Effect of crystalloid administration on oxygen extraction in endotoxemic pigs

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Effect of crystalloid administration on oxygen extraction in endotoxemic pigs. J. Appl. Physiol. 85(5): 1667–1675, 1998.—We asked whether crystalloid administration improves tissue oxygen extraction in endotoxosicosis. Four groups of anesthetized pigs (n = 8/group) received either normal saline infusion or no saline and either endotoxin or no endotoxin. We measured whole body (WB) and gut oxygen delivery and consumption during hemorrhage to determine the critical oxygen extraction ratio (ERO2crit) just after onset of ischemia (critical oxygen delivery rate), gut was removed for determination of area fraction of interstitial edema and capillary hematocrit. Radiolabeled microspheres were used to determine erythrocyte transit times from 0.33 to 0.55 ± 0.08, P < 0.05) and gut ERO2crit (0.77 ± 0.07 to 0.52 ± 0.06, P < 0.05). Unexpectedly, saline administration also decreased WB ERO2crit (0.82 ± 0.06 to 0.62 ± 0.08, P < 0.05) and gut ERO2crit (0.77 ± 0.07 to 0.67 ± 0.06, P < 0.05) in nonendotoxin pigs. Saline administration increased the area fraction of interstitial space (P < 0.05) and resulted in arterial hemodilution (P < 0.05) but not capillary hemodilution (P > 0.05). Saline increased the relative dispersion of erythrocyte transit times from 0.33 ± 0.08 to 0.72 ± 0.53 (P < 0.05) and resulted in increased heterogeneity of microvascular erythrocyte transit times.

The splanchnic circulation has been thought to be particularly susceptible to ischemic injury (10, 24, 35). The susceptibility has been attributed to specific regulation of organ perfusion (35), to increased overall oxygen requirements by the metabolically active splanchnic organs (24), and to the countercurrent flow in the intestinal villi whereby there is shunting of oxygen away from the tips of the villi (8, 31, 32). Furthermore, it has been proposed that ischemia of the splanchnic circulation may predispose to decreased mucosal integrity and lead to bacterial translocation, which may then lead to multiple organ dysfunction syndrome (4, 10). Thus we were particularly interested in intestinal oxygen extraction and the splanchnic circulation.

Accordingly, we sought to determine whether crystalloid administration in a porcine model of endotoxin shock would improve or impair oxygen extraction capacity of the whole body (WB) and specifically of the splanchnic bed. To better understand the mechanism of this effect, we measured the dispersion of erythrocyte transit times in the gut, the gut capillary hematocrit, and the area fraction of interstitial space as a measure of tissue edema.

METHODS

Surgical preparation. This study was approved by the Animal Care Committee of the University of British Columbia and conforms to National Institutes of Health standards for animal experimentation. We used the experimental preparation of Humer et al. (20), which was relevant to the goals of the present study because these investigators have previously demonstrated an oxygen extraction defect after endotoxin infusion in this preparation. Thirty-two pigs, weighing 25.7 ± 2.8 kg, were fasted overnight and then sedated with 0.5 mg/kg im of midazolam (Hofman la Roche, Mississauga, Canada) and anesthetic agents (ketamine, 2 mg/kg iv, and xylazine, 0.5 mg/kg iv). After anesthetization, arterial blood pressure (AP) was measured via a 22-gauge catheter inserted into the femoral artery and monitoring of heart rate (HR) and end-tidal CO2 (ETCO2) was performed. After crush of the aorta, the pigs received lactated Ringer’s solution at 20 ml/kg/h for 30 min. The pigs were then randomized into four groups (n = 8/group) to receive either saline solution or no saline and either endotoxin or no endotoxin. The endotoxin was a heat-killed suspension of E. coli serotype 0111:B4 (lipopolysaccharide [LPS] concentration, 10 mg/kg iv). The endotoxin solution was infused into the femoral artery at a rate of 2 ml/kg/h for 1 h. The saline solution was given at the same rate in the control group. During endotoxin infusion, the pigs were maintained at mild hypothermia (Tb, 34–35°C) with forced air warming.

