British Journal of Pharmacology (1998) 124, 1689–1697
http://www.stockton-press.co.uk/bjp

Effect of increased cardiac output on liver blood flow, oxygen exchange and metabolic rate during longterm endotoxin-induced shock in pigs

[1]Borislav Šantak, [1,2]Peter Radermacher, [1]Jens Adler, [1]Thomas Iber, [1]Karen M. Rieger, [1]Ulrich Wachter, [1]Josef Vogt, [1]Michael Georgieff & [1]Karl Träger

[1]Sektion Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Universitätsklinik für Anästhesiologie, Klinikum der Universität, D-89073 Ulm, Germany

1 We investigated hepatic blood flow, O_2 exchange and metabolism in porcine endotoxic shock (Control, $n=8$; Endotoxin, $n=10$) with administration of hydroxyethylstarch to maintain arterial pressure (MAP) > 60 mmHg.

2 Before and 12, 18 and 24 h after starting continuous i.v. endotoxin we measured portal venous and hepatic arterial blood flow, intracapillary haemoglobin O_2 saturation (Hb-O_2%) of the liver surface and arterial, portal and hepatic venous lactate, pyruvate, glyercol and alanine concentrations. Glucose production rate was derived from the plasma isotope enrichment during infusion of [6,6-$^2H_2$]-glucose.

3 Despite a sustained 50% increase in cardiac output endotoxin caused a progressive, significant fall in MAP. Liver blood flow significantly increased, but endotoxin affected neither hepatic O_2 delivery and uptake nor mean intracapillary Hb-O_2% and Hb-O_2% frequency distributions.

4 Endotoxin nearly doubled endogenous glucose production rate while hepatic lactate, alanine and glycerol uptake rates progressively decreased significantly. The lactate uptake rate even became negative ($P<0.05$ vs Control). Endotoxin caused portal and hepatic venous pH to fall significantly concomitant with significantly increased arterial, portal and hepatic venous lactate/pyruvate ratios.

5 During endotoxic shock increased cardiac output achieved by colloid infusion maintained elevated liver blood flow and thereby macro- and microcirculatory O_2 supply. Glucose production rate nearly doubled with complete dissociation of hepatic uptake of glucogenic precursors and glucose release. Despite well-preserved capillary oxygenation increased lactate/pyruvate ratios reflecting impaired cytosolic redox state suggested deranged liver energy balance, possibly due to the O_2 requirements of gluconeogenesis.

Keywords: Endotoxin; septic shock; hepatic blood flow; hepatic O_2 exchange; capillary haemoglobin O_2 saturation; remission spectrophotometry; hepatic glucose precursor uptake; gluconeogenesis; stable isotope infusion; lactate/pyruvate ratio

Introduction

Deterioration of organ function ultimately leading to multiple organ failure and death (Vincent *et al*., 1996) is a common feature in patients with endotoxaemia, sepsis and septic shock (Danner *et al*., 1991). Since elevated cardiac output was associated with improved survival (Tuchschmidt *et al*., 1992) impaired nutrient blood flow to the tissues is regarded as a key determinant for the progression of the syndrome (Vincent, 1996). In this context, the perfusion of the hepato-splanchic region, in particular the gut (Carrico *et al*., 1986) and the liver (Hawker, 1994), seems to play a pivotal role (Dantzker, 1993). In fact, liver mitochondrial phosphorylative activity was closely correlated to cardiac index in pigs with peritonitis induced by caecal ligation and perforation (Hirai *et al*., 1984). Moreover, when normotensive systemic haemodynamics with increased cardiac output were maintained by volume resuscitation with dextran both total liver blood flow and O_2 extraction were preserved in a porcine model of septic shock (Rasmussen *et al*., 1992). Therefore maintaining or even rising hepatic blood flow by increasing cardiac output, in particular with fluid administration, has been advocated to provide adequate substrate delivery to the liver and thereby to preserve hepatic metabolic function in patients with sepsis (Pastor *et al*., 1995). It should be noted, however, that the approach of maintaining systemic and hepato-splanchnic O_2 availability by increased cardiac output due to volume resuscitation alone failed to prevent hepatic ultrastructural lesions in pigs with peritonitis (Tighe *et al*., 1995).

Therefore the objective of the present study was to investigate liver blood flow, O_2 exchange and metabolism during a longterm endotoxin-induced shock in pigs. Normotensive haemodynamics with a sustained increase in cardiac output were achieved by volume resuscitation with hydroxyethylstarch, and the rate of gluconeogenesis, a highly O_2 consuming metabolic pathway accounting for 50–80% of total hepatic O_2 uptake (Jungas *et al*., 1992) was determined to estimate hepatic metabolic rate. The pig model was chosen because of its value as a large human-like biology (Douglas, 1972) and the possibility to assess liver haemodynamics, O_2 transport and metabolism without interference of other splanchnic organs. We hypothesized that increased hepatic metabolic rate associated with endotoxic shock causes derangements of liver energy balance despite maintenance of liver perfusion and oxygenation.

[2]Author for correspondence at: Universitätsklinik für Anästhesiologie, Sektion Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Klinikum der Universität, Parkstrasse 11, D-89073 Ulm (Donau), Germany.

