Gut Mucosal Damage during Endotoxic Shock is due to Mechanisms other than Gut Ischemia

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ABSTRACT

Whether the gut alterations seen during sepsis are caused by microcirculatory hypoxia or disturbances in cellular metabolic pathways associated with mitochondrial respiration remains controversial. We hypothesized that hypoperfusion or hypoxia and local production of nitric oxide might play an important role in the development of gut mucosal injury during endotoxic shock, and investigated their roles using differing levels of fluid resuscitation and occlusion of the superior mesenteric artery (SMA). Anesthetized New-Zealand rabbits were allocated to: Group I (Sham, n=8); Group II, low dose endotoxin (LPS, E. coli-055:B5, 150µg/kg)/fluid resuscitation (12 ml/kg/h) (n=8); Group III, high dose LPS (1mg/kg)/fluid resuscitation (12 ml/kg/h) (n=8); Group IV, high dose LPS (1 mg/kg)/hypovolemia (4 ml/kg/h fluids) (n=8); and Group V, SMA ligation/fluid resuscitation (12 ml/kg/h) (n=4). Luminal gut lactate concentrations and PCO2-gap increased in groups IV and V (p<0.05), reflecting alterations in gut perfusion. Interestingly, significant histological alterations were observed in all LPS groups but not in Group V. Blood and luminal gut nitrate/nitrite concentrations increased only in group IV. The mechanism of gut injury in endotoxic shock seems unrelated to hypoxia and release of nitric oxide. Gut dysfunction may occur as a result of so-called "cytopathic hypoxia".
INTRODUCTION

The gut injury seen during sepsis has been associated with the development of organ dysfunction and multiple organ failure (MOF) (10, 27). However, the pathophysiological mechanisms underlying sepsis-induced gut injury are unclear, and whether it is caused by microcirculatory hypoxia or disturbances in cellular metabolic pathways associated with mitochondrial respiration remains controversial. Indeed, blood flow redistribution may result in regional ischemia in the gastrointestinal tract during sepsis, despite a globally increased systemic oxygen delivery (DO₂). Gut alterations, including ileal mucosal acidosis (1, 12, 37, 45), increase in mucosal permeability (13, 37) and gut mucosal injury (6, 20, 30, 49), have often been reported after administration of lipopolysaccharide (LPS) or live bacteria and in experimental peritonitis. Vallet et al. (44) reported a decrease in ileal mucosal PO₂ in endotoxic dogs and Hasibeder et al. (18) noted a decrease in jejunal mucosal PO₂ in endotoxic pigs. Others have made similar observations in various animal models (1, 48). Recently, Theisen et al. (41) reported alterations in microvascular blood flow to the gut mucosa in a porcine model of hyperdynamic endotoxic shock, despite mesenteric blood flow being maintained.

However, other studies have suggested normal or elevated blood flow or tissue PO₂ in endotoxemia or experimental sepsis. VanderMeer et al. (45) reported ileal mucosal acidosis despite maintained mucosal perfusion and oxygenation in endotoxic pigs in association with normal or even higher mucosal PO₂ levels as measured with multiwired PO₂ electrodes. Using colored microspheres, Revelly et al. (32) showed an increased mucosal flow to the gut in endotoxic pigs and Crouser et al. (6) demonstrated that mucosal gut flow was maintained despite mucosal acidosis and alterations in gut oxygen metabolism in a feline ileal preparation. Following cecal ligation and puncture in rats, there was a 42% increase in
microvascular flow to the midjejunum in early sepsis, shown using laser-Doppler flow measurements and colloidal carbon infusion (46).

