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Oxygen extraction in pigs subjected to low-dose infusion of endotoxin after major abdominal surgery

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Background: Sepsis may impair O_2 extraction due to blood flow redistribution or decreased utilization of the available oxygen.
Methods: We assessed the effect of endotoxemia on systemic and regional O_2 extraction and lactate handling in pigs, randomized to receive either endotoxin ($0.4\,\mu g\,kg^{-1}\,h^{-1}$; n = 10) or saline infusion (controls; n = 9) for 12 h.
Results: High baseline regional and systemic O_2 extraction in the endotoxin group (median 56%, range 45–77%) and in the controls (67%, 49–72%) was maintained until the end of the experiment (endotoxin group: 60%, 50–71%; controls: 60%, 50–74%) despite hypotension and a decrease in stroke volume in endotoxic animals. Hepatic lactate exchange decreased during endotoxemia from 14 $\mu mol\,kg^{-1}\,min^{-1}$ (range 10–28 $\mu mol\,kg^{-1}\,min^{-1}$) to 10 (range 3–15) $\mu mol\,kg^{-1}\,min^{-1}$; $P < 0.01$), but remained stable in the controls, with 13 $\mu mol\,min^{-1}$ (4–18 $\mu mol\,min^{-1}$) at baseline and 7 $\mu mol\,min^{-1}$ (3–17 $\mu mol\,min^{-1}$) after 12 h of saline infusion.
Conclusions: The high and sustained oxygen consumption and oxygen extraction in this endotoxemic model speak against any major impairment of hepatosplanchnic or systemic oxygen extraction and oxidative metabolism. The reduced hepatic lactate exchange despite an unchanged hepatic lactate influx suggests altered metabolic activities independent of oxygen consumption.

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Key words: Gastric tonometry; hemodynamics; jejunal tonometry; lactate; oxygen consumption; oxygen delivery; septic shock.

IMPAIRED oxygen utilization is assumed to play a major role in the development of multiple organ failure (MOF) in septic shock (1–4). The increase in oxygen requirements in hypermetabolic conditions is not always accompanied by an increase in blood flow. As a consequence, oxygen extraction has to increase. Beyond a certain threshold, oxygen consumption becomes dependent on delivery (5). Indirect evidence that tissue perfusion may not meet metabolic demands – despite a hyperdynamic circulation – includes high lactate concentrations (6), gastrointestinal mucosal acidosis (7), and abnormalities in the microcirculation (8). The mechanisms and consequences of these abnormalities are not known. In sepsis, metabolic demands are increased, blood flow is redistributed, and oxygen delivery decreases in certain tissues (2). Due to the heterogeneity of blood flow, hypoxic tissue units may exist next to normoxic and well-perfused ones (8–10). Under these conditions, treatment with vasoactive agents can even worsen the maldistribution of blood flow (11–14).

Recent studies have suggested that impairment of oxygen utilization at the mitochondrial level leads to cytopathic hypoxia (15–19), characterized by impaired adenosine triphosphate (ATP) production (15), despite normal or supranormal tissue pO_2 levels (20). If prolonged and quantitatively relevant, this should be accompanied by decreased oxygen extraction and tissue oxygen consumption, provided that blood flow is adequate.

A hypermetabolic and hyperthermic response to general anesthesia and to stress is a well-known phenomenon in pigs (21–23). If endotoxemia-induced impairment of oxygen extraction limited oxygen consumption, it should be more evident when metabolism is increased by stress associated with major surgery. This study assesses the evolution of systemic and regional oxygen consumption and extraction during prolonged low-dose endotoxemia after major abdominal surgery in pigs.

Research was performed at the Surgical Research Unit, Department of Clinical Research and Clinic for Large Animals, University of Berne, Berne, Switzerland.

Materials and methods

Anesthesia and monitoring

The study was performed in accordance with the National Institutes of Health guidelines for the care and use of experimental animals and with the approval of the Animal Care Committee of the County of Berne, Switzerland.