Methods

Animal preparation

The study protocol was approved by the University Animal Care Committee as well as the federal authorities for animal research of the Regierungspräsidium Tübingen, Baden-Württemberg, Germany. Eighteen domestic pigs (41 ± 4 kg) were fasted for 24 h with water *ad libitum*. The animals were anaesthetized with intramuscular atropine (2.5 mg; Atropinsulfat®, Braun, Melsungen, Germany) and azaperone (150–200 mg; Stresnil®, Janssen, Neuss, Germany) followed by cannulation of an ear vein and i.v. administration of sodium pentobarbital (10 mg kg^{-1}; Nembutal®, Sanofi Wintrop, Munich, Germany) and ketamine (1.5–2.0 mg kg^{-1}; Ketavet®, Parke-Davis, Berlin, Germany). The pigs were orally intubated, and their lungs were mechanically ventilated (PEEP 5 cmH_2O; Servo 900B, Siemens, Erlangen, Germany) with a tidal volume of 15 ml kg^{-1} at a respiratory rate of 10–12 breaths min^{-1} adjusted to maintain $PaCO_2$ between 35–40 mmHg. During the surgical preparation the inspired gas mixture consisted of N_2O and O_2 (FiO_2 0.35), during the observation period of air and O_2 with the FiO_2 adjusted to provide an arterial haemoglobin O_2 saturation $>90\%$. Anaesthesia was maintained with continuous i.v. pentobarbital (300 mg h^{-1}), and depth of anaesthesia was controlled by continuous EEG monitoring (Neurotrac, Interspec Inc., Cornshohocken, PA, U.S.A.). The spectral edge frequency was always below 15 Hz, the median power frequency was 5–10 Hz. In previous experiments this pentobarbital infusion rate allowed provision of full anaesthesia without any additional muscle relaxation (Šantak *et al*., 1997). Buprenorphine (0.3 mg; Temgesic®, Boehringer, Mannheim, Germany) i.v. was added every 4 h and prior to any surgical or noxious stimuli in order to prevent a rise in heart rate and arterial pressure due to inadequate anaesthesia. Muscle paralysis was obtained with alcuronium (14 mg h^{-1}; Alloferin®, Hoffmann-La Roche AG, Basel, Switzerland). The right and left jugular veins and a submandibular vein as well as the right and left femoral arteries were surgically exposed. A central venous catheter for drug, isotope and fluid infusion was inserted into the superior V. cava, a balloon-tipped thermodilution pulmonary artery catheter (93A 754 7F, Baxter Healthcare, Irvine, CA, U.S.A.) was placed for the measurement of central venous, mean pulmonary artery (PAP) and pulmonary artery occluded pressure (Medex MX 80 pressure transducers, Medex Inc., Hillard, OH, U.S.A.). In one femoral artery a catheter was placed for continuous blood pressure recording and blood sampling, in the other one a 3 Fr thermistor-tipped fibreoptic catheter for thermal-dye double indicator dilution measurements (FT-Pulsiocath PV 2023, Pulsion, Munich, Germany). Ringer's lactate (10 ml kg^{-1} h^{-1}) was infused i.v. as maintenance fluid. A midline laparotomy was performed, and precalibrated ultrasonic flow probes (Transonic Systems, Ithaca, NY, U.S.A.) were placed around the portal vein and the common hepatic artery distal to the takeoff of the gastroduodenal artery. Care was taken to minimize dissection of the common hepatic artery to avoid disrupting the nervous sheath. A 4 Fr catheter (CS-16402, Arrow, Reading, PA, U.S.A.) was then introduced into the portal vein, and an angiography catheter (7F Multipurpose A-1, Cordis, Roden, The Netherlands) was introduced via the right jugular vein into a hepatic vein under ultrasound guidance. For the assessment of the ascites formation a drainage tube was placed through the abdominal wall, and a glass tube was inserted through the abdominal wall onto the liver surface for the placement of the remission spectrophotometry lightguide. A stabilization period of at least 8 h was allowed before baseline measurements were recorded.

Measurements and calculations

Cardiac output was determined by thermodilution (66S Monitor, Hewlett Packard, Palo Alto, CA, U.S.A.), the data reported being the mean of 4–5 injections of 10 ml ice-cold saline randomly spread over the respiratory cycle. The intrathoracic blood volume was measured by arterial thermal-green dye double indicator dilution (COLD Z-021, Pulsion, Munich, Germany) after injection of 12 ml of ice-cold indocyanine-greene (2.5 mg ml^{-1} in distilled water). The continuously recorded portal venous and hepatic arterial blood flow rates were measured using the precalibrated ultrasonic flow probes and summed to obtain the total hepatic blood flow. Arterial, mixed venous as well as portal and hepatic venous blood samples were analysed for PO_2, PCO_2, and pH (NOVA Stat 5, Biomedical, Waltham, MA, U.S.A.) as well as total haemoglobin and haemoglobin O_2 saturation (IL 482 CO-Oximeter, Instrumentation Laboratories, Lexington, MA, U.S.A., calibrated for pig blood). Systemic O_2 delivery was calculated as the product of cardiac output and arterial O_2 content. Hepatic O_2 delivery and O_2 uptake were calculated as the product of portal venous and hepatic arterial blood flow times the portal venous and the arterial O_2 content, respectively, and the portal-hepatic venous and the arterial-hepatic venous O_2 content differences where appropriate. Arterial blood glucose levels were measured every 2 h using an automatic enzymatic glucose analyzer (Glucometer Elite®, Bayer AG, Leverkusen, Germany). Systemic CO_2 production was continuously measured directly from the respiratory gases and recorded minute by minute using a Deltatrac® Metabolic Monitor (Datex, Helsinki, Finland). Since progressive respiratory failure developed during endotoxic shock the FiO_2 had to be continuously increased in order to maintain arterial haemoglobin O_2 saturation $>90\%$ in these animals. Therefore, it was not possible to keep the $FiO_2 < 0.6$ which is mandatory for the reliable measurement of O_2 uptake using the Deltatrac® device (Takala *et al*., 1989). Systemic O_2 uptake, hence, was calculated as the product of cardiac output times the arterial-mixed venous O_2 content difference. Body temperature was kept within 0.5°C of the baseline value using a heating mattress or external ice cooling as necessary.

Capillary haemoglobin O_2 saturation (Hb-O_2%) on the liver was measured using remission spectrophotometry (Frank *et al*., 1989; Hasibeder *et al*., 1996) using the Erlanger Microlightguide Spectrophotometer device (EMPHO, Bodenseewerk Gerätetechnik, Überlingen, Germany). Briefly, light emitted from a xenon-lamp is radiated into the tissue via a single highly flexible micro-lightguide. The remitted light is collected via six identical recipient light conductors which are arranged around the circumference of the illuminating fibre. Both the collecting and the emitting micro-lightguides are encased in a flexible rubber tube. The remitted light passes through a rapidly rotating filter-disc serving as as monochromating unit, and extinction signals for a wavelength range from 502 to 628 nm are recorded allowing for the construction of haemoglobin absorption spectra. The mean as well as the frequency distribution of the capillary Hb-O_2% are then calculated by using an algorithm (Frank *et al*., 1989) based on the fact that the characteristics of the remitted light are determined by the basic tissue absorption and scattering, the respective concentrations of oxygenated and deoxygenated haemoglobin and wavelength-dependent scattering (Frank *et*

al., 1989). For this purpose the micro-lightguide was placed on the liver surface through the glass tube which had been passed through the abdominal wall. It was carefully checked that gentle contact with the liver surface was continuously achieved without exerting pressure which would *per se* influence tissue microcirculatory blood flow and thereby intracapillary haemoglobin content and Hb-O_2. The reported values of the capillary Hb-O_2% are the mean of 300 spectra recorded at six different measurement sites, i.e. a total of 1800 spectra was analysed for each data point in every animal.