In addition to antibiotic therapy, fluid administration is a key component of the management of sepsis and septic shock (27). The goal of fluid administration is to restore intravascular volume to maintain an adequate blood pressure and cardiac output. Restoring a normal cardiac output requires intravascular replacement of third-space losses, compensation for venous pooling of blood, and a sufficiently high left ventricular filling pressure to compensate for decreased ventricular contractility during sepsis (26). Despite limited data in humans and appropriate uncertainty in the literature, a number of studies suggest that sepsis is accompanied by impaired tissue oxygen extraction (8, 20, 23, 33) so that even a normal cardiac output may not necessarily be adequate during sepsis. That is, higher oxygen delivery (D˙O2; equals cardiac output × arteriovenous oxygen content) may be required to prevent evidence of unmet oxygen demand (13, 33), including decreasing oxygen consumption (V˙O2) (20, 23), rising lactate levels (20, 23), low gastric intramucosal pH (14), and organ system dysfunction (8, 13, 33). In addition to the direct hemodynamic benefit of fluid administration, there is evidence that microvascular flow distribution and tissue oxygen extraction may be improved (36). Conversely, tissue edema observed during sepsis and fluid administration (9, 15) could conceivably impair tissue oxygen extraction. Considering the multiple effects on intravascular volume, cardiac function, microvascular flow, and tissue edema, fluid administration in sepsis is a complex intervention. Our goal was to determine whether fluid administration in sepsis improved tissue oxygen extraction, possibly because of improved microvascular flow distribution (34, 37), or whether fluid administration impaired tissue oxygen extraction associated with tissue edema.

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Ontario). Thirty minutes later the animals were anesthetized with the use of 500 mg im of ketamine (MTC Pharm, Cambridge, Ontario) followed by 125–250 mg iv of thiopenal (Abbott, Montreal, Quebec), titrated to effect. Anesthesia was maintained throughout the experiment with the use of 5 ml·kg⁻¹·h⁻¹ iv of ketamine infusion and 0.5% inspired isoflurane (Anaquest, Mississauga, Ontario). To avoid changes in WB oxygen demand, skeletal muscle relaxation was maintained with intravenous pancuronium bromide infusion (Organon, Scarborough, Ontario) at 6 mg/h, titrated to effect.

A tracheotomy was performed, and an 8.0-mm endotracheal tube (Portex, Wilmington, MA) was inserted and secured. During instrumentation and experimentation, the animals were mechanically ventilated (dual-phase control respirator pump, model 613; Harvard Apparatus, Mills, MA) with 30% oxygen. A low-compliance catheter was inserted into the right carotid artery for arterial pressure measurement and arterial blood sampling. A catheter was inserted into the left external jugular vein for saline infusion and administration of medications. A pulmonary artery catheter (Criticath model DSP5105H; Ohmeda Medical Devices, Oxnard, CA) was placed via the right external jugular vein for measurement of right atrial pressure and pulmonary artery occlusion pressure, for mixed venous blood sampling, and for cardiac output measurement in triplicate (thermodilution cardiac output monitor, Edwards model 9250; Baxter Health Care, Irvine, CA). A catheter was inserted into the left carotid artery for controlled hemorrhage.

An anterolateral thoracotomy was performed through the fifth or sixth intercostal space on the left. The pericardium was entered, and a catheter inserted and secured in the left atrial appendage. This catheter was used for injection of radiolabeled red blood cells and radioactive microspheres for gut blood volume and flow measurements, respectively.

Through a midline laparotomy, the pancreaticoduodenal vein at the second part of the duodenum and the superior rectal vein at the promontory of the sacrum were tied off. After this, the splenic artery and vein were tied off to prevent autotransfusion, and a catheter was inserted via the splenic vein stump to sample portal vein blood. Ligation of these vessels ensured that the gastrointestinal circulation was isolated such that venous drainage passed through the portal vein. Portal venous flow was measured by placement of a 1.5-cm ultrasonic flow probe connected to a Transonic T201 ultrasonic blood flowmeter (Transonic, Ithaca, NY) around the portal vein. An orogastric tube was inserted to allow drainage of the gastric secretions. A 60-cm length of jejunum was isolated 30 cm distal to the duodenal-jejunal junction for later resection for morphometric analysis. Umbilical tapes were brought around the mesentery to allow ligation of the vasculature after injection of radiolabeled microspheres (see below). The mesentery was otherwise not disrupted. The abdomen was loosely closed to facilitate later removal of the jejunal segment.