In addition to the possible effects of hypoperfusion on endotoxin-induced gut injury, LPS may induce alterations in gastrointestinal oxygen metabolism. Changes in ileal oxygen metabolism have been correlated with the severity of mitochondrial injury in feline ileum (7). Cellular alterations referred to as ‘cytopathic hypoxia’ may be implicated in MOF in which case production of adenosine triphosphate (ATP) may be decreased despite normal PO2 values in the vicinity of mitochondria within cells (11). A number of different biochemical mechanisms or mediators have been suggested to account for cellular dysfunction and cytopathic hypoxia in sepsis including inactivation of pyruvate dehydrogenase, reversible inhibition of cytochrome oxidase by nitric oxide (NO), inhibition of mitochondrial respiratory complexes by peroxynitrite, and activation of the nuclear enzyme, poly-(ADP-ribosyl)-polymerase (PARP) (3, 13, 28, 29, 43, 51).

We developed a rabbit model to investigate the role of these factors - blood flow alterations, tissue hypoxia, and NO release - in the development of gut mucosal injury during endotoxic shock.

MATERIALS AND METHODS

Animal model. Thirty-six specific pathogen free New-Zealand rabbits (2.7 to 3.4 Kg body weight) were handled according to the rules of the local Animal Care Committee after institutional approval for animal investigations was obtained. Induction doses of ketamine (20 mg/kg) and xylazine (4 mg/kg) were given intramuscularly for sedation and anesthesia and followed by a continuous IV infusion of ketamine (15-35 mg/kg/h) started 3 hours after induction. An intravenous catheter was inserted into an ear vein for venous access (Surflo I.V. catheter, 18 G x 2”) and 20 ml of saline solution was given as a bolus. Cefazoline (50 mg/kg)
and chloramphenicol (12.5 mg/kg) were administered intravenously. Tracheotomy was performed and the animals were ventilated (Servo ventilator, Siemens, Solnd, Sweden) with 40-60 % of FiO$_2$, tidal volume of 7 to 10 ml/kg and a respiratory rate of 40/min further adjusted to maintain a PaO$_2$ > 80 mmHg and a PaCO$_2$ between 35-45 mmHg.

**Surgical procedure.** A 16-G polyethylene catheter was inserted into the right carotid artery and connected to a pressure transducer to enable continuous recording of arterial pressure. Another catheter (Surflo I.V. catheter, 22 G x 2”) was placed in the jugular vein for recording of right atrial pressure (RAP) and venous access. A midline laparotomy was performed and ultrasonic flow probes (Transonic System Inc., Ithaca, NY) were placed around the superior mesenteric artery (QSMA) and the abdominal aorta (Q$_a$orta) just above the origin of the celiac trunk to continuously measure flow. A bowel segment was delineated in the mid ileum. An antimesenteric enterotomy was performed and inflow (Foley catheter 8) and outflow (Foley catheter 10) catheters were placed to delimit a 5 cm segment. After positioning the catheters in the ileum, the balloons were gently inflated with water. Once inflated, they permitted the perfusion of a closed area of the gut lumen with gut luminal perfusate (GLP) solution. A larger catheter size was used for the outflow to facilitate perfusate withdrawal. In a second gut segment, a tonometric catheter (TRIP® Tonometry Catheter, Datex, Finland) was placed through a minimal antimesenteric wall incision and secured with a purse-string suture. Body temperature was maintained with a heating lamp. The animals were allowed to recover for 60 min before starting the experimental protocol. Hemodynamic measurements and blood samples were obtained at baseline and every hour up to 4 hours after LPS administration.

**Gut luminal perfusion.** The bowel segment was carefully rinsed with heated (37° C) physiologic saline, before a perfusate solution (RPMI-1640 medium; SIGMA, St Louis, MI)
was infused at a rate of 6ml/h. In addition, 10,000 KIU/ml of aprotinin (Trasylol®, Bayer, Germany) was added per 10 ml of perfusate solution to inhibit proteolytic activity. The lumen of the proximal catheter was used for continuous infusion and the lumen of the distal catheter was used to recover the perfusate every hour. GLP samples were taken hourly, centrifuged at 3000 rpm/min, filtered through a 0.22 µ filter (Millex®-OR, Bedford, USA) to eliminate bacteria, and stored at -80°C.