Arterial, portal and hepatic venous lactate and glycerol concentrations were enzymatically measured in duplicate using an automatic whole blood lactate analyzer (YSI Modell 2300 STAT, Schlag Wissenschaftliche Messinstrumente, Bergisch-Gladbach, Germany) and a commercially available enzymatic test kit (Boehringer, Mannheim, Germany), respectively. Arterial, portal and hepatic venous alanine levels were assessed in duplicate with the ninhydrine-reaction after separation with high-performance liquid chromatography (Amino Acid Analyzer LC 3000, Biotronik, Hamburg, Germany). For the determination of the arterial and portal as well as hepatic venous lactate/pyruvate ratios (Control group $n=5$, Endotoxin group $n=7$) the lactate and pyruvate concentrations were measured in duplicate spectrophotometrically (Czok & Lamprecht, 1970; Hohorst, 1970). The coefficients of variation for the lactate, pyruvate, glycerol and alanine measurements were 1.8, 9.2, 2.2 and 6.3%. Hepatic lactate, glycerol and alanine fluxes were subsequently calculated as the product of portal venous and hepatic arterial blood flow times the portal-hepatic venous and the arterial-hepatic venous concentration differences, respectively.

Endogenous glucose production rate was determined as described previously (Reinelt *et al*., 1997). Briefly, stable, non-radioactive isotope-labelled [6,6-$^2H_2$]-glucose (Mass Trace, Woburn, MA, U.S.A.) was dissolved in physiologic saline (50 mg ml^{-1}) and continuously infused for 120 min (0.1 mg kg^{-1} min^{-1}) after a priming dose of 8.0 mg kg^{-1}. The glucose rate of appearance (Ra) was derived from the arterial plasma isotope enrichment, and the endogenous glucose production rate was subsequently calculated as the difference between Ra and the infusion rate of unlabelled glucose. The plasma isotope enrichment used for the computation of Ra was the corresponding mean of triplicate blood samples obtained within 10 min each and analysed by gas chromatography-mass spectrometry (Hewlett Packard GC 5890, MS 5970; Hewlett Packard, Palo Alto, CA, U.S.A.) in the selected-ion monitoring-mode using electron impact ionization after derivatization of glucose to 1,5-penta-acetate.

Protocol

The animals were were randomly assigned to two groups: Control ($n=8$) and Endotoxin ($n=10$). After recording baseline measurements an endotoxin (*E. Coli* lipopolysaccharide B 0111:B4, DIFCO Laboratories, Detroit, MC, U.S.A.; 20 mg l^{-1} in 5 % dextrose) (4 ml h^{-1}), or saline infusion was started. The endotoxin infusion rate was incrementally increased every 20 min until mean PAP reached 50 mmHg and then subsequently adjusted to result in moderate pulmonary hypertension with mean PAP $\approx$ 35–40 mmHg. This endotoxin infusion rate was maintained until the end of the experiment. Hydroxyethylstarch (Infukoll® HES 6%, Serumwerk, Bernburg, Germany) was administered as required to maintain mean arterial pressure (MAP) > 60 mmHg, and a mixture of 10% glucose and xylitol each (GX 20%, Pharmacia, Erlangen, Germany) was infused to maintain arterial blood glucose levels between 5–7 mmol l^{-1}. Further haemodynamic, gas exchange and metabolic measurements were recorded 12, 18, and 24 h after the start of the endotoxin or saline infusion, respectively. After the last set of data had been obtained the animals were killed by KCl injection.

Table 1 Time-dependent variables of systemic haemodynamic and O_2 exchange parameters

		Baseline	*12 h after start of endotoxin*	*18 h after start of endotoxin*	*24 h after start of endotoxin*
MAP	Control	82±13	87±10	81±9	84±13
mmHg	Endotoxin	84±7	77±7	76±7#	64±9#§
PAP	Control	19±3	23±3	20±2	21±3
mmHg	Endotoxin	20±3	30±4#§	35±4#§	36±4#§
PAOP	Control	7±3	7±3	6±3	7±3
mmHg	Endotoxin	5±2	10±3	9±2	10±4
CVP	Control	6±2	7±2	6±2	7±2
mmHg	Endotoxin	5±2	10±3	9±2	9±4
CO	Control	103±14	116±23	109±12	122±32
ml min^{-1} kg^{-1}	Endotoxin	103±13	157±28#§	184±27#§	159±11#§
ITBV	Control	23±3	23±3	24±3	24±3
ml kg^{-1}	Endotoxin	24±5	30±2#§	31±3#§	30±4#§
DO_2sys	Control	12.4±1.8	12.2±1.6	11.6±1.5	12.8±1.8
ml min^{-1} kg^{-1}	Endotoxin	12.1±1.6	17.3±2.8#§	18.3±3.7#§	15.1±5.6#
VO_2sys	Control	4.6±0.5	4.5±0.7	3.9±0.6	4.1±0.7
ml min^{-1} kg^{-1}	Endotoxin	4.2±0.7	4.2±0.8	4.7±0.6	4.7±0.7
VCO_2sys	Control	4.1±0.6	4.0±0.4	4.2±0.6	4.0±0.4
ml min^{-1} kg^{-1}	Endotoxin	4.0±0.3	4.5±0.5	4.6±0.4#	4.7±0.4#§
$PaCO_2$	Control	39±3	40±2	41±3	40±3
mmHg	Endotoxin	39±2	44±5	44±5	46±7
pHa	Control	7.47±0.03	7.47±0.02	7.47±0.03	7.47±0.03
	Endotoxin	7.48±0.02	7.40±0.05#§	7.40±0.04#§	7.38±0.08#§

MAP mean arterial pressure; PAP mean pulmonary artery pressure; PAOP pulmonary artery occluded pressure; CVP central venous pressure; CO cardiac output; ITBV intrathoracic blood volume; DO_2sys systemic O_2 delivery; VO_2sys systemic O_2 uptake; VCO_2sys systemic CO_2 production; $PaCO_2$ arterial CO_2 partial pressure; pHa arterial pH. Data are mean±s.d. (Control $n=8$, Endotoxin $n=10$). # designates significant difference within each group vs baseline, § designates significant difference between Control and Endotoxin groups.