Twenty-eight pigs (37–42 kg) were deprived of food but not water 24 h before the experiments. They were premedicated with ketamine (20 mg kg^{-1}) and xylazin (xylazinum; G. Streuli & Co. AG, Uznach, CH) (2 mg kg^{-1} intramuscularly), followed by cannulation of an ear vein and intravenous administration of midazolam (0.5 mg kg^{-1}) and atropine (0.02 mg) for endotracheal intubation 10 min later. Anesthesia was maintained with thiopental (7 mg kg^{-1} h^{-1}) and fentanyl (30 mg kg^{-1} h^{-1} until the end of the operation, then 5 mg kg^{-1} h^{-1}). No muscle relaxants were used. The animals were ventilated with a volume-controlled ventilator (Servo Ventilator 900 C, Siemens Elema, Solna, Sweden) with 5 cm H_2O end-expiratory pressure. FiO_2 was adjusted to keep PaO_2 levels between 13.3 kPa (100 mmHg) and 20 kPa (150 mmHg). Tidal volume was kept at 10 ml kg^{-1} body weight and the minute ventilation was adjusted to maintain $PaCO_2$ levels between 4.5 and 5.5 kPa (34–41 mmHg). A transesophageal Doppler probe (CardioQ, Deltex Medical, Inc., Chichester, CT) was inserted orally. A carotid arterial catheter and a pulmonary artery catheter (CO/SVO2 Catheter, Edwards Lifesciences, Irvine, CA, USA) with an optical module for the measurement of O_2 saturation at the tip of the catheter via the left internal jugular vein were inserted. A second Swan-Ganz catheter was inserted into a liver vein through the right internal jugular vein. A sudden drop in the continually measured oxygen saturation helped to identify the correct position, which was confirmed visually at the end of the experiment. When difficulties in positioning occurred, the catheter was manually guided into the liver vein through an incision in the diaphragm after laparotomy. A femoral vein catheter was inserted for fluid administration. During surgery the animals received normal saline at a rate of 8 ml kg^{-1} h^{-1}.

Animal preparation

The abdominal cavity was exposed by a midline abdominal incision. A drainage catheter was inserted into the urinary bladder. A gastric tonometer (Tonometrics® Datex-Engström für Tonometrics Division, Helsinki, Finland) was inserted into the stomach with a small incision. A jejunal tonometer (Tonometrics® Datex-Engström für Tonometrics Division) was inserted 80 cm distal to the ligament of Treitz through a small antimesenteric incision. The superior mesenteric, hepatic, splenic, and kidney arteries, the celiac trunk and the portal vein were exposed, and ultrasound Doppler flow probes (Transonic® System Inc., Ithaca, NY) were placed around the vessels after *in-vitro* calibration. Signals from the flowmeters (T206 and T106, Transonic®) were recorded with a computer program for further analysis (Dataq Instruments, Windaq 1.60, Dataq Instruments Inc., Akron, OH).

A hepatic artery catheter was inserted via the left gastric artery. Two fluid-filled catheters were inserted into a mesenteric vein. The tip of the first catheter was placed into the portal vein while the tip of the second catheter remained in the mesenteric vein. When all surgical procedures were completed, the abdominal wall was reapproximated, and towels were placed on the surface to minimize heat loss. The preparation time was 280 ± 36 min.

Experimental protocol

After preparation, 180 min were allowed for hemodynamic stabilization. Twenty-two animals were initially randomized into two groups (n = 11/group): a control group with saline infusion, and an experimental group with endotoxin infusion. Three animals died early and were excluded from the protocol: one animal in the endotoxin group due to severe hyperthermia and shock during the stabilization phase, and two animals in the control group (one due to major bleeding after 12 h of the experiment, and one due to leakage from the jejunal suture with peritonitis and severe hypotension after 10 h of the experiment). Therefore, the protocol was applied in 19 animals (10 animals with endotoxin infusion and 9 controls).

A separate group of six animals was prepared and treated identically (three with endotoxin infusion and three controls), and used for the assessment of mitochondrial function.