Measurements and calculations. We measured arterial, mixed venous, and portal vein pO₂, pCO₂, and [Hb] (ABL30; Radiometer, Copenhagen, Denmark), oxygen content (IL 482 CO-oximeter; Instrumentation Laboratories, Lexington, MA), and lactate concentration (YSI 2300STAT lactate analyzer; Yellow Springs Instruments, Yellow Springs, OH) at baseline and every 20 min during progressive hemorrhage. Heart rate, mean arterial pressure, pulmonary arterial pressure, central venous pressure, pulmonary capillary occlusion pressure, cardiac output, portal vein flows, and WB VO₂ were also recorded at 20-min intervals by using a metabolic cart (mean of 5 measures at 1-min intervals; MBM-1000, Deltatrac, Helsinki, Finland). A complete blood count (Coulter counter, Coulter, Miami Lakes, FL) was measured every 40 min.

Blood oxygen content was calculated as hemoglobin × 1.39 × blood oxygen saturation + 0.003 × pO₂. WB DO₂ was calculated as cardiac output (thermodilution measurements) × arterial oxygen content. WB VO₂ was measured by using the metabolic cart (Deltatrac). Gut DO₂ was calculated as portal vein flow × arterial oxygen content. Gut VO₂ was calculated as portal vein flow × the difference between arterial and portal venous oxygen content. The oxygen extraction ratio (ERp) for both WB and gut was calculated as VO₂ divided by DO₂. From the multiple DO₂-VO₂ points obtained during progressive hemorrhage, the WB and gut critical ERp (ERp crítica) was determined with the use of Samsel and Schumacker’s dual-line regression analysis (30).

Protocol. After instrumentation, the animals were allowed to stabilize for 40 min, and a baseline data set was measured. Then the animals were randomized to one of the following groups: Control-Fluid (n = 8), Control-No Fluid (n = 8), Endotoxin-Fluid (n = 8), and Endotoxin-No Fluid (n = 8). Endotoxin groups received 50 µg/kg of Escherichia coli endotoxin (0111:B4; Sigma Chemical, St. Louis, MO) in 60 ml of normal saline over 30 min immediately after the baseline data set. Control groups received an infusion of 60 ml of normal saline without endotoxin. Fluid groups received an infusion of normal saline at 48 ml·kg⁻¹·h⁻¹ from the baseline measurement set until the end of the experiment. No Fluid groups did not receive any further saline infusion. After the animals were randomized to their respective treatment groups, controlled hemorrhage was undertaken at 3 ml/min by using a constant-withdrawal pump from the left carotid catheter until the animal died. Before death, when WB VO₂ had fallen by 25%, suggesting that DO₂ was inadequate to meet demand, we infused radiolabeled red blood cells, injected radiolabeled microspheres (see below), and rapidly excised the previously prepared segment of jejunum.

Erythrocyte transit times. Gut blood flow and red blood cell volume were determined in the isolated segments of gut after they had been removed and fixed for 24 h in 6% phosphate-buffered gluteraldehyde (18). After fixation, the mesenteric vessels were discarded and therefore not included in the analysis of transit times. Before removal of the vessels, 30-cm segments of gut were rapidly injected radiolabeled microspheres (see below), and then injected rapidly (1 s) into the left atrium. At the time of microsphere injection, blood was withdrawn from the left common carotid artery at 10 ml/min for 2 min into weighed vials for later radiation counting. Next, the arterial and venous vasculatures of the gut segment were simultaneously cross-clamped, and the segment of gut was rapidly excised and immersed in 6% phosphate-buffered gluteraldehyde. The 60-cm segments of gut were divided into 30 2-cm pieces, and each was placed into preweighed vials containing 6% phosphate-buffered gluteraldehyde. Each segment was weighed and counted by using a Beckman 8000 gamma counter for 3 min (1). The red blood cell volume of each 2-cm piece of gut was calculated as counts per minute of each piece divided by counts per minute per milliliter of the reference blood sample multiplied by the ratio of capillary hematocrit (see below) to systemic arterial hematocrit. Blood flow to each 2-cm piece of gut was calculated as microsphere counts per minute from that piece of gut divided by microsphere counts per minute per milliliter of the reference arterial withdrawal sample. Average transit time for each piece of gut was calculated as blood volume divided by blood flow, giving units of time (18). After calculation of individual transit times, a distribution of
transit times for all of the pieces of gut taken together was then determined for each gut segment.