Statistical analysis

All values recorded are mean ± s.d.. After exclusion of normal distribution differences between baseline values and those during endotoxin or placebo infusion within each group were tested using a Friedman rank sign analysis of variance and a subsequent Wilcoxon-Wilcox test for multiple comparisons. A $P<0.05$ was regarded as significant. Differences between the Control and the Endotoxin group were analysed using a Mann-Whitney U-test for independent samples with α-adjustment according to Bonferroni. A $P<0.0125$ (0.05 : 4) was regarded as significant.

Results

The systemic haemodynamic, O_2 exchange and acid-base responses to the endotoxin infusion are summarized in Table 1. There was no change over time in any of these parameters in the Control animals. Endotoxin caused a sustained increase of mean PAP ($P<0.05$ vs baseline and vs Control), and due to the colloid infusion intended by the protocol the intrathoracic blood volume was higher than in the Control group ($P<0.05$ vs baseline and vs Control) resulting in an increase of cardiac output by about 50% ($P<0.05$ vs baseline and vs Control). Despite the increased cardiac output MAP progressively dropped over time ($P<0.05$ vs baseline) in the Endotoxin group reaching significantly lower values than in the Control group at the end of the experiment ($P<0.05$ vs Control). The increased cardiac output was paralleled by a higher systemic O_2 delivery than in the Control animals ($P<0.05$ vs baseline and vs Control), but there was no significant effect on systemic O_2 uptake in either of the two groups. In contrast, systemic CO_2 production significantly increased in the Endotoxin animals ($P<0.05$ vs baseline) while there was no change in the Control group. These calorimetry results yielded a 15% increase of metabolic energy expenditure as calculated from O_2 uptake and CO_2 production, respectively (Ferrannini 1988). Endotoxin caused a significant fall of pHa over time ($P<0.05$ vs baseline and vs Control) without change of the arterial PCO_2.

The hepatic haemodynamic, O_2 transport and metabolic responses are summarized in Table 2. There was no change in any of these parameters in the Control group. While hepatic arterial flow was not significantly influenced in the Endotoxin group portal venous flow increased ($P<0.05$ vs baseline) resulting in increased total liver blood flow ($P<0.05$ vs baseline). Hepatic O_2 delivery, however, did not change. This unaltered macrocirculatory liver O_2 supply was associated with constant microcirculatory O_2 availability as documented by the unchanged mean capillary Hb-O_2% and Hb-O_2% frequency distribution (Figure 1). While hepatic O_2 uptake did not change either (Table 2) endogenous glucose production rate nearly doubled in the Endotoxin group (Figure 2c) ($P<0.05$ vs baseline and vs Control) despite the progressive fall in hepatic glycerol (Table 2), alanine (Figure 2a) and lactate (Figure 2b) uptake rates until the end of the experiment ($P<0.05$ vs baseline). Hepatic gycerol and lactate uptake rates ($P<0.05$ vs Control) even became negative at the end of the experiment. The reversal of hepatic lactate balance was accompanied by increased arterial, portal and hepatic venous lactate/pyruvate ratios (Figure 3) ($P<0.05$ vs baseline) and a decrease in both portal and hepatic venous pH (Table 2) ($P<0.05$ vs baseline and Control).

Discussion

The present study was performed to investigate liver blood flow, O_2 exchange and metabolism during a long-term endotoxin-induced shock in the pig in which appropriate colloid infusion had allowed us to obtain normotension and a sustained increase in cardiac output. The rate of gluconeogenesis was measured to estimate hepatic metabolic rate since *de novo* glucose synthesis is a highly O_2 consuming metabolic pathway accounting for 50–80% of total hepatic O_2 uptake (Jungas *et al*., 1992). The major finding was that, although increasing cardiac output allowed to achieve elevated total liver blood flow and thereby to maintain hepatic macro and

Table 2 Time-dependent variations of liver haemodynamic and O_2 exchange parameters

		Baseline	*12 h after start of endotoxin*	*18 h after start of endotoxin*	*24 h after start of endotoxin*
Qpv	Control	971 ± 422	1073 ± 330	1085 ± 259	1070 ± 320
ml min^{-1}	Endotoxin	793 ± 299	967 ± 321#	1079 ± 320#	1128 ± 323#
Qha	Control	140 ± 78	145 ± 96	148 ± 87	142 ± 83
ml min^{-1}	Endotoxin	157 ± 89	132 ± 72	227 ± 101	177 ± 96
Qhep	Control	1111 ± 416	1218 ± 333	1234 ± 289	1212 ± 362
ml min^{-1}	Endotoxin	950 ± 262	1098 ± 326	1306 ± 340#	1305 ± 386#
DO_2hep	Control	92 ± 45	95 ± 31	94 ± 29	99 ± 30
ml min^{-1}	Endotoxin	81 ± 26	92 ± 35	100 ± 31	96 ± 18
Liver	Control	53 ± 7	49 ± 7	54 ± 7	50 ± 7
cap-Hb-O_2%	Endotoxin	55 ± 5	52 ± 7	52 ± 7	47 ± 10
VO_2hep	Control	21 ± 7	21 ± 12	26 ± 10	25 ± 13
ml min^{-1}	Endotoxin	28 ± 17	28 ± 16	22 ± 10	20 ± 12
VGlyc-hep	Control	0.6 ± 0.2	0.2 ± 0.5	−0.2 ± 1.4	−0.2 ± 0.8
μmol min^{-1} kg^{-1}	Endotoxin	−0.3 ± 0.6	0.3 ± 2.1	−1.0 ± 3.3	−3.7 ± 7.1#
pHpv	Control	7.42 ± 0.02	7.43 ± 0.02	7.42 ± 0.03	7.42 ± 0.03
	Endotoxin	7.42 ± 0.03	7.35 ± 0.05#§	7.36 ± 0.04#§	7.36 ± 0.08#§
pHhv	Control	7.44 ± 0.02	7.43 ± 0.02	7.43 ± 0.02	7.42 ± 0.03
	Endotoxin	7.45 ± 0.02	7.35 ± 0.02#§	7.36 ± 0.04#§	7.34 ± 0.08#

Qpv, Qha and Qhep portal venous, hepatic arterial and total hepatic blood flow; DO_2hep hepatic O_2 delivery; Liver cap-Hb-O_2 mean hepatic capillary haemoglobin O_2 saturation; VO_2hep liver O_2 uptake; VGlyc-hep hepatic glycerol balance; pHpv and pHhv portal and hepatic venous ph. Data are mean ± s.d. (Control $n=8$, Endotoxin $n=10$). # designates significant difference within each group vs baseline, § designates significant difference between Control and Endotoxin groups.