Endotoxin was infused into the right atrium (*Escherichia coli* lipopolysaccharide B0111:B4 [Difco Laboratories, Detroit, MI], 20 mg l^{-1} in 5% dextrose). The initial infusion rate was 0.4 μg kg^{-1} h^{-1} until the mean pulmonary artery pressure reached 40 mmHg. The infusion was then stopped and subsequently adjusted to maintain moderate pulmonary artery hypertension (mean PAP 25–30 mmHg). The adjusted endotoxin infusion rate was maintained constant until the end of the experiment, unless the mean arterial pressure decreased below 50 mmHg with no response to additional fluids. If the hypotension persisted, the infusion was temporarily stopped. Physiogel (Gelatin

4%, Braun, Emmenbrücke, Switzerland) was administered as required to maintain pulmonary artery occlusion pressure between 5 and 8 mmHg. While the primary target variable in fluid management was the pulmonary artery occlusion pressure, additional volume boluses of 50 ml were given as long as the stroke volume increased if hypovolemia was suspected despite target-filling pressure being reached (hypotension, tachycardia, oliguria, increase in arterial lactate concentration). Glucose 50% was administered when blood glucose concentration was less than 3.5 mmol l^{-1}.

At the end of the experiment, the animals were sacrificed with an overdose of intravenous potassium chloride. In the separate group of six animals used for assessment of mitochondrial function, liver biopsies were taken at the end of the experiment.

Hemodynamic monitoring

Carotid and pulmonary arterial, central venous, hepatic venous, and portal venous pressures and pulmonary artery occluded pressure were recorded with quartz pressure transducers, and displayed continuously on a multimodular monitor (Datex-Ohmeda S/5 Compact Critical Care monitor, Datex-Ohmeda, Helsinki, Finland) and recorded. All pressure transducers were calibrated simultaneously and zeroed to the level of the heart. Cardiac output (CO, l min^{-1}) was measured by the thermodilution technique (mean value of three measurements, cardiac output module, Datex-Engström®). Central venous blood temperature (°C) was recorded from the thermistor in the pulmonary artery catheter. Regional blood flows (ml min^{-1}) were recorded by ultrasound transit time flow probes (Transonic®). Heart rate and ECG were continuously monitored. Signals from the laser Doppler flowmeter device were visualized continuously on a computer monitor during probe installation, and the position of the probes could be corrected immediately in case of inadequate signal or motion artefacts. Once the experiment was started, manipulation was avoided to minimize the possibility of probe displacement.

Isolation of liver mitochondria

Isolation was performed at 4°C. A piece of liver (6–8 g) was excised, placed in ice-cold isolation buffer (mannitol 220 mmol l^{-1}, sucrose 70 mmol l^{-1}, morpholinopropane sulfonic acid 5 mmol l^{-1}), and weighed. Tissue was minced with scissors and homogenized with additional homogenization media (isolation buffer plus ethylendinitrilo tetraacetic acid disodium salt dihydrate 2 mmol l^{-1}) in a Potter Elvehjem homogenizer with a loose-fitting pestle (four up and down passes). The homogenate was then centrifuged for 10 min at 700 g. The supernatant was collected and centrifuged again for 10 min at 7000 g. At this time the supernatant was discarded, and the pellet was resuspended with isolation media and centrifuged twice again for 10 min at 7000 g, in order to eliminate organelles and suborganelles of similar weight to mitochondria. The pellets were then suspended in buffer at a final concentration of 50–100 mg of mitochondrial protein per ml. The protein concentration was determined spectrophotometrically with the Biuret method and by comparing the results with standard known concentrations.

Respiratory function with Clark electrode

Mitochondria were incubated in a 3-ml incubation chamber at 30°C, and the respiratory functions were assayed in a medium consisting of KCL 25 mmol l^{-1}, morpholinopropane sulfonic acid 12.5 mmol l^{-1}, ethylene glycol-bis N,N,N′,N′-tetraacetic acid 1 mmol l^{-1}, and phosphate buffer 5 mmol l^{-1}, pH 7.4.

Oxygen consumption was determined using a Clark Type electrode (Yellow Springs Instruments, Yellow Springs, OH) in the presence of glutamate 20 mmol l^{-1}.

State 3 respiration rates were determined using ADP 200 μmol l^{-1}. State 3 represents the phase where oxygen is utilized to transform ADP into ATP, hence to produce energy. The rates that can be measured when ADP has been consumed are called state 4 respiration rates and reflect ADP-independent, nonenergy producing oxygen consumption. Oxygen consumption (O_2 min^{-1} mg protein^{-1}) and the res piratory control ratio (RCR: State 3/State 4) as a marker of the efficiency of oxygen utilization were calculated according to Estabrook (24).