**Experimental protocol.** The animals were randomized to four groups and a fifth group (group V) was added after preliminary analysis of the results of the original four groups (Table 1): Group I (Sham), received placebo and 12 ml/kg/h fluids (Ringer’s lactate); Group II (low dose LPS/fluid resuscitation): intravenous LPS (E. coli-055:B5, DIFCO laboratories, Detroit, USA) 150 µg/kg and 12 ml/kg/h fluids; Group III (high dose LPS/fluid resuscitation): LPS 1mg/kg + 12 ml/kg/h fluids; Group IV (high dose LPS/hypovolemia): LPS 1 mg/kg + 4 ml/kg/h fluids; and Group V (gut ischemia/fluid resuscitation): ligation of SMA and 12 ml/kg/h fluids. All animals received intravenous lactated Ringer’s solution as fluid resuscitation throughout the experimental protocol and a 20 ml IV bolus of hydroxyethyl starch (HES 6% - MW 200.000 - D/0.5, Haes-Steril, Fresenius) at the end of the surgical instrumentation. A continuous intravenous infusion of HES was started after the bolus, in all animals except Group IV, to provide a total volume of 20 ml/kg over 5 hours. Immediately after baseline measurements, LPS was diluted in normal saline 1mg/ml and administered intravenously over 3 minutes to Groups II-IV. Group V had the peritoneum reopened for ligation of the SMA at this time point.

**Analytical methods.** Blood gas analyses were performed on arterial samples (ABL-30, Radiometer, Copenhagen, Denmark). Samples for lactate analysis were stored on ice and
analyzed (ABL-30, Radiometer) within 10 to 30 min. Blood samples were centrifuged immediately (3000 x g for 20 minutes) and plasma stored at -80° C.

**Nitric oxide.** Stable end products of NO metabolism, nitrate (NO$_3^-$) and nitrite (NO$_2^-$) concentrations were determined spectrophotometrically from serum and GLP using the Griess reaction. Briefly, for nitrite measurements the absorbance was measured at 540 nm in a microplate enzyme-linked immunosorbent assay (ELISA) reader (Titertek multiscan MCC/340, MKII, Eflab, Finland). Nitrate level was determined after stoichiometrical reduction to nitrite.

**Tonometry.** For tonometry measurements, 1 ml of 0.9% saline was placed in the silicone balloon of the tonometer and allowed to equilibrate for 30 min. The first 0.7 ml aspirated was discharged and analysis was performed in the remaining 0.3 ml immediately in a blood gas analyzer (ABL 500 radiometer, Copenhagen, Denmark). Ileum PCO$_2$ was corrected for incomplete equilibration time during the 30-min sampling periods multiplying PCO$_2$ by 1.24 (5). Ileal mucosal-arterial PCO$_2$ gradient (PCO$_2$-gap) was calculated as the difference between ileal and arterial PCO$_2$.

**Grading of mucosal damage.** At the end of the experiment each animal received a lethal injection of sodium pentobarbital and the ileum was immediately removed. A segment was taken for optical microscopy, fixed in 10% formaldehyde-saline followed by sectioning and staining with hematoxilin and eosin. Mucosal histology was graded as previously described (4) using the following scale: grade 0, normal mucosa; grade 1, sub epithelial space formation; grade 2, extension of the sub epithelial space with moderate lifting of the epithelial layer from the lamina propria; grade 3, massive epithelial lifting down the sides of the villi; grade 4, denuded villi with lamina propria and dilated capillaries exposed; and grade 5, digestion and disintegration of the lamina propria, hemorrhage and ulceration.
Data analysis. Results are presented as mean ± SD. Significance was tested by analysis of variance for repeated measurements (ANOVA). Bonferroni adjustment was used for multiple comparisons. Linear regression was used to test the relation between gut lactate release and PCO2-gap and QSMA. The grade of histologic damage was evaluated using a Mann-Whitney rank sum test. A p value < .05 was considered statistically significant.