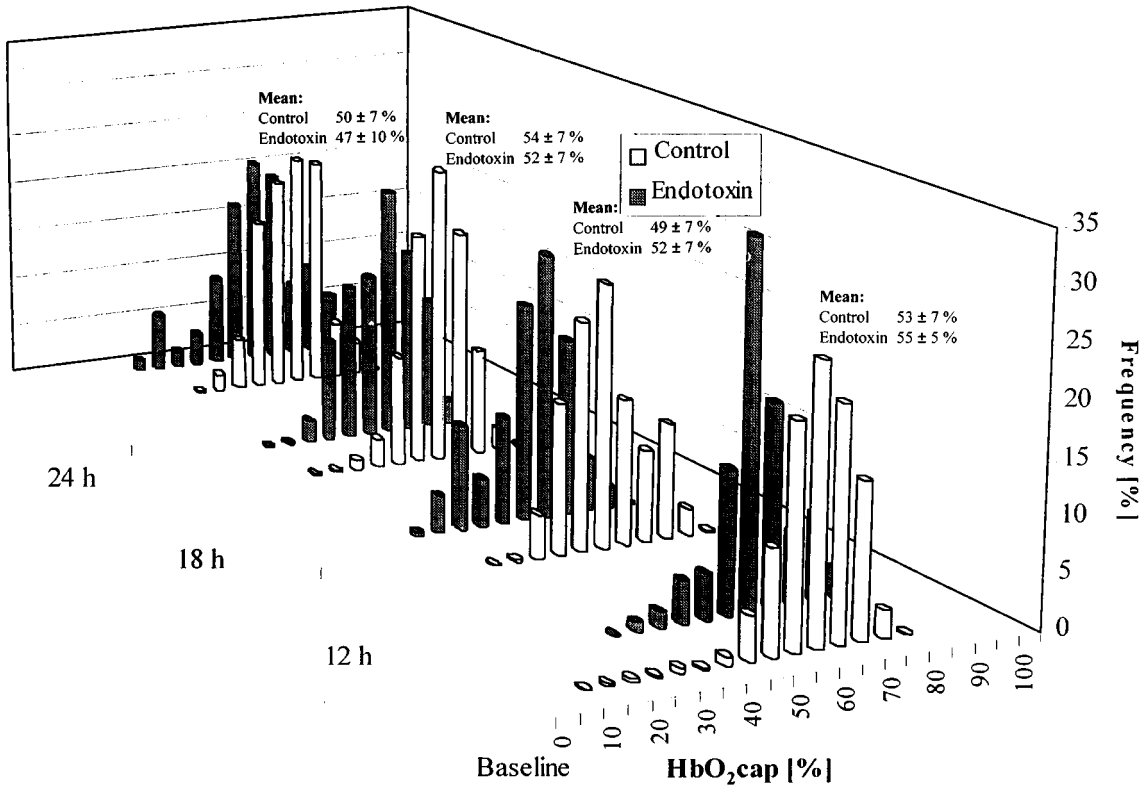

Figure 1 Mean ± s.d. and frequency distribution of the capillary haemoglobin O_2 saturation (Hb-O_2%) of the liver in the Control (light columns) and Endotoxin (hatched columns) animals. All data are derived from 300 Hb-O_2% spectra each recorded at six different measurement sites.

microcirculatory O_2 supply, endotoxic shock caused derangements of liver energy balance possibly due to increased hepatic metabolic rate.

Although cardiac output increased by 50–80% in the Endotoxin group, total liver blood flow was only augmented by about 30–40% suggesting that blood flow was redistributed away from the splanchnic organs. This finding is in sharp contrast to numerous reports on patients with sepsis or septic shock (Wilmore *et al*., 1980; Dahn *et al*., 1987; 1995; Ruokonen *et al*., 1993; Steffes *et al*., 1994; Reinelt *et al*., 1997) indicating that the increased cardiac output is paralleled by a comparable rise in hepato-splanchnic blood flow so that the fractional contribution of splanchnic blood flow to the total output remains constant. We can only speculate about this difference, but it is noteworthy that a similar disproportionate response of blood flow to the liver or other splanchnic organs has been observed in other porcine models of septic shock in which output was increased without using vasoactive drugs (Imamura & Clowes, 1975; Rasmussen *et al*., 1992; Tighe *et al*., 1995).

Since total haemoglobin concentrations progressively fell due to the repetitive blood sampling hepatic O_2 delivery remained constant in the Endotoxin group despite the increased total liver blood flow. This finding of unaltered macrocirculatory liver O_2 supply was underscored by the results of the remission spectrophotometry measurements: mean capillary Hb-O_2% remained unchanged as well suggesting that O_2 availability was not impaired on the microcirculatory level either. Our results confirm data reported by VanderMeer *et al*. (1995) that volume resuscitation with colloids to maintain normodynamic haemodynamics in porcine endotoxic shock allowed to prevent ileal mucosal hypoperfusion and tissue hypoxia as assessed with laser-Doppler flowmetry and Clarke-type PO_2 microelectrodes. It has to be noted, however, that our findings are in sharp contrast to previous data by Hasibeder *et al*. (1996) who reported decreased ileal mucosal Hb-O_2% using remission spectrophotometry during short-term endotoxaemia in pigs. This discrepancy is probably due to the endotoxin-induced fall in cardiac output and, hence, systemic O_2 delivery in that study: in fact, when systemic O_2 delivery was restored to comparable levels as in our experiment by infusion of dopamine mucosal Hb-O_2% and tissue PO_2 were not different from the control animals (Hasibeder *et al*., 1996). Interestingly, not only was the mean Hb-O_2% unaffected in the Endotoxin animals in our study but the frequency distribution remained unaltered as well suggesting that capillary heterogeneity was not significantly aggravated. In contrast, other authors have demonstrated both a reduction of the perfused capillary density and increased capillary heterogeneity in experimental septic shock (Lam *et al*., 1994). We can only speculate about this discrepancy but the sustained supranormal cardiac ouput achieved by volume resuscitation with colloids may have assumed importance in this context: in septic rats in which output was maintained at higher levels than in sham control animals, skeletal muscle tissue PO_2 frequency distributions were restored to normal (Astiz *et al*., 1988).