Blood sampling

Blood samples for the measurement of hemoglobin (Hb), lactate, and blood gases (ABL 520 and OSM3, Radiometer, Copenhagen, Denmark) were taken at baseline and after 1.5, 3, 6, and 12 h of endotoxin or saline infusion from the pulmonary and carotid arteries and portal, hepatic, and mesenteric veins.

Oxygen transport variables

The following equations were used.

Oxygen content (ml l^{-1}):

$$\text{Hemoglobin (g } l^{-1}) \times \text{oxygen saturation} \times 1.39 + 0.003 \times pO_2(\text{mmHg}) \quad [1]$$

Systemic oxygen delivery (DO_2) (ml min^{-1} kg^{-1}):

Cardiac index (CI; $l\,min^{-1}kg^{-1}$)
×arterial oxygen content ($ml\,l^{-1}$) [2]

Hepatosplanchnic oxygen delivery ($ml\,min^{-1}\,kg^{-1}$):

(hepatic arterial blood flow [HABF; $l\,min^{-1}kg^{-1}$]
+ portal venous blood flow [PVBF; $l\,min^{-1}kg^{-1}$])
×arterial oxygen content [$ml\,l^{-1}$]) [3]

Systemic oxygen consumption (VO_2) ($ml\,min^{-1}\,kg^{-1}$):

CI ($l\,min^{-1}kg^{-1}$)
×(arterial oxygen content [$ml\,l^{-1}$]
− venous oxygen content [$ml\,l^{-1}$]) [4]

Hepatosplanchnic oxygen consumption ($ml\,min^{-1}\,kg^{-1}$):

(HABF + PVBF) × (arterial oxygen content [$ml\,l^{-1}$]
− hepatic vein oxygen content [$ml\,l^{-1}$]) [5]

Oxygen extraction ratio:

VO_2/DO_2 [6]

Lactate exchange was calculated as:
Hepatosplanchnic lactate exchange ($\mu mol\,min^{-1}\,kg^{-1}$):

(arterial lactate − hepatic vein lactate)
×(HABF + PVBF) [7]

Hepatic lactate exchange ($\mu mol\,min^{-1}\,kg^{-1}$):

([arterial lactate × HABF] + [portal vein lactate
×PVBF]) − (hepatic vein lactate
×[PVBF + HABF]) [8]

Pre-hepatic lactate exchange ($\mu mol\,min^{-1}\,kg^{-1}$):

(arterial lactate − portal vein lactate) × PVBF [9]

Hepatic lactate influx ($\mu mol\,min^{-1}\,kg^{-1}$):

(arterial lactate × HABF) + (portal vein lactate
×PVBF) [10]

Hepatic lactate extraction ratio:

Hepatic lactate exchange/hepatic lactate influx [11]

Carbon dioxide gradients (PCO2 gap) were calculated as the difference between tonometric and arterial PCO2 (kPa).

Statistics

For statistical analysis the SPSS 11.0 software package (SPSS Inc., Chicago, IL, USA)was used. Due to the small size of our groups and the variability of the data, the values were not normally distributed; for that reason, non-parametric tests were used. Results are given as median (range). Groups were compared at baseline by the Mann–Whitney *U*-test. Changes within groups were assessed by the Friedman test. Statistical significance was considered at $P \leq 0.05$.

Results

One of the animals subjected to infusion of endotoxin died at 11 h due to severe septic shock.

Fluid administration ($ml\,kg^{-1}\,h^{-1}$) was similar in both groups (11.1 [8.6–30.3] in controls vs. 10.9 [8.6 –31.7] in endotoxemic pigs; $P = 0.28$). This total included the maintenance infusion of $8\,ml\,kg^{-1}\,h^{-1}$ and the boluses given to achieve the hemodynamic end points during the whole experiment, according to the protocol. Central temperature (°C) was high in both groups at baseline (40.6 [39.7–41.0] in controls and 40.3 [39.5–40.6] in endotoxemic pigs), and did not change over time (Table 1). Hemodynamic variables are shown in Table 1. As shown in Fig. 1, peak pulmonary artery pressures were reached within the first 90 min after the start of endotoxin infusion. The maximal response of the pulmonary artery pressure during endotoxin infusion was 37 mmHg (19–51 mmHg) as compared to 19 mmHg (16–22 mmHg) in the controls. Endotoxin produced an early decrease in stroke volume, while the heart rate increased (Table 1). In contrast, in the control pigs the cardiac performance remained stable (Table 1). Systemic blood pressure decreased over time in both groups; in the endotoxin group the dose of endotoxin had to be reduced due to progressive hypotension in two animals and stopped briefly in five animals.