Area fraction of interstitial space. We used a morphometric technique to quantify the area fraction of interstitial space. This approach has been validated by Weibel (38) and others and has been used by a number of investigators (2, 11) to quantify edema volume fraction. J ejunum from five animals in each group was randomly selected. From each of these animals, 6 of the 30 2-cm sections of jejunum were randomly selected and fixed overnight in 2.5% gluteraldehyde in 0.1 M cacodylate buffer. The tissue was postfixed for 2 h in 1% osmium tetroxide, then stained en bloc for 1 h in 5% aqueous uranyl acetate, and embedded in Eufapoxy resin. Thin sections, selected from a 1-µm toluidine blue-stained section, were cut on a Reichert Ultracut ultramicrotome, mounted on 200-mesh copper grids, and stained with lead citrate. Five capillaries (<10 µm) were selected from random layers of the bowel wall for each section (30 capillaries per jejunal segment) and were photographed at ×5,000 magnification on a Zeiss 10CR transmission electron microscope. Capillary plasma and erythrocyte volume fractions were determined by point-counting by using a 400-point grid. The capillary hematocrit was calculated as the quotient of erythrocyte volume fraction and total capillary volume fraction.

Statistical analysis. The relationship between \( V\dot{O}_2 \) and \( D\dot{O}_2 \) for WB and gut was determined for each animal by finding two best-fit linear-regression lines (30) from a plot of \( V\dot{O}_2 \) and \( D\dot{O}_2 \). The \( E\dot{R}_2 \) was determined at the point of intersection of the two lines (Fig. 1). To determine whether intravascular volume expansion altered \( E\dot{R}_2 \) during endotoxia, we used a two-way ANOVA for the effect of volume and endotoxin. We calculated the mean (\( \mu \)), second moment (\( \mu^2 \)), and relative dispersion (\( r^2 \)) of the distribution of gut erythrocyte transit times using standard formulas (20). We also used a two-way ANOVA to test for differences due to fluid administration and endotoxin in the relative dispersion of gut erythrocyte transit times and in the area fraction of interstitial space. A one-way ANOVA was used to test for effect of saline administration on arterial and capillary hematocrit. When a significant difference was found, we used a sequentially rejective Bonferroni test procedure to identify individual differences. Results are presented as means ± SD.

RESULTS

Effect of endotoxin. Endotoxin infusion significantly reduced the \( E\dot{R}_{crit} \) in the WB (0.55 ± 0.08 vs. 0.82 ± 0.06, Endotoxin-No Fluid vs. Control-No Fluid group,

Table 1. Critical \( D\dot{O}_2 \), \( V\dot{O}_2 \), and \( E\dot{R}_2 \) for wholebody and gut