Both human volunteers after injection of endotoxin (Fong *et al*., 1990) as well as patients with sepsis or septic shock exhibit pronounced increases in endogenous glucose production rate (Wilmore *et al*., 1980; Dahn *et al*., 1987, 1995; Reinelt *et al*., 1997) while the opposite has been described in experimental endotoxicosis (Filkins & Cornell, 1974; Wolfe *et al*., 1977, 1978; Wichterman *et al*., 1980; Kober *et al*., 1985),

possibly due to impaired hepatocellular capacity to maintain gluconeogenesis from precursors (Wolfe *et al*., 1977, 1978). In sharp contrast to that, in our experiment endotoxic shock was associated with an endogenous glucose production rate about twice as high as in the Control animals. We cannot definitely explain these different findings but Wolfe *et al*. (1978) had already shown that there are factors which operate *in vivo* that enable hepatic glucose production to be maintained despite a direct inhibitory effect of endotoxin on the isolated organ. The sustained normotensive shock state with increased cardiac output and therefore well-preserved macro- and microcirculatory O_2 supply may have played an important role in this context: Indeed, 24 h after injection of endotoxin gluconeogenesis was well-preserved in haemodynamically stable dogs (Shaw & Wolfe, 1984). Furthermore, the experimental design using a continuous i.v. infusion of endotoxin may have assumed importance in this context since continuous long-term endotoxin infusion in rats lead to a pronounced increase in *de novo* synthesis of glucose from glucogenic precursors (Lang & Spitzer, 1987). Moreover, a 6-h infusion of endotoxin in pigs caused the rate of gluconeogenesis to increase (Chandrasena et al., 1983), even when lethal doses of endotoxin were used (Hand *et al*., 1983a) which severely depressed hepatic blood flow (Hand *et al*., 1983b).

Although endogenous glucose production rate was nearly doubled in the Endotoxin group throughout the whole period of septic shock the hepatic uptake of glucose precursors, i.e. lactate, alanine and glycerol, continuously dropped. The

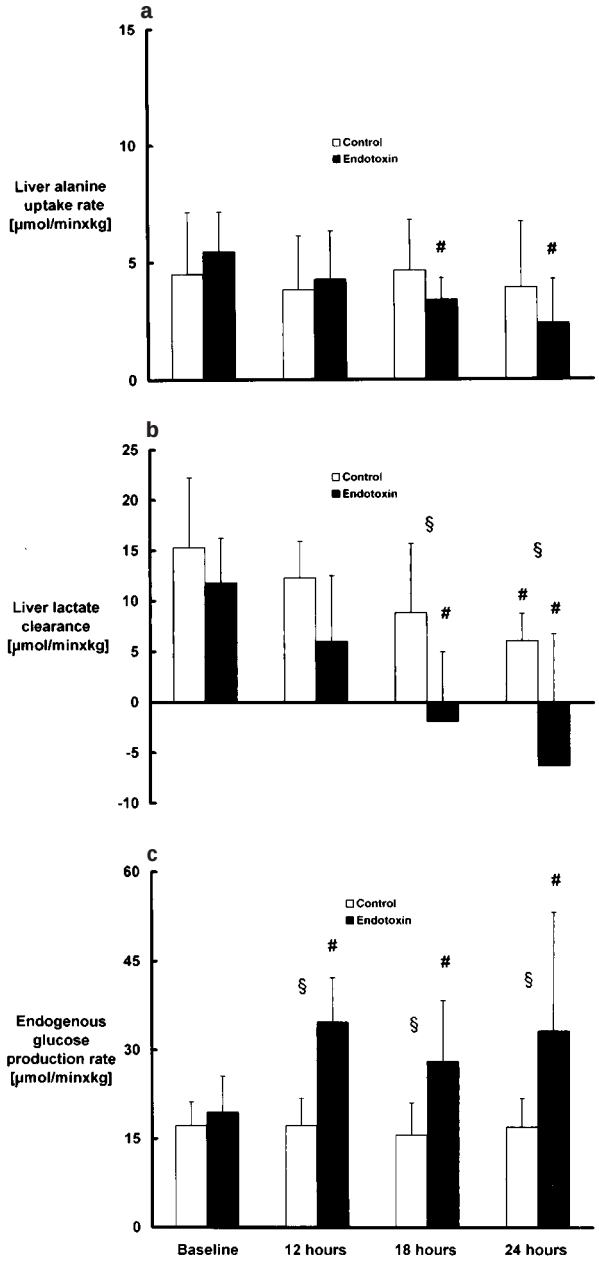

Figure 2 Liver alanine (a) and lactate (b) uptake rates as well as endogenous glucose production rate (c) in the Control (open columns) and Endotoxin (black columns) animals. All data are mean ± s.d., # denotes significant difference versus baseline, § denotes significant difference between the two groups.