RESULTS

All groups received a similar amount of fluids, except the hypovolemic group (Group IV) (Table 1). Body temperatures were maintained between means of 37.8 and 38.5°C.

Hemodynamics. After LPS administration, mean arterial pressure (MAP) decreased significantly in Group II (low LPS, high fluid; from 76 ± 11 to 52 ± 12 mmHg at 3 h; p<0.05), Group III (high LPS, high fluid; from 69 ± 6 to 52 ± 7 mmHg at 3 h p<0.05) and, especially, in Group IV (from 68 ± 8 to 38 ± 8 mmHg at 3 h; p<0.05) (Figure 1). At 1, 2 and 3 h, group III had significantly lower MAP than group I (56 ± 9 mmHg vs 72 ± 7 mmHg at 1 h; 51 ± 9 mmHg vs 80 ± 14 mmHg at 2 h; and 52 ± 7 mmHg vs 77 ± 18 mmHg at 3 h; p<0.05 for all). QAO was significantly lower at 1 h in Group III (49 ±9 ml/min) and Group IV (45 ± 10 ml/min) than in Group I (82 ± 37 ml/min) (p<0.05 for all). After this transient decline, QAO returned to baseline in all groups (p=NS). QSMA increased significantly above baseline in Group III (from 52 ± 9 to 84 ± 12 ml/min at 2 h, 88 ± 17 ml/min at 3 h and 81 ± 17 ml/min at 4 h; p<0.05 for all) (Figure 1).

Tonometry-derived measurements. PCO2-gap increased significantly in groups IV (from 1.7 ± 6.6 to 17.2 ± 11.0 torr at 3 h and to 19 ± 9 torr at 4 h) and V (from 6.2 ± 4.0 to 62 ± 38 torr at 1 h, 64 ± 35 torr at 2 h and 69 ± 22 torr at 4 h; p<0.05 for all) (Figure 1). PCO2-gap was significantly higher in group V compared to all other groups at 1, 2, 3 and 4 h (p<0.05 for all).
**Lactate measurements.** An increase in arterial lactate concentrations occurred in groups III (from $2.2 \pm 0.6$ to $4.3 \pm 2.0$ mEq/l at 4 h, p<0.05) and IV (from $2.0 \pm 0.7$ to $6.7 \pm 1.3$ mEq/l at 4 h, p<0.05) (Figure 2). Lactate concentrations were considerably higher in group IV than in the other groups at all time points (Figure 2). Luminal gut lactate concentrations increased significantly in group IV (from $0.2 \pm 0.1$ to $1.1 \pm 1.3$ mEq/l at 4 h, p<0.05) and, especially, in group V (from $0.3 \pm 0.3$ to $5.6 \pm 0.7$ mEq/l at 4 h; p<0.05) (Figure 2). In group V, but not in groups II, III and IV, there was a significant relation between gut lactate release and Q_{SMA} (r=0.47, p=0.0008) and between gut lactate and PaCO2-gap (r=0.62, p=0.0001) (Figure 3).

**Serum and gut concentrations of NO^2-/NO^3- (NOx).** After LPS administration, NOx concentrations significantly increased in serum and decreased in the GLP in group IV (from $149 \pm 32$ to $223 \pm 55$ µmol, and from $675 \pm 161$ to $422 \pm 224$ µmol, respectively, both p<0.05) (Figure 4). These levels were unaltered in all other groups.

**Histological damage.** All ileum specimens obtained in control animals were normal (degree 0). Mild to severe alterations were observed in all endotoxin groups. No histological alterations were detected after prolonged occlusion of the superior mesenteric artery (group V) (Figure 5).

