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ORIGINAL

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Different effects of early endotoxaemia on hepatic and small intestinal oxygenation in pigs

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Abstract *Objective:* Study on simultaneous O_2 supply/uptake relationships in liver and gut during endotoxaemia, to determine whether signs of dysoxia develop uniformly in the splanchnic region.
Design: Animal study to assess the early effects of endotoxaemia on oxygenation of both liver and small intestine.
Interventions: Eight anaesthetized pigs received a continuous portal venous infusion of lipopolysaccharide ($0.5\ \mu g \cdot kg^{-1} \cdot h^{-1}$) for 6 h. Systemic, pulmonary and splanchnic haemodynamics as well as systemic and splanchnic O_2 supply/uptake relationships were determined.
Results: There was a multiphasic haemodynamic response pattern characterized by an early (within the 1st h) and a subsequent more prolonged phase (between the 2nd and 6th h) of decreases and recovery of hepatic arterial, portal venous and superior mesenteric arterial blood flows (electromagnetic flow probes) and splanchnic O_2 deliveries. Unrelated to perfusion pressure and O_2 delivery, there were early and sustained decreases in ileal mucosal surface partial pressure of oxygen (PO_2) (multiwire PO_2 electrode) and pH (tonometry). This was not reflected by ileal serosal surface PO_2, O_2 uptake and arteriomesenteric venous pH and partial pressure of carbon dioxide (PCO_2) gradients. There was little evidence of concomitant hepatic dysoxia as evaluated by surface PO_2.
Conclusions: The study demonstrates early and sustained regional (mucosa) intestinal hypoxia with little evidence of simultaneous hepatic dysoxia during initial endotoxaemia.

Key words Lipopolysaccharide · Liver · Small intestinal serosa and mucosa · Tissue oxygen tension · Acid–base balance

Introduction

Despite improved diagnostic monitoring and new therapeutic modalities, sepsis remains a serious cause of mortality in most intensive care units [1]. Many reports confirm the role of endotoxin in the clinical manifestation of septic shock [2]. It is generally accepted that endotoxaemia is associated with an imbalance between organ oxygen (O_2) delivery and O_2 demands [3–5]. The observed abnormal dependency of O_2 uptake on O_2 delivery may be due to an alteration in regional blood flow, and therefore limited O_2 delivery, or to an increase in O_2 demand. The risk of such an imbalance occurring during endotoxaemia seems to be especially high in the splanchnic organs [3–5]. Endotoxin-induced early splanchnic dysoxia may be an important factor in the development of subsequent multiple organ failure. Therefore, early detection of splanchnic organ dysoxia is essential for early therapeutic interventions to prevent persistent splanchnic ischaemia.

Early detection of critical O_2 deprivation in so-called “silent” organs (i.e. organs such as the small intestine and the liver that may become ischaemic without showing immediate clinical signs) is particularly problematic. Various parameters for recognizing O_2 deprivation of silent organs have been suggested, such as the relationship between O_2 supply and uptake [6,7], tissue partial pressure of oxygen (PO_2), arteriovenous pH and partial pressure of carbon dioxide(PCO_2) gradients [6], blood lactate concentrations [6,7], tonometrically determined intestinal intramucosal pH or PCO_2 [5,8] or the hepatic venous β-hydroxybutyrate/acetoacetate concentration ratio [7]. Studies looking at the early effects of endotoxaemia have focused on either the liver [9] or the gut [3,5,10–12]. It is, however, important to assess simultaneously O_2 supply/uptake relationships in liver and gut to determine whether signs of dysoxia develop uniformly in the splanchnic region or whether there are quantitatively or even qualitatively different responses of liver and gut to early endotoxaemia. If present, such differences may have therapeutic implications.

Accordingly, this study was performed to assess simultaneously the early effects of endotoxaemia on oxygenation of both liver and small intestine. Since information on the global intestinal O_2 supply/uptake relationship does not necessarily reflect the state of mucosal oxygenation [3,10,13], we evaluated oxygenation of both intestinal mucosa and intestinal serosa.

Materials and methods

Animal preparation

Following approval by our local Ethics Committee on Animal Research, these studies were performed in eight 3-month-old healthy domestic pigs (weight 22–26 kg) of both sexes in conformity with the German Law on the Protection of Animals. After overnight fasting, the animals were premedicated with intramuscular flunitrazepam (0.1 mg/kg). Anaesthesia was induced with intravenous (i.v.) ketamine (5 mg/kg) and flunitrazepam (0.1 mg/kg) administered via the ear vein. Following i.v. injection of vecuronium (0.4 mg/kg), the trachea was intubated with a cuffed tube. Anaesthesia was maintained by continuous i.v. infusions of ketamine ($6\,mg \cdot kg^{-1} \cdot h^{-1}$) and flunitrazepam ($0.04\,mg \cdot kg^{-1} \cdot h^{-1}$). Mechanical ventilation was provided by a constant-volume ventilator (Siemens, SV 900 B, Stockholm, Sweden) and facilitated by a continuous i.v. infusion of vecuronium ($1.5\,mg \cdot kg^{-1} \cdot h^{-1}$). Respiratory rates and inspired O_2 concentratin were adjusted to maintain arterial PCO_2 between 38 and 42 mmHg and arterial PO_2 between 95 and 120 mmHg. All animals were in the supine position. Body temperature was continuously monitored by a thermistor of a flow-directed thermodilution catheter (model 93A-131-7Fr, Edwards Laboratory), and was maintained by placing the animals on a heating pad and by warming the inspired gases. Catheters were inserted into the abdominal aorta, pulmonary artery and superior vena cava as previously described [14,15]. Following midline laparotomy, the left hepatic vein (16-G radiopaque polyurethane indwelling catheter, Arrow, Reading, Penna., USA) and the portal vein (4 Fr × 51/8-inch two-lumen indwelling catheter, Arrow, Reading, Penna., USA) were cannulated using Seldinger's technique. The superior mesenteric vein was cannulated (16 G × 8-inch polyurethane catheter, Arrow, Reading, Penna., USA) via a distal tributary in the ileal mesentery.