Oxygen transport variables are shown in Table 2. No differences were found in systemic and hepatosplanchnic oxygen delivery, either at baseline between groups or within groups over time. During the whole experiment, systemic and regional oxygen consumption remained stable in both groups (Table 2).

Metabolic markers are displayed in Table 3. No alterations in the arterial lactate values were found during the whole experiment, and the prehepatic lactate production did not change over time. The hepatic lactate exchange decreased significantly in pigs infused with endotoxin (Table 3). While the hepatic influx did not change significantly during endotoxemia, the hepatic lactate extraction decreased [baseline: 56% (41–66%); end of experiment: 43% (7–62%), $P = 0.05$].

The gastric and jejunal mucosal-arterial pCO_2 gradients – though variable – remained stable and similar in both groups (Table 3).

No correlation was found between hepatic lactate exchange and regional oxygen consumption (Fig. 2). Table 4 shows the effects of endotoxin infusion on

Table 1

Summary of hemodynamic data of the endotoxin-infused group and the control group.

	Baseline	1.5	3	6	12
Mean arterial pressure (mmHg)					
E	68 (53–84)*	65 (51–87)	62 (48–78)	60 (54–80)	56 (39–72)
C	73 (64–80)*	69 (64–78)	63 (58–74)	67 (53–81)	58 (45–87)
Hear rate (beat min^{-1})					
E	107 (89–144)*	125 (98–230)	154 (95–169)	141 (101–204)	127 (90–171)
C	120 (81–150)	122 (92–162)	133 (92–171)	133 (90–162)	133 (107–162)
Pulmonary artery pressure (mmHg)					
E	15.5 (14–23)	20.5 (17–59)	18.5 (14–28)	18 (15–25)	19 (15–22)
C	17 (16–22)	18 (16–20)	17 (15–19)	18 (14–19)	17 (13–21)
Cardiac output (ml kg^{-1} min^{-1})					
E	89 (67–133)	84 (51–149)	74 (46–104)	94 (60–142)	98 (80–126)
C	106 (80–189)	99 (80–139)	97 (72–115)	116 (87–151)	131 (103–161)
Stroke volume (ml kg^{-1})					
E	0.78 (0.58–1.22)**	0.68 (0.34–1.03)	0.55 (0.36–0.67)	0.72 (0.59–1.00)	0.75 (0.48–1.11)
C	0.91 (0.54–2.3)	0.81 (0.57–1.31)	0.74 (0.56–1.20)	0.92 (0.62–1.16)	0.96 (0.67–1.23)
Temperature (°C)					
E	40.3 (39.5–40.6)	40.4 (39.8–41.4)	40.4 (40.0–41.0)	40.4 (39.8–41.3)	40.1 (39.9–40.6)
C	40.6 (39.7–41.0)	40.6 (39.7–41.2)	40.7 (39.3–41.0)	40.2 (39.0–40.6)	40.0 (39.2–40.7)
Hematocrit (%)					
E	26 (22–33)	27 (24–31)	29 (22–31)	24 (18–32)	24 (21–28)
C	27 (20–30)	26 (24–30)	26 (23–33)	24 (21–32)	22 (20–30)

E: endotoxic animals; C: controls.
Freidman test: $*P < 0.05$, $**P < 0.01$.
Values are expressed as median (range).

mitochondrial respiration: a severe decrease in ADP-dependent oxygen utilization was accompanied by an increase in oxygen consumption in the absence of ADP. Consequently, the efficiency of the mitochondrial respiration was impaired

