagic shock.

Several underlying mechanisms could explain tissue hypoxia in the face of preserved intestinal VO$_2$. First, blood flow might have been redistributed to the muscularis mucosae, rendering mucosal layers hypoxic, but with low impact on whole VO$_2$. However, Lundgren et al. demonstrated that decreases in perfusion pressures redistributed flow towards the mucosa [15]. Second, countercurrent irrigation might induce a functional shunt that could place distal microvilli segments at risk [16]. Rozenfeld et al. suggested that during progressive ischemia, mucosa and serosa might become simultaneously hypoxic with a “patchy” distribution [14]. Our experimental design might have produced hypoxic areas large enough to cause intramucosal acidosis and lactate generation, but not to lower global intestinal VO$_2$.

Last, we cannot disregard the fact that surgical instrumentation or mucosal barrier ischemic injury could result in bacterial translocation, endotoxemia and subsequent cellular disturbances, which could account for the metabolic derangement observed. In this setting, aerobically generated hyperlactatemia produced by inhibition of pyruvate dehydrogenase, without changes in VO$_2$, may occur [17]. However, ours is an acute experimental model, whose main feature is hypoperfusion.

The other key finding of this study was that the effects of ischemia were equally reflected in stomach, jejunum and ileum. There was no difference in the behavior of ΔPCO$_2$ and pHi in the three segments and the variation coefficients were similar under basal and ischemic conditions. However, there has been much controversy about pHi correlations along the gastrointestinal tract. Poole et al. found, in dogs, that reduction of celiac and mesenteric flows to less than 50% of the basal values resulted in intramucosal acidosis in both territories [18] which was greater in the intestine than in the stomach (7.11 versus 7.30). Hartmann et al. [5] and Montgomery et al. [6] studied hemorrhage effects on gastric, small intestine and colonic pHi in pigs. The extent of the hemorrhage was similar to that of our model (16.9 ml/kg), but only intestinal pHi fell. Larger bleedings reduced pHi in the three segments. Walley et al. exposed pigs to progressive hemorrhage and found
that gastric $\Delta$PCO$_2$ at critical gut DO$_2$ was extremely high and variable (63 ± 40 mmHg) compared to jejunal $\Delta$PCO$_2$ (17 ± 15 mmHg). They concluded that gastric tonometry is noisy and rather inaccurate [19]. Differences in experimental designs or the species involved could account for the variations observed. For instance, reductions of regional blood flow during cardiopulmonary bypass, evaluated by laser Doppler velocimetry, were equal in rabbit stomach, jejunum and ileum [20]. In addition, despite a larger blood supply, gastric blood flow is more decreased than intestinal blood flow during hemorrhagic shock in dogs [21]. Also, a study of the vascular pattern of the intestinal villi performed in different species showed a more uniform histologic structure in the relatively shorter gastrointestinal tract of the dog [22].

In summary, our data show that, at least in this model of hemorrhagic shock, tissue and metabolic markers of hypoxia such as lactic acidosis and intramucosal acidosis may appear early, even in the absence of dependence of oxygen consumption on oxygen transport. In addition, $\Delta$PCO$_2$ and pH$_i$ showed a similar response to hemorrhage in the three gastrointestinal segments studied. These findings support the value of gastric tonometry as a useful indicator of splanchnic perfusion.

REFERENCES