Precalibrated electromagnetic flow probes (Stölzer Messtechnik, Waldkirch, Germany) of appropriate sizes to ensure a snug fit were placed around the hepatic artery, the portal vein and the superior mesenteric artery close to its origin [14]. Care was taken to preserve the periarterial nerve plexus. Zero flow readings were checked repeatedly during the experiment. The superior gastroduodenal artery was ligated to ensure that true hepatic arterial blood flow was measured.

A tonometric catheter (TRIP NGS Catheter, Tonometrics, Worcester, Mass., USA) was inserted into the lumen of the terminal ileum through a small antimesenteric enterotomy and secured in place with a purse-string suture. Proximal to this site, an additional 3-cm transmural longitudinal antimesenteric incision was made for subsequent intermittent placement of a multiwire surface PO_2 electrode onto the gut mucosa (see below). Care was taken not to interfere with the blood supply of this gut segment. To prevent intestinal secretions from contaminating the site of mucosal PO_2 measurement, a silastic drain was inserted into the bowel lumen through a small incision proximal to the site of mucosal PO_2 measurements.

After the preparation had been completed, the midline laparotomy was loosely closed with sutures except for 5–10 cm in the upper abdomen to allow access to liver and gut for subsequent determinations of hepatic and intestinal surface PO_2 (see below). The gap was covered with saline-soaked gauze and wet cellophane to prevent drying out and heat loss.

Measurements and calculations

All intravascular catheters were connected to pressure transducers (Senso Nor, type 840, Horten, Norway), which were zeroed to ambient pressure. A multichannel recorder (Hellige, Freiburg, Germany) was used for the recording of signals. Cardiac output was determiend by the thermodilution technique (Siemens, CO Computer model 404-1, Erlangen, Germany). The mean value of triplicate injections of 5 ml of ice-cold temperature-monitored saline was considered to reflect actual cardiac output if the measurements were within a range of $\pm 5\%$ from the calculated mean. Total hepatic blood flow was calculated as the sum of hepatic arterial and portal venous blood flow. Vascular resistances (systemic, pulmonary arterial, hepatic arterial, portal venous and superior mesenteric arterial) were calculated using standard and previously described formulae [14].

Blood gas tensions (PO_2, PCO_2) and blood pH values were determined using an ABL 505 blood gas autoanalyser (Radiometer, Copenhagen, Denmark). Haemoglobin concentrations and O_2 saturations were measured by an OSM 3 haemoximeter (Radiometer, Copenhagen, Denmark), which had been calibrated for pig's blood. Haematocrit was determined from centrifuged (Bayer AG, Compur Microspin, Leverkusen, Germany) arterial blood sampled in capillary tubes. O_2 deliveries, uptakes and extractions were derived using standard and previously described formulae [14].

Lactate concentrations were determined enzymatically–photometrically [15]. Small-intestinal lactate production was calculated as:

$$[(Lac)_a - (Lac_{SMV})] \times Q_{SMA}$$

where $(Lac)_a$ and $(Lac)_{SMV}$ are systemic arterial and superior mesenteric venous lactate concentrations, respectively, and Q_{SMA} is superior mesenteric arterial blood flow.

Surface PO_2 of liver and of the serosa and mucosa of the small intestine was measured using multiwire platinum electrodes [14–17]. During each measurement at each location, approximately 100 individual PO_2 values were noted, from which the mean value was calculated. The distribution of these values is presented as summary surface PO_2 histograms.

Intestinal mucosal pH (pH_i) was determined tonometrically [8]. The tonometer consists of a gas-impermeable polyester tube with a gas-permeable balloon close to its tip. At the time of tonometer placement, the balloon was filled with 2.5 ml of 0.9% saline and allowed to equilibrate for 1 h. At the time of measurement, the first 1 ml of saline withdrawn was discarded, as it represents the catheter's deadspace. The remaining 1.5 ml was analysed for PCO_2 in an ABL 505 blood gas autoanalyser. pH_i was calculated using the Henderson–Hasselbach equation:

$$pH_i = 6.1 + \log(HCO_3^-)_a / P_iCO_2 \times 0.03$$

with 6.1 being the dissociation constant of bicarbonate, 0.03 the solubility of CO_2 in plasma, $(HCO_3^-)_a$ the arterial bicarbonate concentration as an estimate of intestinal (HCO_3^-) and P_iCO_2 the intestinal PCO_2 as derived from the PCO_2 measured in the saline-filled balloon and time-corrected for the equilibration period [8].

Experimental protocol

After completion of the surgical preparation, at least 60 min were allowed for haemodynamic stabilization. The amount of Ringer's solution necessary to maintain cardiac filling pressures during this stabilization period was subsequently administered unchanged throughout the experiment.

After baseline measurements had been obtained, purified lipopolysaccharide (LPS) [18] was infused into the portal vein at a rate of 0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ over a period of 6 h. Haemodynamic data were recorded, and parameters of O_2 supply/uptake and of acid–base balance were determined every 20 min during the 1st h of LPS infusion and then hourly during the following 5 h. Tissue surface PO_2, pH_i and concentrations of lactate were measured hourly. At the end of the experiment the animals were killed by a rapid i.v. injection of potassium chloride.

In three additional animals, the effect of time on the stability of the surgical preparation was evaluated during baseline conditions (laparotomy, mechanical ventilation, flunitrazepam/ ketamine/ vecuronium anaesthesia). The surgical preparation was performed as described above. No interventions were undertaken after baseline values had been obtained, and repeat measurements were made at 1-h intervals during the following 6 h.

Statistical analysis

The data were first analysed by Friedman's statistic. When positive ($p < 0.05$), the Wilcoxon signed-rank test was used for comparisons between baseline values and data of the different experimental periods. A p-value of < 0.05 was considered statistically significant. Values are presented as means $\pm$ standard errors of the means (SE).