Discussion

The main finding in this study was the preservation of systemic and hepatosplanchnic oxygen consumption at a high extraction rate during prolonged endotoxemia. The increased systemic oxygen extraction maintained stable oxygen consumption even during the hypodynamic phase of shock. Maintenance of hepatic oxygen extraction in both early sepsis and septic shock has recently been described by others (25). Hart et al. found no alterations in the concentrations of adenine nucleotides or NAD (+)/NADH within the liver in early sepsis, while hepatic concentrations of ATP and NADH decreased during septic shock. In our study, the main metabolic alteration was a progressive decrease in the ability of the liver to extract lactate in the presence of well-preserved oxygen uptake. This suggests that the metabolic utilization of lactate in the liver was impaired independent of oxygen utilization. The mitochondrial respiratory efficiency was severely impaired during endotoxin infusion, suggesting that the oxygen use shifted from being potentially efficient (ATP production) to being futile (without energy production); a condition defined as partial uncoupling of mitochondrial oxidative phosphorylation. Since we did not measure ATP production and ATP-ase activity, as others did (26), we cannot exclude that other mechanisms could be involved. On the other hand, the lack of correlation between hepatic lactate derangements and oxygen consumption is consistent with the uncoupling hypothesis. This further calls into question the value of VO_2 monitoring during endotoxemia.

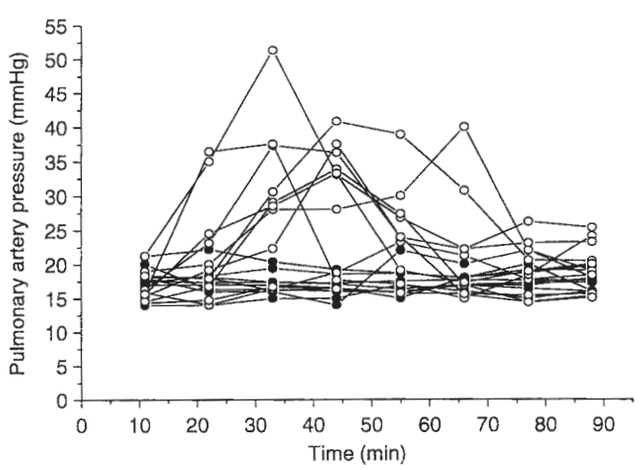

Fig. 1. Pulmonary artery pressure in the control animals (●) and the endotoxin-infused animals (○) during the first 90 min of endotoxin or placebo infusion.

Table 2

Systemic and hepatosplanchnic oxygen delivery, consumption and extraction.

	Baseline	1.5 h	3 h	6 h	12 h
Systemic DO2 (ml kg^{-1} min^{-1})					
E	11.2 (7.7–17.1)	9.9 (7.0–17.4)	8.9 (6.6–14.1)	10.7 (7.7–20.6)	10.7 (9.1–14.8)
C	13.7 (10.9–17.8)	12.1 (10.0–15.0)	11.3 (8.7–14.6)	12.9 (9.9–15.8)	13.7 (9.7–16.0)
Hepatosplanchnic DO2 (ml kg^{-1} min^{-1})					
E	2.6 (1.9–4.5)	2.4 (2.0–4.4)	2.1 (1.1–4.2)	2.5 (1.9–5.0)	2.8 (1.9–3.9)
C	3.0 (2.2–8.0)	3.0 (2.1–5.0)	3.1 (2.0–4.4)	3.0 (2.9–4.5)	3.3 (2.0–4.5)
Systemic VO2 (ml kg^{-1} min^{-1})					
E	6.5 (4.4–8.4)	6.8 (5.0–9.3)	6.1 (4.9–7.9)	6.9 (5.8–9.7)	6.7 (5.1–8.9)
C	8.0 (7.3–11.6)	8.1 (6.4–9.1)	7.7 (6.0–9.5)	8.1 (6.4–8.8)	7.8 (5.2–9.5)
Hepatosplanchnic VO2 (ml kg^{-1} min^{-1})					
E	1.9 (1.6–3.3)	1.9 (1.4–2.7)	1.8 (1.1–2.6)	2.1 (1.5–3.5)	2.1 (1.7–2.7)
C	2.2 (1.5–3.4)	2.1 (1.5–3.1)	2.1 (1.7–3.1)	2.1 (1.7–3.0)	2.3 (1.9–3.0)
Systemic oxygen extraction (%)					
E	56 (45–77)*	64 (47–75)	72 (48–84)	64 (47–79)	60 (50–71)
C	67 (49–72)	67 (57–68)	67 (60–76)	60 (51–76)	60 (50–74)
Hepatosplanchnic oxygen extraction (%)					
E	80 (54–84)	75 (60–88)	86 (63–95)	83 (67–88)	78 (58–88)
C	76 (58–87)	72 (63–86)	70 (59–90)	72 (54–85)	69 (49–91)

E = endotoxic animals; C = controls; DO2 = oxygen delivery; VO2 = oxygen consumption.
Freidman test: $*P < 0.05$.
Values are expressed as median (range).