**DISCUSSION**

Cellular dysfunction and injury secondary to different types of shock are explained usually by a diminished cellular oxygen availability resulting from decreased arterial oxygen tension (hypoxic hypoxia), hemoglobin concentration (anemic hypoxia), or blood flow (stagnant hypoxia). In sepsis however, a number of studies have suggested that tissue injury can occur despite adequate tissue oxygenation (11), and our findings show convincingly that endotoxin can induce histological lesions by mechanisms other than tissue hypoxia. Our observations are consistent with the development of so-called "cytopathic hypoxia", i.e., altered oxygen
utilization despite adequate oxygen availability in the vicinity of the cell mitochondria. Hypoxia is unlikely to have played a major contribution to the histological lesions observed in our study for several reasons. First, hypoxemia and anemia were absent, and mesenteric blood flow was even increased after generous fluid resuscitation. Even though LPS administration induced an early hypotensive state, mesenteric oxygen delivery was well preserved. Second, mucosal PCO₂ did not increase, and this can reasonably exclude a reduction in regional blood flow (5). Finally, the absence of luminal lactate release in fluid resuscitated animals suggests the absence of gut ischemia. It was only when hypovolemia was purposely maintained (Group IV) or when ischemia was induced by ligation of SMA (Group V) that increases in PCO₂-gap and luminal gut release of lactate occurred.

Our observations are in accord with results from other studies. Fink et al. (13) showed that gut permeability alterations occurred after endotoxin administration but not after a similar mechanically-induced reduction in mesenteric flow, suggesting that hypoperfusion by itself is not responsible for increased permeability. In a feline ileum preparation, epithelial necrosis occurred early after LPS administration, despite unaltered ileal tissue oxygen content, blood volume, and blood flow (8). Revelly et al. (33) reported that marked decreases in mucosal ATP content related to permeability and translocation alterations induced by LPS were not improved by massive resuscitation. In an isolated pig hindlimb preparation, the LPS-induced decrease in the skeletal muscle cytochrome aa₃ (Caa₃) redox status did not depend on oxygen delivery (14). Finally, in vitro studies indicate that cell dysfunction occurs within minutes of exposure to endotoxin and that a major functional derangement involves the inhibition of pyruvate dehydrogenase activity, pyruvate and lactate accumulation in the cell, and deranged oxidative phosphorylation (11).

Our observations indicate that these changes can occur as early as 4 hours after LPS administration. Other studies have also reported that LPS or bacteria can rapidly induce tissue
injury. Hersch et al. (20) reported that tissue injury, including the gut, antedated multiple organ dysfunction in a normotensive sheep model with cecal ligation and perforation. Yu and Martin (49), using the same scoring system for mucosal damage that we used (4), demonstrated gut mucosal injury in normotensive septic rats 24 hours after tracheal instillation of *Pseudomonas aeruginosa*. Apoptosis is likely to be involved in histological lesions in association with necrosis as a result of cytotoxic mediators. Crouser et al. (8) reported that ileal necrosis and apoptosis could occur as early as 2 and 4 h, respectively, after LPS administration. Hotchkiss et al. (21) found extensive focal crypt epithelial and lymphocyte apoptosis in intestinal tissues obtained intraoperatively from patients with acute traumatic injuries.

A key finding in our study was the absence of histological lesions after prolonged mechanical occlusion of the SMA. In this group, gut hypoxia must have been present, and was reflected by the marked rise in PCO2-gap and in gut lactate production, known to be secondary to gut ischemia (40). The well-maintained integrity of the gut mucosal morphology in this group after prolonged ligation of the SMA may be explained by several factors. Initially, the collateral vessels from the caudal mesenteric and celiac axis were not ligated, so that some flow could be maintained. Indeed, we confirmed this finding by infusing 5 ml of methylene blue (MB) into the right atrium 2 and 3 h after SMA ligation in another rabbit; there was a small amount of MB distributed in a heterogeneous manner in the jejunum and ileum showing that the collateral vessels maintained some flow. The vascular bed can also compensate for the flow reduction by increasing the oxygen extraction rate. Indeed, blood flow can be decreased to less than 50% without significantly altering gut VO2 (25). In this range, ileal VO2 could be maintained independent of blood flow. Finally, there was no reperfusion possible, and much histological damage actually occurs during reperfusion. In
addition, other cell protective mechanisms, such as induction of heat shock proteins, may have taken place (23).