Results

Systemic and splanchnic haemodynamic variables and parameters of systemic and splanchnic oxygenation and of small intestinal metabolism are presented in Tables 1–3 and in Figs 1–4. The amount of Ringer's solution necessary to maintain cardiac filling pressures during the stabilization period was 17–24 $ml \cdot kg^{-1} \cdot h^{-1}$. This same amount of fluid was administered throughout the experiment.

Systemic and splanchnic haemodynamics

Portal venous infusion of endotoxin induced a multiphasic haemodynamic response pattern. An early phase (at approximately 40 min) was characterised by intense generalized vasoconstriction as reflected, in part, by pronounced increases in systemic, pulmonary, hepatic and superior mesenteric arterial vascular resistances (Table 1, Fig. 1), accompanied by decreases in cardiac output (Table 1) and splanchnic blood flows (Fig. 1). During this time, mean arterial pressure remained unchanged (Table 1). However, pulmonary arterial, central venous, pulmonary capillary wedge, portal and hepatic venous pressures increased (Table 1).

By 60 min, most variables had either markedly improved (pulmonary arterial and pulmonary capillary wedge pressure) or returned to baseline values (cardiac output, splanchnic flows and resistances, with the exception of portal venous vascular resistance) (Table 1, Fig. 1). Only mean arterial pressure now decreased for the first time (Table 1).

This first phase of early vasoconstriction and rapid partial or complete recovery was followed by a similar, though extended, second phase of vasoconstriction and partial or complete recovery. All vascular resistances and pulmonary artery pressure increased, and cardiac output, stroke volume and all splanchnic blood flows decreased again, with peak effects occurring between 180 and 240 min (Fig. 1, Table 1). The second rise in superior mesenteric arterial vascular resistance did not reach statistical significance.

By 360 min, there was either a tendency towards (pulmonary artery pressure and pulmonary vascular resistance) or normalization of most systemic and splanchnic haemodynamic variables.

Systemic and regional O_2 supply/uptake relationships

The behaviour of systemic, total hepatic and superior mesenteric O_2 deliveries reflected that of cardiac output and regional blood flow. Early decreases at 40 min were followed by recoveries at 60 min (Fig. 2). A second, prolonged, decrease in O_2 delivery lasting 2–3 h was followed by further recovery at 360 min. Respective changes in portal venous O_2 saturation and delivery and in hepatic arterial O_2 delivery (Table 2) were responsible for the observed changes in total hepatic O_2 delivery (Fig. 2).

Table 1 Haemodynamic variables. Values are means ± SE

Variables	Baseline	20 min	40 min	60 min	120 min	180 min	240 min	300 min	360 min
Heart rate (min^{-1})	96 ± 6	99 ± 8	122 ± 7*	124 ± 6*	152 ± 7*	138 ± 7*	134 ± 8*	136 ± 8*	152 ± 17*
Intravascular pressure (mmHg)									
Mean arterial	106 ± 3	108 ± 3	105 ± 5	86 ± 3*	82 ± 4*	98 ± 5	101 ± 4	102 ± 6	93 ± 5
Mean pulmonary arterial	17 ± 1	23 ± 4*	42 ± 3*	24 ± 3*	27 ± 3*	34 ± 2*	29 ± 2*	27 ± 2*	26 ± 5*
Central venous	2.3 ± 0.3	3.0 ± 0.3	5.9 ± 0.9*	3.1 ± 0.9*	3.4 ± 0.3*	3.5 ± 0.4*	3.4 ± 0.5*	3.4 ± 0.5*	3.7 ± 0.5*
Pulmonary capillary wedge	4.4 ± 0.6	5.0 ± 0.5	6.5 ± 0.7*	5.6 ± 0.7*	5.5 ± 0.8*	5.6 ± 0.7*	6.0 ± 0.6*	5.9 ± 0.7*	5.6 ± 0.7*
Portal venous	6.4 ± 0.8	7.0 ± 1.0	8.9 ± 0.9*	7.5 ± 0.6	10.0 ± 1.0*	11.0 ± 1.0*	10.0 ± 1.0*	10.0 ± 0.8*	9.6 ± 0.9*
Hepatic venous	4.1 ± 0.5	4.0 ± 0.3	6.4 ± 0.9*	4.2 ± 0.5	4.5 ± 0.4	4.6 ± 0.5	4.9 ± 0.5	4.9 ± 0.4	5.0 ± 0.2
Blood flow									
Cardiac ouput (l/min)	3.0 ± 0.2	2.9 ± 0.2	2.4 ± 0.3*	2.8 ± 0.2	2.7 ± 0.3	2.3 ± 0.2*	2.1 ± 0.2*	2.5 ± 0.2*	3.0 ± 0.2
Stroke volume (ml)	31 ± 2	30 ± 2	20 ± 2*	23 ± 4	18 ± 2*	17 ± 2*	16 ± 2*	18 ± 2*	20 ± 2*
Total hepatic (ml/min)	595 ± 49	546 ± 42	449 ± 51*	526 ± 34	486 ± 40*	435 ± 40*	441 ± 40*	510 ± 39	578 ± 48
Vascular resistance (units)									
Pulmonary arterial	35 ± 2	39 ± 3	44 ± 3*	30 ± 3	32 ± 4	42 ± 3*	50 ± 7*	41 ± 2*	32 ± 3
Systemic arterial	5 ± 2	7 ± 2	14 ± 2*	7 ± 1	10 ± 2	13 ± 1*	12 ± 2*	9 ± 1*	7 ± 1