Table 3

Arterial lactate, regional lactate exchange and influx, mucosal-arterial PCO2 gradients.

	Baseline	1.5 h	3 h	6 h	12 h
Arterial lactate (mmol l^{-1})					
E	1.0 (0.7–1.7)	1.1 (1.0–1.4)	1.2 (0.6–1.7)	1.1 (0.9–1.6)	0.8 (0.5–1.3)
C	1.0 (0.8–1.6)	0.9 (0.7–1.4)	0.9 (0.7–1.4)	0.8 (0.5-1.5)	0.7 (0.5–1.4)
Pre-hepatic lactate exchange (μmol kg^{-1} min^{-1})					
E	−3.1 (−5.9–0.0)	−2.9 (−4.6–0.0)	−2.5 (−9.0–0.0)	−3.8 (−8.3–0.0)	−4.0 (−7.3–0.0)
C	−1.5 (−5.3–0.0)	−5.1 (−7.5–0.0)	−3.9 (−9.9– −1.5)	−4.3 (−8.2–4.1)	−(−13.0–2.8)
Hepatosplanchnic lactate exchange (μmol kg^{-1} min^{-1})					
E	10.9 (5.9–24.7)*	9.1 (0.0–18.7)	6.0 (−10.3–10.2)	6.4 (4.4–13.7)	3.9 (0.0–12.7)
C	10.6 (1.6–16.5)	5.9 (−4.4–10.8)	6.8 (−5.8–13.2)	7.9 (0.0–15.8)	4.1 (−6.0–9.5)
Hepatic lactate extraction (%)					
E	56 (41–66)**	44 (14–66)	43 (−5.0–65)	40 (19–54)	43 (7–62)
C	41 (13–60)	38 (3–58)	39 (−9–56)	42 (14–63)	31 (10–49)
Hepatic lactate influx (μmol kg^{-1} min^{-1})					
E	24.9 (19.1–41.9)	26.0 (20.4–37.4)	24.8 (13.7–29.2)	28.1 (19.7–47.3)	21.7 (16.4–51.0)
C	30.1 (15.6–67.9)	29.0 (17.0–34.8)	28.7 (16.4–38.1)	28.1 (16.6–37.7)	24.2 (13.3–41.2)
Hepatic lactate exchange (micromol min^{-1})					
E	14.5 (10.8–27.8)***	12.3 (3.7–18.7)	9.6 (−1.3–17.3)	10.5 (4.4–19.8)	12.5 (3.9–18.3)
C	9.7 (3.3–14.9)	11.0 (0.9–16.5)	12.1 (−2.3–16.6)	10.8 (3.3–17.2)	6.9 (2.7–17.1)
Gastric mucosal-arterial PCO2 gradient (kPa)					
E	3.6 (1.7–16.4)	3.8 (2.1–16.5)	4.5 (0.5–17.5)	5.8 (1.7–17.0)	4.2 (2.8–12.2)
C	2.6 (1.1–17.5)	3.0 (0.4–17.1)	2.9 (1.0–17.2)	2.9 (0.8–17.4)	6.3 (0.0–11.3)
Jejunal mucosal-arterial PCO2 gradient (kPa)					
E	3.8 (−0.6–6.4)	4.3 (−4.2–7.3)	3.6 (−3.9–9.0)	4.4 (−3.6–9.8)	4.1 (−3.0–7.5)
C	4.1 (2.2–5.1)	3.4 (2.4–5.6)	3.4 (2.5–5.6)	3.0 (2.2–4.9)	3.3 (2.8–9.3)

E = endotoxic animals; C = controls.
Freidman test: $*P < 0.05$, $**P = 0.05$, $***P < 0.01$.
Values are expressed as median (range).