<table>
<thead>
<tr>
<th>Group</th>
<th>( D\dot{O}_2 ) (ml O2/min·kg gut wt(^{-1}))</th>
<th>( V\dot{O}_2 ) (ml O2/min·kg gut wt(^{-1}))</th>
<th>( E\dot{R}_2 )</th>
<th>( D\dot{O}_2 ) (ml O2/min·kg gut wt(^{-1}))</th>
<th>( V\dot{O}_2 ) (ml O2/min·kg gut wt(^{-1}))</th>
<th>( E\dot{R}_2 )</th>
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</thead>
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<tr>
<td>No fluid</td>
<td>9.1 ± 1.7</td>
<td>7.5 ± 1.3</td>
<td>0.82 ± 0.06</td>
<td>23.5 ± 14.8</td>
<td>19.6 ± 12.0</td>
<td>0.77 ± 0.07</td>
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<tr>
<td>Fluid</td>
<td>10.3 ± 2.2</td>
<td>6.3 ± 1.3</td>
<td>0.62 ± 0.06*</td>
<td>20.5 ± 6.8</td>
<td>14.0 ± 5.6</td>
<td>0.67 ± 0.06*</td>
</tr>
<tr>
<td>Endotoxin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No fluid</td>
<td>12.2 ± 3.8†</td>
<td>6.5 ± 1.5</td>
<td>0.55 ± 0.08†</td>
<td>24.3 ± 9.0</td>
<td>12.9 ± 16.0</td>
<td>0.52 ± 0.05†</td>
</tr>
<tr>
<td>Fluid</td>
<td>15.3 ± 3.2†</td>
<td>7.4 ± 1.2</td>
<td>0.49 ± 0.04†</td>
<td>22.9 ± 11.7</td>
<td>11.8 ± 5.2</td>
<td>0.52 ± 0.09†</td>
</tr>
</tbody>
</table>

Values are means ± SD. WB, whole body; \( D\dot{O}_2 \) and \( V\dot{O}_2 \), oxygen delivery and consumption, respectively; \( E\dot{R}_2 \), oxygen extraction ratio.

*Significant difference between No Fluid and Fluid Control groups (\( P < 0.05 \)); †significant difference between Control and Endotoxocosis groups (\( P < 0.05 \)).
P < 0.05) and gut (0.52 ± 0.05 vs. 0.77 ± 0.07, Endotoxin-No Fluid vs. Control-No Fluid group, P < 0.05) (Table 1, Fig. 2). A decrease in critical oxygen extraction was accompanied by a significant increase in critical $\dot{D}_O_2$ and unchanged baseline $\dot{V}_O_2$ for WB. However, gut baseline oxygen was decreased (but not significantly), whereas gut critical $D_O_2$ was unchanged. Relative dispersion of erythrocyte transit times between

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**Fig. 2.** Raw $\dot{D}_O_2$ and $\dot{V}_O_2$ data points are shown for Control-No Fluid (A), Control-Fluid (B), Endotoxin-No Fluid (C), and Endotoxin-Fluid (D) groups. Left, whole body (WB) figures; right, gut figures. Points include all those for the 8 animals in each group and therefore display significant scatter. However, steep linear downslope in Control-No Fluid WB relationship (A, left) suggests surprisingly uniform and efficient oxygen extraction characteristics during anaerobic metabolism.
segments of jejunum was not affected by endotoxin (Fig. 3). This measurement of erythrocyte transit time distribution is not a measure of the capillary distribution, as it includes transit time through all jejunal wall vessels but not mesenteric vessels. Endotoxin had no significant effect on the area fraction of interstitial space (Table 2, Fig. 4). Endotoxin did not affect arterial hematocrit (P > 0.05) or capillary hematocrit (P > 0.05; Table 3).

Initial oxygen transport (Table 4) and hemodynamic variables did not differ between the Endotoxin and Control groups (P > 0.05). At the critical D2, the Endotoxin-No Fluid group had lower mean arterial blood pressure (44 ± 14 vs. 59 ± 11 mmHg, Endotoxin-No Fluid vs. Control-No Fluid, P < 0.05), lower systemic vascular resistance (516 ± 375 vs. 1,651 ± 408 dyn·s·cm⁻³, Endotoxin-No Fluid vs. Control-No Fluid, P < 0.05), and higher pulmonary artery occlusion pressure (7.7 ± 2.5 vs. 4.3 ± 1.8 mmHg, Endotoxin-No Fluid vs. Control-No Fluid, P < 0.05). In addition, the Endotoxin-Fluid group had lower arterial oxygen tension (74 ± 20 vs. 109 ± 39 Torr, Endotoxin-Fluid vs. Control-Fluid, P < 0.05; Table 4) and survived for a shorter period of hemorrhage (171 ± 81 vs. 369 ± 105 min, Endotoxin-Fluid vs. Control-Fluid, P < 0.05; Table 6).