* $p < 0.05$ compared to baseline

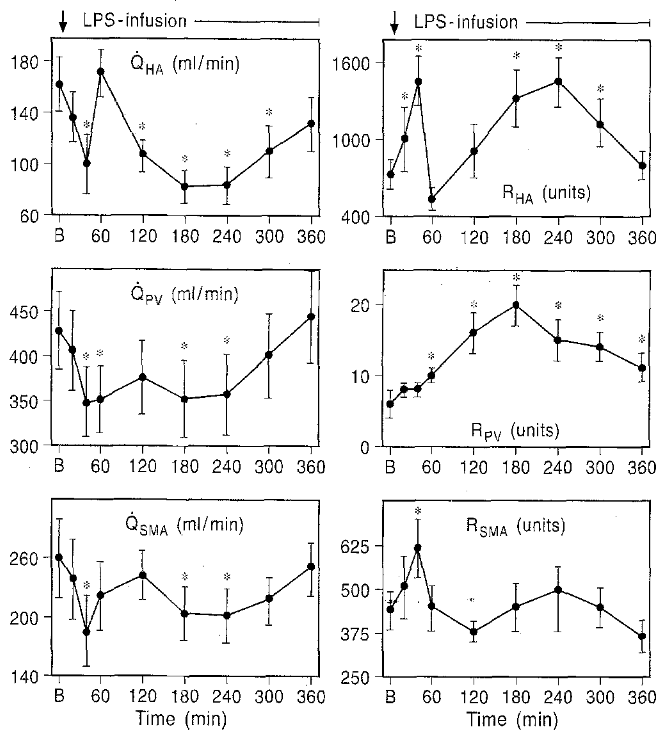

Fig. 1 Changes in splanchnic haemodynamics. Values are means ± SEM. $\dot{Q}_{HA}$ hepatic arterial blood flow, $\dot{Q}_{PV}$ portal venous blood flow, $\dot{Q}_{SMA}$ superior mesenteric arterial blood flow, R_{HA} hepatic arterial vascular resistance, R_{PV} portal venous vascular resistance, R_{SMA} superior mesenteric arterial vascular resistance, B baseline. * $p < 0.05$ compared with baseline values

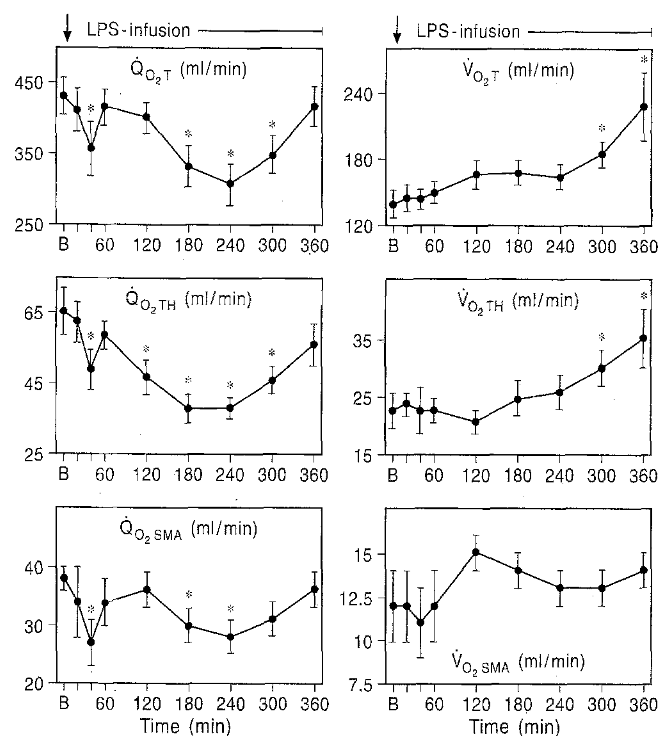

Fig. 2 Changes in systemic and splanchnic O_2 supplies and uptakes. Values are means ± SEM. $\dot{Q}_{O2T}$ total systemic O_2 delivery, $\dot{Q}_{O2\,TH}$ total hepatic $\dot{Q}_2$ delivery, $\dot{Q}_{O2\,SMA}$ superior mesenteric arterial O_2 delivery, $\dot{V}_{O2\,T}$ total systemic O_2 uptake, $\dot{V}_{O2\,TH}$ total hepatic O_2 uptake, $\dot{V}_{O2\,SMA}$ superior mesenteric arterial O_2 uptake. * $p < 0.05$ compared with baseline

Whereas total systemic and hepatic O_2 uptakes increased at 300 and 360 min, small intestinal O_2 uptake did not change significantly throughout the experiment (Fig. 2). Total systemic, hepatic and small intestinal O_2 extraction ratios increased at 40 min, and normalized at 60 min (Table 2). Starting at 120 min, there was a sustained increase in O_2 extraction ratios despite normalized O_2 deliveries at 360 min.

Tissue oxygenation

There were marked differences between intestinal mucosal and serosal surface oxygenation. At baseline, mean mucosal surface PO_2 was already considerably lower (19 ± 5 mmHg) than mean serosal surface PO_2 (62 ± 5 mmHg) (Table 2). In addition (and in contrast to the serosa), mucosal summary surface PO_2 histograms were shifted leftward, with some PO_2 values occurring in the hypoxic range of 0–5 mmHg (Fig. 3).

By 60 min, mean mucosal PO_2 had decreased by 55% (Table 2), and the surface PO_2 histogram had shifted to the left with approximately 50% of all PO_2 values now occurring in the hypoxic range (Fig. 3). The pronounced decreases in mean surface PO_2 and the marked leftward shift of the PO_2 histograms persisted throughout the rest of the experiment. In contrast, mean serosal surface PO_2 values decreased significantly by just 25% only at 120 and 180 min, and no values below 15 mmHg were registered.