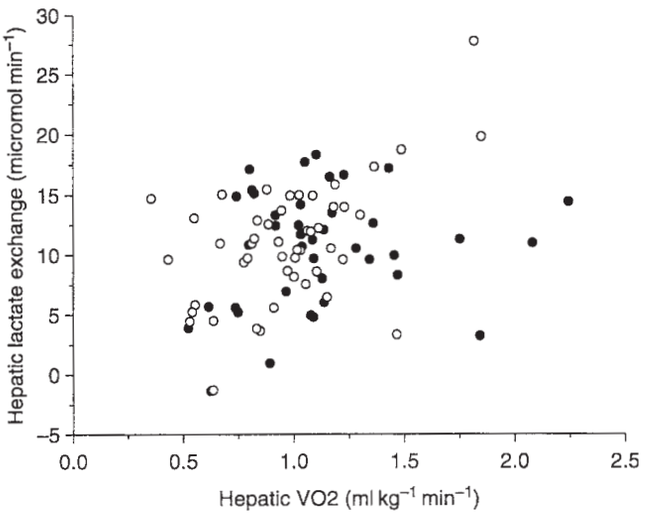

Fig. 2. No significant correlation was found in the control animals (●) and the endotoxin-infused animals (○) between hepatic lactate exchange and hepatic oxygen consumption (hepatic VO_2).

Since one of the goals of the experiment was that the animals survive for 12 h, a low dose of endotoxin was administered in order to avoid sudden or early death. Nevertheless, the quantity given to each pig was in the range of the endotoxin amount required to induce 'hyperdynamic hypotensive shock' (27).

A low dose of endotoxin produced the characteristic hemodynamic response (28–30), with a rapid increase in pulmonary artery pressure (31), followed by an early hypodynamic phase, with a decrease in stroke volume and an increase in heart rate. This was followed by a gradual recovery of stroke volume and progressive hypotension. Despite the characteristic hemodynamic changes, endotoxin had no effect on oxygen consumption, and the high systemic and regional oxygen extraction was sustained.

Volume management in experimental sepsis may markedly modify the metabolic and hemodynamic responses. Our strategy, based on target-filling pressures and assessment of response of stroke volume to additional fluid boluses, resulted in relatively low total volumes of fluid administration as compared to some other models (14, 32) Nevertheless, it corresponds to more than 10 l of fluid/24 h for an adult patient. Had this been too restrictive, any impairment in oxygen utilization should have been more evident.

Table 4

Liver mitochondrial glutamate-dependent oxygen consumption.	
State 3 (nanoatom O2 min^{-1} mg protein^{-1})	
E	29.7 (24.5–35.6)
C	41.2 (39.0–44.6)
State 4 (nanoatom O2 min^{-1} mg protein^{-1})	
E	10.8 (8.9–12.2)
C	7.4 (6.7–8.9)
RCR (State 3/State 4)	
E	2.8 (2.4–3.2)
C	5.6 (5.0–6.0)

Values are expressed as median (range).

Our model has some limitations: the high body temperature in all animals suggests that the animals were susceptible to a pathological thermal response to stress and anesthesia. A hyperthermic reaction to anesthesia and stress in pigs is a well-known entity (21–23). It is associated with hypermetabolism and increased mortality, and is also known as porcine stress syndrome (PSS). The main characteristics are a rapid rise in heart rate and cardiac output, accompanied by an increase in oxygen consumption and carbon dioxide production, and an extremely high oxygen extraction ratio, at around 70–80%. Later findings are low blood pressure, decreased cardiac output, and eventually death. Animals suffering from PSS exhibit significantly higher exo-NADH oxidase activity compared with normal animals, while other mitochondrial parameters seem to be unaffected by the PSS condition (33). A major impairment in oxygen utilization induced by endotoxin should be readily evident in such a condition of increased metabolic demands. Alternatively, given the complex operation without using prophylactic antibiotics, the increase in temperature could raise concern of an early infection. However, we have performed similarly complex surgical procedures in other strains of animals before without using prophylactic antibiotics, and did not observe an increase in temperature with them (34). Furthermore, we used a fully sterile technique throughout the experiment.

Effects of analgesic and anesthetic drugs may influence systemic and hepatic blood flow. However, the fentanyl dose administered (35) and the type of anesthetic used (36) are known to have minimal effect on liver perfusion.

In summary, our results indicate that in this model of prolonged endotoxemia, there is no evidence of a major impairment of oxygen extraction. We cannot exclude the possibility that changes in oxygen utilization could be present in other septic models or in clinical sepsis. We assume that the observed changes in hepatic function indicate that impairment in organ function develops during sepsis in the presence of normal oxygen consumption.

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