Effect of fluid administration. Fluid administration significantly reduced the ER₂crit in the WB (0.62 ± 0.08 vs. 0.82 ± 0.06, Control-Fluid vs. Control-No Fluid group, P < 0.05) and gut (0.67 ± 0.06 vs. 0.77 ± 0.07, Control-Fluid vs. Control-No Fluid group, P < 0.05; Table 1, Fig. 2). There was no significant difference in the already impaired ER₂crit between the Endotoxin-Fluid and the Endotoxin-No Fluid groups for the WB or gut. Fluid administration significantly increased the area fraction of interstitial space in both the Endotoxin and Control groups in all bowel wall layers, except for serosa in Endotoxin groups; however, the effect was significant only for Control groups (Table 2). In particular, the average area fraction of interstitial space almost doubled in the metabolically active mucosa (Fig. 4).

The total amount of fluid administered and total urine output are shown in Table 6. The Control-Fluid group received significantly more fluid because of a significantly longer survival time than did the Endotoxin-Fluid group. Arterial hematocrit decreased with

### Table 3. Arterial and gut capillary hematocrit

<table>
<thead>
<tr>
<th>Group</th>
<th>Arterial Hct at Baseline</th>
<th>Arterial Hct at Onset of Ischemia</th>
<th>Final Arterial Hct</th>
<th>Gut Capillary Hct at Onset of Ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.0 ± 3.2</td>
<td>25.0 ± 3.5</td>
<td>21.6 ± 3.6</td>
<td>27.5 ± 8.6</td>
</tr>
<tr>
<td>Fluid</td>
<td>22.0 ± 3.3</td>
<td>16.1 ± 3.5†</td>
<td>10.4 ± 3.4</td>
<td>29.2 ± 14.3</td>
</tr>
<tr>
<td>Endotoxin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No fluid</td>
<td>24.0 ± 3.5</td>
<td>24.4 ± 4.2</td>
<td>19.6 ± 3.7</td>
<td>25.3 ± 7.7</td>
</tr>
<tr>
<td>Fluid</td>
<td>22.6 ± 2.6</td>
<td>19.8 ± 3.8†</td>
<td>15.2 ± 4.5</td>
<td>25.3 ± 5.3</td>
</tr>
</tbody>
</table>

Values are means ± SD in % Hct, hematocrit. Onset of ischemia refers to those values taken just after the critical oxygen delivery. *Significant difference between No Fluid and Fluid groups (P < 0.05); † significant difference between arterial hematocrit at baseline and at onset of ischemia (P < 0.05).
fluid administration in Control and Endotoxin groups (P < 0.05, Table 3). However, capillary hematocrit at the onset of ischemia was unchanged from baseline by fluid administration in control and endotoxemic animals (P > 0.05, Table 3).

The initial oxygen transport (Table 4) and hemodynamic (Table 5) variables did not differ between the Fluid and No Fluid groups (P > 0.05). The lower arterial PO₂ and higher arterial PCO₂ values at later time points in both saline groups could possibly reflect fluid overload with pulmonary edema. At the critical D0₂, the Control-Fluid group had a higher mean arterial pressure (77 ± 12 vs. 59 ± 11 mmHg, Fluid vs. No Fluid, P < 0.05), higher cardiac output (5.7 ± 2.7 vs. 2.8 ± 0.7 l/min, Fluid vs. No Fluid, P < 0.05), lower systemic vascular resistance (1,183 ± 604 vs. 1,651 ± 408 dyn·s·cm⁻², Fluid vs. No Fluid, P < 0.05), higher pulmonary artery occlusion pressure (6.9 ± 1.1 vs. 4.3 ± 1.8 mmHg, Fluid vs. No Fluid), and a lower hemoglobin (55 ± 11 vs. 84 ± 13 g/l, Fluid vs. No Fluid, P < 0.05). The Control-Fluid group had a greater time of survival after onset of hemorrhage (369 ± 105 min) compared with the resuscitated No Fluid group (202 ± 60 min, P < 0.05; Table 6).