Table 2 Parameters of oxygenation. Values are mean ± SE

Variables	Baseline	20 min	40 min	60 min	120 min	180 min	240 min	300 min	360 min
Oxygen pressure (mmHg)									
Arterial	111 ± 1	117 ± 10	103 ± 7	120 ± 7	112 ± 4	104 ± 2	110 ± 5	110 ± 4	114 ± 3
Liver	60 ± 3	–	–	51 ± 4	47 ± 3*	41 ± 7*	41 ± 4*	41 ± 6*	49 ± 4*
Ileal serosa	62 ± 5	–	–	54 ± 6	46 ± 6*	46 ± 6*	54 ± 4	53 ± 3	51 ± 5
Ileal mucosa	19 ± 5	–	–	9 ± 2*	8 ± 2*	5 ± 1*	4 ± 1*	6 ± 2*	10 ± 4*
Haemoglobin (g/100 ml)	0.2 ± 0.3	10.2 ± 0.3	10.7 ± 0.4	10.5 ± 0.4	10.4 ± 0.4	10.3 ± 0.3	10.2 ± 0.3	10.0 ± 0.3	10.0 ± 0.3
Haemoglobin saturation (%)									
Mixed venous	68 ± 2	64 ± 3	57 ± 4*	64 ± 2	57 ± 3*	48 ± 3*	46 ± 3*	47 ± 1*	47 ± 3*
Portal venous	70 ± 4	68 ± 3	62 ± 2*	64 ± 3	56 ± 4*	49 ± 2*	50 ± 2*	53 ± 3*	57 ± 3*
Hepatic venous	51 ± 3	47 ± 5	38 ± 4*	46 ± 3	36 ± 4*	19 ± 2*	19 ± 2*	23 ± 2*	26 ± 2*
Superior mesenteric venous	68 ± 3	66 ± 2	60 ± 2*	62 ± 3	59 ± 2*	53 ± 2*	52 ± 3*	53 ± 2*	59 ± 3
Arterial	99 ± 1	99 ± 1	97 ± 2	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1
Oxygen delivery (mol/min)									
Hepatic arterial	23 ± 3	20 ± 3*	14 ± 3*	26 ± 3	16 ± 2*	12 ± 2*	12 ± 2*	15 ± 3*	19 ± 3
Portal venous	46 ± 7	42 ± 6	35 ± 5*	38 ± 4	31 ± 5*	26 ± 4*	27 ± 4*	32 ± 5	38 ± 7
Oxygen extraction ratios (%)									
Total systemic	33 ± 2	36 ± 2	42 ± 3*	37 ± 2	44 ± 3*	52 ± 3*	55 ± 2*	53 ± 1*	54 ± 3*
Total hepatic	36 ± 3	40 ± 5	48 ± 5*	39 ± 3	46 ± 2*	69 ± 3*	69 ± 2*	65 ± 3*	62 ± 4*
Small intestine	33 ± 3	36 ± 2	39 ± 2	40 ± 2	41 ± 2*	48 ± 3*	48 ± 3*	42 ± 3*	41 ± 2*

* $p < 0.05$ compared to baseline

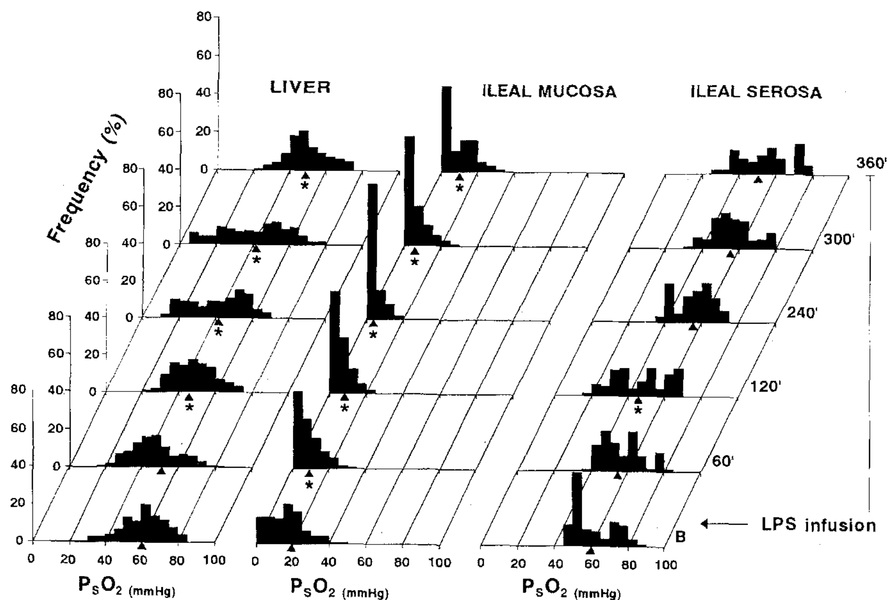

Fig. 3 Summary surface PO_2 histograms of liver and of ileal mucosa and serosa. Each histogram consists of approximately 800 individual PO_2 measurements. P_sO_2 surface PO_2, ▲ mean tissue PO_2. * $p < 0.05$ compared with baseline

Changes in hepatic surface oxygenation were moderate. Mean surface PO_2 values decreased at 120 min by 20–30%, and remained so until the end of the experiment (Table 2). The surface PO_2 histograms demonstrated only a slight leftward shift, with no values in the hypoxic range (Fig. 3).