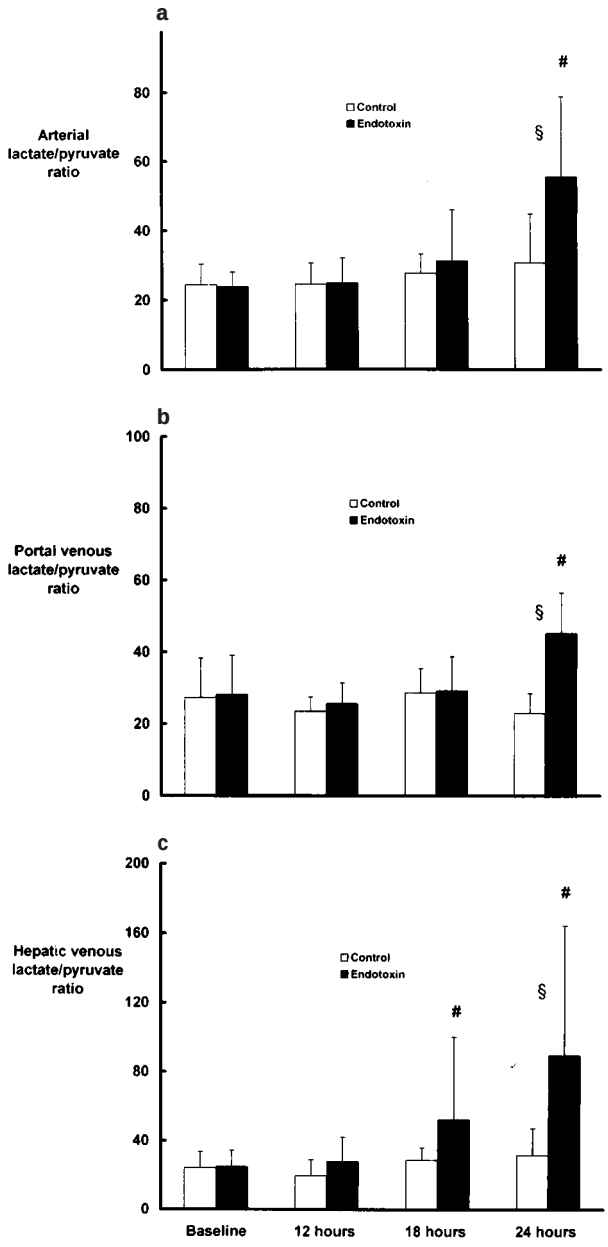

Figure 3 Arterial (a), portal (b) and hepatic venous (c) lactate/pyruvate ratios in the Control (open columns) and Endotoxin (black columns) animals. All data are mean ± s.d., # denotes significant difference vs baseline, § denotes significant difference between the two groups.

lactate and glycerol balances across the liver were even negative at the end of the experiment, i.e. the liver became a lactate and glycerol producing organ. Since glycogenolysis probably did not significantly contribute to endogenous glucose release (Hand *et al*., 1983b; Lang *et al*., 1984; Lang & Spitzer, 1987; Dahn *et al*., 1995) this result suggests that there was a remarkable dissociation between the rate of gluconeogenesis in the liver and the hepatic uptake rate of glucose precursors. This result is in sharp contrast to previous reports in both patients with sepsis (Wilmore *et al*., 1980; Shaw *et al*., 1985; Dahn *et al*., 1987, 1995) and awake pigs with peritonitis (Imamura *et al*., 1975). In particular, in the latter report the authors found that the sum of the glucogenic precursors, i.e. lactate, pyruvate, alanine and glycerol, taken up by the liver was directly proportional to the rate of hepatic glucose release. Several phenomena have to be taken into account to explain this striking discrepancy: first, in the previous studies shock was not present, and, in fact, Wilmore *et al*. (1980) reported disproportionate variations in hepato-splanchnic alanine and lactate uptake and glucose release rates in bacteraemic patients in whom complications developed when compared to patients without complications. Moreover, vanLambalgen *et al*. (1988) also reported net lactate production in the liver in canine endotoxic shock. Second, the contribution of renal glucose formation to total glucose release may have substantially varied (Stumvoll *et al*., 1995) and thereby influenced the relationship between hepatic uptake of glucogenic precursors and total endogenous glucose production. Although gluconeogenesis in the kidney has been shown to be reduced in rats with peritonitis (Ardawi *et al*., 1990) it may account for up to 30–40% of systemic glucose appearance in stress states (Stumvoll *et al*., 1995). Our method of estimating endogenous glucose production rate does not allow to discriminate between the metabolic sites, and, hence, we could only speculate on this factor. Finally, it has to be noted that both Imamura *et al*. (1975) and Wilmore *et al*. (1980) calculated substrate balances based on the Fick principle, i.e. as the product of blood flow times the arterial-hepatic venous and portal-hepatic venous concentration differences, respectively. This approach is particularly susceptible to errors when the concentration difference is small when compared to the absolute blood levels, e.g. for glucose (Wolfe, 1982). Furthermore, mathematical coupling of shared variables cannot be excluded when both precursor uptake and glucose release rates are calculated as the product of flow times the content differences across the liver (Phang *et al*., 1994). Our method of deriving endogenous glucose production from the plasma isotope enrichment of [6,6-$^2H_2$]-glucose during constant i.v. isotope infusion completely avoids these problems (Wolfe, 1982).

The net lactate production in the liver was associated with a significant increase of the hepatic venous lactate/pyruvate ratio indicating impaired cytosolic redox state (Hotchkiss & Karl, 1992). Astiz *et al*. (1988) found a similar derangement of oxidative metabolism and energy production as documented by increased lactate/pyruvate ratios in rat skeletal muscle although tissue PO_2 distributions had been well-preserved by volume resuscitation with albumin. It is noteworthy that we found the most pronounced increase in the lactate/pyruvate ratios in the hepatic venous blood. Taking into consideration the unchanged hepatic O_2 uptake it is tempting to speculate that the increased glucose production rate may assume importance in this context: Based on the stoichiometry of gluconeogenesis the synthesis of 1 mol of glucose in the liver requires 6 mol of ATP from the oxidation of fatty acids equivalent to 1.07 moles O_2 for every mole of glucose (Hand *et al*., 1983b; Dahn *et al*., 1995). Assuming that the liver accounted for approximately 60–70% of endogenous glucose production (Stumvoll *et al*., 1995) the mean hepatic glucose formation of approximately 1.0 mmol min^{-1} in the Endotoxin animals would have required about 24 ml min^{-1} of O_2, or in other words, theoretically the total hepatic O_2 uptake. Based on this calculation, any other energy requiring metabolic pathway in the liver, hence, would have theoretically been dependent on anaerobic ATP production. It should be noted, however, that an alternative mechanism could also explain the increased hepatic venous lactate/pyruvate ratios which clearly document impaired hepatocellular energy balance (Hotchkiss & Karl, 1992); endotoxin administered i.v. either as a bolus (Thiemermann *et al*., 1995) or as a continuous infusion (Gardiner *et al*., 1995) causes a several fold increase in nitric oxide formation. This excess formation of nitric oxide inhibits mitochondrial aconitase (Gardner *et al*., 1997), in particular in hepatocytes (Stadler *et al*., 1991), which results in derangements of energy metabolism and, hence, impaired aerobic formation of ATP. Consequently, the increased hepatic venous lactate/pyruvate ratios might have also been due to suppressed mitochondrial oxidative phosphorylation. In our *in vivo* model we could not directly determine mitochondrial function independently from cellular O_2 availability. Therefore we can only speculate about the respective role of O_2 supply limitations resulting from increased metabolic O_2 demands on the one hand and potentially impaired intracellular O_2 utilization on the other hand.