**DISCUSSION**

The major finding from our study is that fluid administration significantly impairs WB and gut oxygen extraction in control pigs, but the small effect of fluid administration in the endotoxin animals, which already had decreased oxygen extraction, was not statistically significant. Impaired oxygen extraction from fluid administration is associated with increased area fraction of interstitial space and increased heterogeneity of transit times. However, impaired oxygen extraction from endotoxin was not associated with a change in area fraction of interstitial space or a change in heterogeneity of transit times.

Sepsis has substantial effects on the microcirculation that may account for impaired tissue oxygen extraction (20, 34). The host defense response against invading
microorganisms can cause the development of the systemic inflammatory response syndrome (5). Endotoxin and other bacterial products trigger macrophages and other inflammatory cells to release tumor necrosis factor-$\alpha$, interleukin-1, interleukin-6, and many other proinflammatory mediators (4, 5). These endogenous mediators of the septic inflammatory response have important effects on the microvasculature, including leukocyte slowing and microthrombi (3, 10, 12), that may lead to decreased capillary flows, increased capillary permeability, i.e., “leaky capillaries” (10, 33) with the resultant interstitial edema (9), and an alteration in vascular tone (13, 20, 28).

Altered microcirculatory regulation of blood flow may lead to mismatching of oxygen supply to demand (20, 21), resulting in impaired tissue oxygenation (19, 37). Morff (22) has suggested that increased capillary recruitment improves tissue oxygenation in rat cremaster muscle. A number of investigators have suggested that, to improve microvascular flow, reduced hematocrits may be useful (7, 36). Hemodilution decreases blood viscosity, may improve capillary red blood cell flux (36), and may improve blood flow distribution within the capillary bed (34, 36). Van der Linden et al. (36) found that hemodilution to a hematocrit of 20 or 30% with the use of colloid infusion, resulting in an increased $\text{ER}_{\text{O}_{2}}$ during progressive hemorrhage, compared with a hematocrit of 40%. Tyml (34) has demonstrated that the heterogeneity of microvascular flow in rat skeletal muscle is reduced by hemodilution. Therefore, our hypothesis was that one potential beneficial effect of fluid administration in septic shock could improve oxygen extraction via the mechanism of decreased heterogeneity of blood flow associated with hemodilution.

However, contrary to our hypothesis, we found that fluid administration resulted in impaired oxygen extraction in Control but did not lower an already impaired oxygen extraction in the Endotoxin groups. We also found that fluid administration was associated with unchanged capillary hematocrit and increased heterogeneity of transit times. Finally, fluid resuscitation resulted in increased interstitial space. Therefore, a possible mechanism for impaired oxygen extraction associated with fluid resuscitation is increased heterogeneity of transit times, possibly resulting from decreased capillary recruitment secondary to altered morphology of the capillary bed from interstitial edema. Our results must be interpreted relative to findings in other studies because our model is not a model of isovolemic hemodilution as is the case in some other studies; rather, our model more resembles the clinical scenario of aggressive fluid resuscitation of septic shock, which results in peripheral edema.

Our findings of increased area fraction of interstitial space in fluid-resuscitated animals is consistent with the clinical observation of tissue edema in septic patients who have had crystalloid fluid administration. Furthermore, because sepsis is characterized by a generalized increase in capillary permeability (10, 33), Starling’s law may provide an explanation for the development of interstitial edema (9). Our finding of decreased oxygen extraction associated with fluid administration could be due to an increased oxygen diffusion distance associated with interstitial edema (16). However, whether oxygen uptake is affected by interstitial edema is unsupported in studies by others (25).