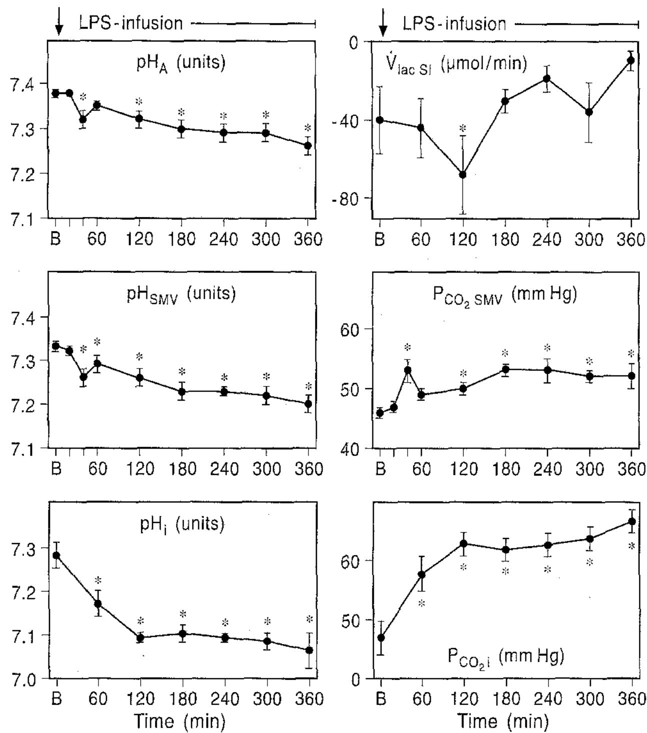

Fig. 4 Changes in small intestinal metabolism. pH_A arterial pH, pH_{SMV} superior mesenteric venous pH, pH_i ileal mucosal pH, $\dot{V}_{lacSI}$ small intestinal lactate uptake, $P_{CO2\,SMV}$ superior mesenteric venous PCO_2, P_{CO2i} ileal mucosal PCO_2. Values are means ± SEM. * $p < 0.05$ compared with baseline

Acid-base balance and metabolic function

Endotoxaemia resulted in decreases in pH values of arterial and superior mesenteric venous blood beginning at 40 min (Fig. 4). However, at no time was there as expansion of the asteriosuperior mesentric venous pH gradient.

There was an increase in intravascular PCO_2 at 40 min and a return to baseline values at 60 min (Table 3, Fig. 4). Subsequently, arterial PCO_2 remained unchanged, but mesenteric venous PCO_2 increased and remained elevated.

As early as 60 min (at a time of no change in arterial pH and PCO_2), small intestinal mucosal pH decreased and PCO_2 increased markedly (Fig. 4). As a result of proportionally greater changes in the mucosa, intramucosal-arterial and intramucosal-superior mesenteric venous PCO_2 gradients had increased by 60 min and remained elevated throughout (Table 3).

Arterial and mesenteric venous lactate concentrations increased only transiently at 120 and 180 min (Table 3). However, all values remained within normal limits. Except at 120 min (when small intestinal lactate production increased), small intestinal lactate production remained unchanged throughout (Fig. 4).

Miscellaneous

In the three control animals, differences between baseline values and those obtained hourly during the following 6 h did not exceed 10%.

Discussion

The principal findings of this study can be summarized as follows: (1) there was early and maintained intestinal

Table 3 Parameters of acid–base balance and lactate metabolism. Values are mean ± SE

Variables	Baseline	20 min	40 min	60 min	120 min	180 min	240 min	300 min	360 min
Carbon dioxide tension (mmHg)									
Arterial	38 ± 1	39 ± 1	43 ± 2*	39 ± 1	38 ± 1	40 ± 1	40 ± 1	40 ± 1	42 ± 2
Superior mesenteric venous	46 ± 1	47 ± 1	53 ± 2*	49 ± 4	50 ± 1	53 ± 1*	53 ± 2*	52 ± 1*	52 ± 2*
PCO_2 gradients (mmHg)									
Superior venous–arterial	8 ± 1	8 ± 1	10 ± 1	10 ± 1	12 ± 1*	13 ± 1*	13 ± 1*	12 ± 1*	10 ± 1
Intramucosal-superior mesenteric venous	1 ± 1	–	–	8 ± 1*	13 ± 2*	10 ± 1*	11 ± 2*	13 ± 2*	15 ± 2*
Intramucosal–arterial	9 ± 2	–	–	20 ± 4*	26 ± 2*	23 ± 3*	22 ± 2*	23 ± 2*	25 ± 5*
Lactate concentration (µmol/ml)									
Arterial	1.1 ± 0.2	–	–	1.1 ± 01	1.5 ± 0.2*	1.6 ± 0.2*	1.4 ± 0.1	1.3 ± 0.1	1.2 ± 0.2
Superior mesenteric venous	1.1 ± 0.1			1.2 ± 0.4	1.7 ± 0.2*	1.7 ± 0.2*	1.4 ± 0.1	1.4 ± 0.2	1.3 ± 0.2

* $p < 0.05$ compared to baseline

mucosal (but not serosal) hypoxia, and mucosal acidosis unrelated to perfusion pressure, mesenteric O_2 delivery and systemic arterial pH; (2) there was little evidence of simultaneous hepatic hypoxia; (3) there was a multiphasic response pattern of splanchnic haemodynamics and oxygenation.

Critique of methods

Both baseline anaesthesia and the invasive surgical preparation might have resulted in spontaneous deterioration of the preparation over time and may thus have influenced the results. However, the data obtained in the three control animals during 6 h of baseline conditions rule out significant spontaneous deterioration of the preparation with time.

The technique of measuring surface PO_2 using O_2-sensitive multiwire surface electrodes is well established [5, 16, 17]. A low-weight electrode (2.2 g), a specially designed electrode holder and loose fixation of the connecting wires to a clamp mounted on a pole directly above the site of measurement were employed to minimize capillary compression when placing the electrodes on the mucosal surface. Baseline surface PO_2 values in the range previously described [16], and the presence of rhythmic oscillations of the surface PO_2 tracings reflecting preserved vasomotion on the microcirculatory level [16], indicate that capillary compression of the villi during measurements was avoided.

Ileal intramucosal pH was determined using tonometry, which has been well established in numerous previous studies [5, 8, 10, 13]. Its validity when compared to the direct measurements of mucosal pH by means of microelectrodes was demonstrated in endotoxic shock [19]. We determined pH_i after an equilibration period of 60 min using appropriate correction factors [8] to calculate the luminal PCO_2 of the partially equilibrated samples. Since preliminary studies had shown that an ABL 505 blood gas analyser measures PCO_2 in saline more accurately than Corning 178 and 278 blood gas analysers, an ABL 505 blood gas analyser was used for luminal PCO_2 determinations.