The involvement of cytopathic hypoxia in the pathogenesis of MOF may account for the poor results of studies on optimization of oxygen delivery in patients with septic shock (16, 19), except for when the intervention takes place very early to correct other factors such as hypovolemia and cardiac dysfunction (34). This is in contrast with high risk surgical patients in whom these therapeutic goals are more likely to improve survival (26, 38, 47). Indeed, all efforts to increase cellular oxygen availability may be in vain in the presence of derangements in mitochondrial oxygen metabolism (31).

Even though luminal gut lactate release was not observed, serum lactate concentrations significantly increased following LPS administration, suggesting that blood lactate comes primarily from other sources. Bellomo et al. (2) reported that lactate is even taken up by the gut in early endotoxemia in dogs. Other important sources of lactate in sepsis may be the lung (2, 9) or leukocytes (17). Lactate production in sepsis may arise from sites of inflammation rather than areas of poor perfusion and is related to augmented glycolytic metabolism by inflammatory cells and mitochondrial dysfunction. As in other studies in endotoxic shock, we showed that gut ischemia was present only when fluid replacement was insufficient (13, 50). Adequate fluid administration was essential to maintain splanchnic perfusion but was unable to prevent endotoxin-induced mucosal injury. In the group with fluid restriction there was more severe hyperlactatemia compared with the other groups indicating that in this circumstance, due to hypovolemia, the liver clears lactate less efficiently. In contrast despite severe mesenteric hypoperfusion, animals in group V had significantly lower lactate concentrations due to better resuscitation.

The pathophysiologic mechanisms underlying mucosal gut injury during septic shock remain poorly understood. LPS either by itself or via the release of various cytokines, is able
to alter cellular function by a NO-dependent pathway (3, 28, 29, 42). Studies with well-controlled conditions of oxygenation and perfusion such as *in vitro* enterocyte monolayers or Ussing chamber models have shown that NO mimics the action of LPS on permeability to both probe and bacteria (15, 28, 36). Salzman et al. (36) have documented that NO donors dilate tight junctions, cause cytoskeletal disruption, and deplete cellular ATP. Changes in the dynamics of NO deplete intracellular levels of ATP, cause marked derangements in the structure of the actin based cytoskeleton, and increase intracellular ionized calcium concentration (28). In the present study, plasma or gut luminal nitrite and nitrate concentrations did not correlate with LPS-induced ileal mucosal injury and there was a significant decrease in ileal luminal Nox concentrations compared to baseline during hypovolemia. Crouser et al. (8) also reported that iNOS induction was not involved in the early ileal epithelial necrosis induced by LPS in a feline model. In pigs with septic shock induced by live bacteria Snygg et al. (39) noted that jejunal mucosal NO production decreased markedly during severe hypovolemia, whereas stable production was observed following *E. coli* sepsis. The mechanisms behind the inhibition of ileal mucosal NOx production are not known. Possible mechanisms include the presence of diminished flow and tissue dysoxia secondary to hypovolemia leading to a state of low availability of the substrate L-arginine and/or oxygen, or direct inhibition of cellular enzymes in the intestinal mucosa (39). Thus a very complex relationship between NO and hypoxia or flow may exist, and it is possible that constitutively formed NO plays a role in these changes.