A key, new observation in the present study is that fluid administration significantly increases the relative dispersion of blood flow transit times throughout the gut wall. This may indicate increased relative dispersion of blood flow transit times in the capillary bed as well as within the larger arterioles and venules within the gut wall. Conceivably, the resultant decrease in capillary diameter due to endothelial edema (15) may impair microvascular blood flow or contribute to leukocyte retention and plugging within the capillary bed (1). In addition, red blood cell rheology may be altered in the septic state (29) and conceivably could be altered by fluid administration, resulting in more heterogeneous microvascular flow. As a result, impaired oxygen extraction may be accounted for by mismatching of oxygen supply to demand. Thus the potential detrimental effects of fluid administration appear to contribute to overall impaired oxygen extraction in the present set of experiments. A potential limitation of the present study, however, is that, although we investigated the overall blood flow transit times in the gut segments, we did not divide the gut segments into small enough sections to determine the degree of heterogeneity between mucosa and muscularis. Investigators such as Connolly et al. (6) have shown that there is a significant spatial heterogeneity of blood flow and capillary transit times in both mucosa and muscularis, with relative dispersions (SD/mean) ranging from 23 to 97%.

In our previous study (20), impaired oxygen extraction in endotoxin pigs was associated with increased heterogeneity of transit times. In that study, endotoxin pigs also received fluid administration, whereas controls did not. Similarly, in this study, impaired oxygen extraction associated with increased heterogeneity of transit times was observed in endotoxic pigs that received fluid administration. Impaired oxygen extraction in endotoxic pigs without fluid administration was not associated with increased heterogeneity of transit times.
times. Therefore, increased heterogeneity of transit times is associated with fluid administration but is not the sole mechanism of impaired oxygen extraction in endotoxia. Similarly, interstitial edema was increased in association with fluid administration rather than with endotoxia. Therefore, whereas interstitial edema with increased heterogeneity of transit times could result in impaired oxygen extraction from fluid administration, this is not sufficient to account for impaired oxygen extraction from endotoxia.

The data in Table 1 show that the decrease in gut ER$_{O2crit}$ from endotoxin was associated with a nonsignificant decrease in critical VO$_2$, whereas critical DO$_2$ was not changed. However, there was no difference in temperature between Control and Endotoxin groups at the critical point (data not shown), which could explain a decrease in baseline gut VO$_2$. Therefore, if a decrease in gut ER$_{O2crit}$ from endotoxin is due to a decrease in VO$_2$, the mechanism is not related to temperature, to heterogeneity of erythrocyte transit times in the whole gut wall, or to interstitial edema. The mechanism for the decrease in gut ER$_{O2crit}$ from endotoxin could possibly be due to heterogeneity of erythrocyte transit times in mucosa or due to mitochondrial effects rather than changes in vascular regulation. Finally, because a decrease in WB ER$_{O2crit}$ is associated with an increase in critical DO$_2$, but not a decrease in baseline VO$_2$, which is unlike the gut, vascular regulation and redistribution of blood flow may play a larger role in ER$_2$ for the WB and other organs than for the gut.

Capillary transit times are more closely related to oxygen extraction than are transit times in the whole gut wall. We did not measure capillary transit times in this study, but, in a study by Humer et al. (20), capillary transit times accounted for about one-half of whole gut wall transit times. In that study, altered heterogeneity of capillary transit times associated with endotoxia and fluid administration was paralleled by altered heterogeneity in whole gut wall transit times. Although it is possible that increased heterogeneity of capillary transit times with unchanged heterogeneity of whole gut wall transit times could occur in endotoxic pigs that did not receive fluid administration, we do not have a basis for suggesting that this possibility could be a mechanism of impaired oxygen extraction in endotoxic, nonfluid-resuscitated animals.

Interestingly, we noted that, although arterial hematocrit decreased as anticipated with saline administration, microvascular hematocrit determined with the use of morphometric measurements was not altered by saline administration. Thus the putative beneficial effect of crystalloid administration on blood viscosity may not extend to the microvasculature. Furthermore, hemodilution does not explain the decreased oxygen extraction seen with fluid administration.

In summary, large-volume crystalloid administration significantly decreases WB oxygen extraction in control pigs but did not significantly change the already decreased oxygen extraction in the endotoxin-treated animals. Although fluid resuscitation is still considered to be an important aspect of therapy of hypovolemia, we have shown that marked saline administration may lead to interstitial edema and increased heterogeneity of erythrocyte transit times. Therefore, we raise the concern that, in humans, excessive crystalloid administration has the potential to impair tissue oxygen extraction.

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