We used purified LPS from *Salmonella abortus equi* to induce endotoxaemia. Administration of this purified LPS has been shown to induce many pathophysiological changes known to occur during infections with gram-negative microorganisms [18]. Preliminary dose-finding studies had shown that a dose of $0.5\ \mu g \cdot kg^{-1} \cdot h^{-1}$ induced reproducible time-dependent haemodynamic changes. We infused LPS continuously into the portal vein to simulate the situation in which endotoxin is translocated from the gut into the portal vein.

The amount of fluid necessary to maintain cardiac filling pressures during the stabilization period was continued throughout the experiment. In this way, ongoing fluid losses unrelated to endotoxaemia were replaced and possible superimposed effects of fluid resuscitation and haemodilution were avoided. Titration of fluid to a desired haemodynamic end-point was considered inappropriate because myocardial function may be impaired during endotoxaemia [20].

Splanchnic O_2 supply/uptake

Small intestinal surface PO_2 measurements revealed a marked O_2 gradient across the intestinal wall. This gradient was present prior to endotoxin infusion and persisted throughout the experiment. In addition, serosal and mucosal surface PO_2 responded differently to endotoxin infusion. Whereas mean serosal PO_2 decreased maximally by 25% (at 120 and 80 min), with only a moderate leftward shift of the summary PO_2 histogram and with no values in the hypoxic range (0–5 mmHg), mean mucosal PO_2 values decreased by up to 80% (at 240 min), with a marked leftward shift of the PO_2 histogram and almost half of the PO_2 values in the hypoxic range. The impairment of mucosal oxygenation cannot be attributed solely to inadequate flow, perfusion pressure or O_2 delivery because superior mesenteric blood flow, mean arterial pressure and small intestinal O_2 delivery had returned to baseline values at a time (360 min) when mean mucosal surface PO_2 was still 50% below baseline value with many values in the hypoxic range.

The pronounced decrease in mucosal surface PO_2 coincided with a marked decrease in intestinal mucosal pH but unchanged small intestinal O_2 uptake. The increase in the arteriointestinal mucosal pH gradient with no such increase in the arteriomesenteric venous pH gradient would suggest that (unlike the decrease in mesenteric venous pH) the decrease in mucosal pH was not simply a reflection of systemic acidosis. The preferential decrease in mucosal surface PO_2 and pH must be taken as evidence of regional intestinal hypoperfusion.

The mean surface PO_2 values of mucosa and serosa found during baseline conditions are in agreement with recent findings also derived from multiwire surface PO_2 electrodes [16]. Other investigators found lower baseline values (8 vs 19 mmHg in the mucosa; 48 vs 62 mmHg at the serosa) [5]. These lower-surface PO_2 values are likely to be the result of lower PaO_2 values because of room-air ventilation. With the use of O_2 microneedle electrodes, comparable tissue PO_2 values in the villus apex of about 14 mmHg have been reported [21]. Considerably lower mucosal than serosal PO_2 values and the resultant PO_2 gradient

across the intestinal wall may be the result of countercurrent shunting of O_2 from arteriole to capillary at the base of the intestinal villus and/or of O_2 consumption along the length of the entire villus [22, 23].

Our finding of predominantly mucosal hypoxia in response to endotoxaemia is in agreement with recently published work [5]. Decreased capillary density in mucosal villi, and/or more pronounced constriction of central villus arterioles in comparison to first- or second-order arterioles, may lead to redistribution of intestinal blood flow resulting in preferential hypoperfusion and subsequent hypoxia of the mucosa [5, 24]. It is of interest that mucosal hypoxia, hypercapnia and acidosis did not result in increasing mesenteric venous lactate concentrations or arteriomesenteric venous pH and PCO_2 gradients. Persistent mucosal hypoperfusion might have prevented an adequate wash-out of lactate and other acid metabolites. Alternatively (or in addition), the mass of intestinal mucosa may not be sufficient noticeably to change the composition of the venous drainage. Preferential and persistent mucosal hypoperfusion and redistribution of flow across the intestinal wall is suggested by the finding of low mucosal surface PO_2 and pH event at times (60, 120, 300, 360 min) of improved serosal surface PO_2, and normalized mesenteric blood flow and O_2 delivery.

In agreement with previous work in endotoxic pigs [3, 10, 13], we observed unchanged mesenteric O_2 uptake. In contrast, in endotoxic dogs intestinal O_2 uptake decreased in a supply-dependent manner [5]. A supply-dependent decrease in O_2 uptake during endotoxaemia has been reported to occur at an intestinal O_2 extraction ratio exceeding 0.47 [4]. In our study, the small intestinal O_2 extraction ratio increased to maximally 0.48.

At first glance, unchanged small intestinal O_2 uptake would seem to indicate that critical O_2 supply had not yet been reached. However, in the presence of likely mucosal hypoperfusion and possible flow redistribution the value of global small intestinal O_2 uptake as a reflection of the adequacy of O_2 supply needs to be questioned. It may well be argued that the lack of an increase in small intestinal O_2 uptake at 300 and 360 min (in contrast to increases in whole body and hepatic O_2 uptakes) actually reflects a supply-dependent decrease in mucosal O_2 uptake. The regional (mucosal) decrease in O_2 uptake might have prevented a net increase in global small intestinal O_2 uptake.

As decreases in splanchnic O_2 deliveries were primarily caused by decreases in blood flows, splanchnic O_2 deliveries closely followed splanchnic blood flows. Normal O_2 deliveries did not necessarily coincide with normal venous O_2 saturations and O_2 extraction ratios. For example, despite recovered O_2 deliveries at 6 h, splanchnic venous O_2 saturations were reduced by 15–50%, and O_2 extraction ratios increased by 25–70%. This might suggest that increased O_2 demands were not entirely being met by O_2 supplies.

Although surface PO_2 values are not necessarily representative of whole organ oxygenation, the only moderate changes in mean liver surface PO_2 and PO_2 histograms make clinically relevant disturbances of the hepatic microcirculation unlikely. Increased hepatic O_2 extraction seems to have compensated for the intermittent decreases in O_2 deliveries. Furthermore, increases in total hepatic O_2 uptake at 5 and 6 h provide evidence that O_2 supply dependency had not been reached. Increased hepatic O_2 demand can be explained on the basis of increased metabolism of activated Kupffer cells, of hepatocytes or of polymorphonuclear neutrophils entrapped in the liver.