Much of the oxidative injury associated with NO production is mediated by peroxynitrite, a toxic oxidant derived from the reaction of NO and superoxide (29). NO-induced increases in intestinal monolayer permeability can be prevented by peroxynitrite scavengers (28). Agents that interfere with the formation of peroxynitrite, such as superoxide dismutase mimetics and peroxynitrite decomposition catalysts, can also reduce endotoxin-
induced cellular injury. In rats, Salvemini et al. (35) found that superoxide dismutase mimetics were able to reduce the LPS-induced increase in microvascular leakage, lipid peroxidation and epithelial cell injury seen in duodenum and jejunum suggesting that superoxide and peroxynitrite play a significant role in the pathogenesis of gut injury during endotoxemia. In sepsis or shock, production of peroxynitrite is increased and it acts as a potent cytotoxic agent producing breaks in cellular deoxyribonucleic acid (DNA). DNA strand-brakes activate the repair enzyme PARS able to induce cell energy depletion and increase gut mucosal permeability. There is now increasing evidence that exaggerated PARS activation contributes to cellular injury in sepsis. Jagtap et al. (22) reported that a phenanthridinone inhibitor of PARS was able to ameliorate LPS-induced gut injury in rats. There are few data on other mechanisms involved in LPS-induced gut injury. The naturally occurring protein lactoferrin, by its binding activity to the lipid A portion of LPS, prevented villus atrophy, edema and vacuolation induced by LPS in the gut of mice; in this model lactoferrin attenuated the lethal effects of LPS (24).

We acknowledge that the technique used to isolate a closed segment of the gut has some limitations. First, the inflation of the balloons inside the bowel may induce local ischemia and pressure damage. However, histological analysis of normal bowel segment and isolated bowel segment did not show major differences. Second, the gut mucosa could have been affected by the continuous washout by the gut perfusate. We do not believe that luminal perfusion affected the gut mucosa as no histological lesions were detected in control animals, and since the preparation was able to detect an increase in lactate release and in mucosal CO₂ in the presence of a decreased mesenteric blood flow.

In summary, this study indicates that LPS can induce early ileal mucosal injury, by mechanisms other than tissue hypoxia and NO release. Fluid resuscitation is fundamental to maintain intestinal blood flow but does not prevent mucosal damage.
References


Table 1. Characteristics of the rabbits.

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>Endotoxin dose (µg/kg) - IV</th>
<th>Crystalloids (ml)</th>
<th>Colloids (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>-</td>
<td>265 (234 - 296)</td>
<td>68 (60 - 76)</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>150</td>
<td>261 (234 - 289)</td>
<td>67 (60 – 74)</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>1000</td>
<td>265 (250 - 273)</td>
<td>67 (64 - 70)</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>1000</td>
<td>88 (83 - 88) *</td>
<td>20 (19 - 20) *</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>-</td>
<td>250 (242 - 273)</td>
<td>64 (62 - 70)</td>
</tr>
</tbody>
</table>

No: number of rabbits. IV: intravenous. Values are median (minimum - maximum).

*: p<0.05 vs all groups.
**Figure legends**

Figure 1. Mean arterial pressure, aortic blood flow, mesenteric blood flow and PCO2-gap at baseline (time 0) and hourly. Values are mean/SD. *: p<0.05 vs baseline. (groups: I: ■; II: △, III: ▼; IV: ●; V: ○).

Figure 2. Serum lactate and luminal gut lactate (GLP) measurements at baseline (time 0) and hourly. Values are mean/SD. *: p<0.05 vs baseline. (groups: I: ■; II: △, III: ▼; IV: ●; V: ○).

Figure 3. Comparison between Q_{SMA} and PaCO2-gap with luminal gut lactate release. (groups: I: ■; II: △, III: ▼; IV: ●; V: ○).

Figure 4. Levels of serum and gut luminal perfusate NO³⁻ / NO²⁻ (Nox) at baseline and 4 hours after LPS. Values are mean/SD. *: p<0.05 vs baseline. (groups: I: ■; II: △, III: ▼; IV: ●; V: ○).

Figure 5. Column scatters represent grades of histological injury in groups. Within group comparisons, a: p < 0.05 vs I, b: p < 0.05 vs II.
Serum lactate (mEq/l)

Gut lactate (mEq/l)