No previous data on liver surface PO_2 or on the simultaneous behaviour of both hepatic and mesenteric O_2 supply/uptake relationships in response to i.v. endotoxin over 6 h are available for direct comparison.

Splanchnic haemodynamics

All splanchnic blood flows exhibited multiphasic response patterns similar to those observed in the systemic and pulmonary circulations. Dissimilar responses of the various flow ratios and of splanchnic vascular resistances indicate that (1) not all changes in splanchnic flows were caused by respective changes in cardiac output and (2) differences in regional vasomotor response probably contributed to the different flow responses. We could demonstrate that during endotoxaemia there is not necessarily a redistribution of cardiac output away from liver and gut (as indicated by unchanged ratios of total hepatic and superior mesenteric arterial blood flows to Q_T). However, at the same time, there may be intermittent intrahepatic redistribution of flow with a proportional decrease in hepatic arterial flow (as indicated by intermittent decreases in the ratio of hepatic arterial to total hepatic blood flow). This is evidence for selective hepatic arterial vasoconstriction.

The hepatic arterial buffer response predicts that changes in portal blood flow lead to opposite changes in hepatic arterial blood flow [25]. Accordingly, as portal blood flow decreased, hepatic arterial blood flow should have increased. However, the decreases in portal flow were accompanied by even larger decreases in hepatic arterial flow, indicating impaired or even abolished hepatic arterial buffer response. This is additional evidence for intense locally-induced vasoconstriction of the hepatic arterial vascular bed. It remains to be determined whether the potent hepatic arterial vasoconstrictor response is due to a locally released vasoconstrictor substance or due to a greater sensitivity of the hepatic arterial circulation to a circulating vasoconstrictor. The

simultaneous marked initial increase in resistance of all vascular beds studied (with the exception of the portal circulation) would argue for specific mediator-induced vasoconstriction of all vascular beds.

Intraportal application of endotoxin resulted in sustained portal hypertension. The increase in portal venous pressure was not secondary to increases in back pressure (as hepatic venous pressure did not change significantly except at 40 min when central venous pressure increased markedly), and it did not correlate with portal venous blood flow. As pressure rather than flow is usually regulated in the portal circulation [26], the increase in portal venous pressure is suggestive of changes in intrahepatic resistance. Regulation of sinusoidal tone is thought to take place primarily in the region of portal venous inflow [27]. Kupffer cells are located in this region, and they are involved in the regulation of the hepatic microcirculation [27]. As they are activated during endotoxaemia [28], swelling of Kupffer (and endothelial) cells may lead to narrowing of the sinusoidal space and in this way contribute to portal venous hypertension. In addition, activated Kupffer cells secrete a variety of mediators [28], all of which may interfere with intrahepatic blood flow regulation. Direct comparison with and between other studies [9, 10, 12, 29, 30] is difficult because of marked differences in methodology (differences in species; in site, amount and method of endotoxin application; in amount of fluid replacement; in intravascular volume status; in baseline anaesthesia; in length of observation time).

Systemic haemodynamics

Phasic responses to endotoxaemia have previously been described [9, 10, 12]. However, in two of these studies [9, 10] observation time was limited to 2 h. The first phase of vasoconstriction has been attributed to direct effects of endotoxin on vascular endothelium [31] and to the release of various vasoactive humoral substances [10, 32]. The second phase of vasoconstriction has been attributed to the action of O_2 free radicals [33] and increased blood concentrations of β-endorphins [11].

Systemic O_2 supply/uptake

Unchanged (during the first 4 h) and increased (during the last 2 h) whole body O_2 uptake suggests that supply dependency of O_2 uptake had not been reached. Our lowest value of systemic O_2 supply of approximately13 $ml \cdot kg^{-1} \cdot min^{-1}$ and the highest value of whole body O_2 extraction ratio of 0.55 just approached critical values [4]. Factors contributing to the increase in whole body O_2 uptake may include the rise in body temperature, metabolic uncoupling of adenosine diphosphate phosphorylation in the mitochondria [34], calorigenic effects of elevated levels of circulating catecholamines [35] and increased hepatic metabolism [36]. Normal, or even elevated, whole body O_2 uptake during endotoxaemia is consistent with previous findings [3, 4, 10].

Since lactate concentrations hardly changed, lactic acidosis cannot have been the source of the systemic acidosis observed. O_2 deprivation may lead to intracellular metabolic acidosis, even in the absence of lactate. This is associated with decreased adenosine triphosphate (ATP) levels and may represent unreversed ATP hydrolysis [37]. The exact origin of the metabolic acidosis cannot be determined on the basis of this investigation.

Conclusions

In an animal model which allows simultaneous assessment of both hepatic and small intestinal perfusion and oxygenation, we could demonstrate that the first 6 h of endotoxaemia are characterized by cyclic changes in hepatic and small intestinal perfusion and O_2 delivery. Whereas there was little evidence of clinically relevant hepatic dysoxia, endotoxaemia caused early and sustained ileal mucosal hypoperfusion. It is of clinical relevance that regional intestinal hypoperfusion existed even at times of restored perfusion pressure, and of small intestinal flow and O_2 delivery, and that it was not reflected by changes in the composition of small intestinal venous drainage. Thus, mucosal hypoperfusion may well go clinically unrecognized and, when persisting, may sustain and augment translocation of endotoxin from the intestinal lumen into the circulation. Tonometry proved to be an effective means of detecting early mucosal hypoxia. Our data also indicate that in the early period of endotoxaemia development of tonometrically diagnosed mucosal acidosis does not necessarily imply equally impaired hepatic oxygenation. Obviously, different regions within the splanchnic area may be affected differently during early endotoxaemia.

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