In summary we investigated hepatic blood flow, O_2 exchange and metabolism during 24 h of endotoxin-induced shock in the pig. Volume resuscitation with colloids allowed to maintain normotensive systemic haemodynamics with a sustained increase in cardiac ouput and total hepatic blood flow. Thus, macro-and microcirculatory O_2 supply to the liver could be well-preserved. Endotoxic shock was associated with a nearly doubled endogenous glucose production rate, and the rates of uptake of glucogenic precursors and glucose release in the liver were completely dissociated. Despite well-preserved capillary oxygenation, cytosolic redox state was impaired suggesting deranged liver energy balance possibly due to the O_2 requirements of *de novo* glucose synthesis.

Presented in part at the 10th Annual Congress of the European Society of Intensive Care Medicine, Paris, September 7–10, 1997. We thank Wolfgang Siegler, Sandra Weber, Ingrid Eble, Rosa Engelhardt and Volker Kurzweg for skilful technical assistance, and we are indebted to E. Marion Schneider, PhD, Professor of Experimental Anaesthesia, Ulm, for critically reviewing the manuscript. Karen M. Rieger, M.D., was supported by a research grant from the Department of Surgery, Shands Hospital, University of Florida, Gainesville, FL, U.S.A.

References

ARDAWI, M.S.M., KHOJA, S.M. & NEWSHOLME, E.A. (1990). Metabolic regulation of renal gluconeogenesis in response to sepsis in the rat. *Clin. Sci. Colch.,* **79,** 483–490.

ASTIZ, M., RACKOW, E.C., WEIL, M.H. & SCHUMER, W. (1988). Early impairment of oxidative metabolism and energy production in severe sepsis. *Circ. Shock,* **26,** 311–320.

CARRICO, C.J., MEAKINS, J.L., MARSHALL, J.C., FRY, D. & MAIER, R.V. (1986). Multiple organ failure syndrome. The gastrointestinal tract: The 'motor' of MOF. *Arch. Surg.,* **121,** 196–208.

CHANDRASENA, L.G., FETTMAN, M.J., HAND, M.S., CLEEK, J.L. & PHILIPS, R.W. (1983). Endotoxin dose. II. Effects on glucose biokinetics in Yucatan minipigs. *Am. J. Physiol.,* **244,** E399–E407.

CZOK, R. & LAMPRECHT, W. (1970). Pyruvat, Phosphoenolpyruvat und D-Glycerat-2-phosphat. In: *Methoden der Enzymatischen Analyse Band II*, edited by Bergmeyer, H.U., Weinheim, Verlag Chemie, p. 1407–1411.

DAHN, M.S., LANGE, P., LOBDELL, K., HANS, B., JACOBS, L.A. & MITCHELL, R.A. (1987). Splanchnic and total body oxygen consumption differences in septic and injured patients. *Surgery,* **101,** 69–80.

DAHN, M.S., MITCHELL, R.A., LANGE, M.P., SMITH, S. & JACOBS, L.A. (1995). Hepatic metabolic response to injury and sepsis. *Surgery,* **117,** 520–530.

DANNER, R.L., ELIN, R.J., HOSSEINI, J.M., WESLEY, R.A., REILLY, J.M. & PARILLO, J.E. (1991). Endotoxemia in human septic shock. *Chest,* **99,** 169–185.

DANTZKER, D.R. (1993). The gastrointestinal tract. The canary of the body? *JAMA,* **270,** 1247–1248.

DOUGLAS, W. R. (1972). Of pigs and men and research: a review of applications and analogies of the pig, sus scrofa, in human medical research. *Space Life Sci.,* **3,** 226–234.

FERRANNINI, E. (1988). The theoretical bases of indirect calorimetry: A review. *Metabolism,* **37,** 287–301.

FILKINS, J.P. & CORNELL, R.P. (1974). Depression of hepatic gluconeogenesis and the hypoglycemia of endotoxin shock. *Am. J. Physiol.,* **227,** 778–781.

FONG, Y., MARANO, M.A., MOLDAWER, L.L., WEI, H., CALVANO, S.E., KENNEY, J.S., ALLISON, A.C., CERAMI, A., SHIRES, G.T. & LOWRY, S.F. (1990). The acute splanchnic and peripheral tissue metabolic response to endotoxin in humans. *J. Clin. Invest.,* **85,** 1896–1904.

FRANK, K. H., KESSLER, M., APPELBAUM, K. & DÜMMLER, W. (1989). The Erlangen micro-lightguide spectrophotometer EMPHO I. *Phys. Med. Biol.,* **34,** 1883–1900.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1995). Cardiac and regional haemodynamics, inducible nitric oxide synthase (NOS) activity, and the effects of NOS inhibitors in conscious endotoxaemic rats. *Br. J. Pharmacol.,* **116,** 2005–2016.

GARDNER, P.R., COSTANTINO, G., SZABO, C. & SALZMAN, A.L. (1997). Nitric oxide sensitivity of the aconitases. *J. Biol Chem.,* **272,** 25071–25076.

HAND, M.S., FETTMAN, M.J., CHANDRASENA, L.G., CLEEK, J.L. & PHILLIPS, R.W. (1983a). Endotoxin dose. I. Hemodynamic, metabolic, and lethal consequences in Yucatan minipigs. *Am. J. Physiol.,* **244,** E385–E398.

HAND, M.S., FETTMAN, M.J., CHANDRASENA, L.G., CLEEK, J.L., MASON, R.A. & PHILLIPS, R.W. (1983b). Reduced hepatic blood flow does not limit gluconeogenesis in awake endotoxic minipigs. *Circ. Shock,* **11,** 269–285.

HASIBEDER, W., GERMANN, R., WOLF, H.J., HAISJACKL, M., HAUSDORFER, H., RIEDMANN, B., BONATTI, J., GRUBER, E., SCHWARZ, B., WALDENBERGER, P., FRIESENECKER, B. & FURTNER, B. (1996). Effects of short-term endotoxemia and dopamine on mucosal oxygenation in porcine jejunum. *Am. J. Physiol.,* **270,** G667–G775.

HAWKER, F. (1994). SIRS: Is it a liver disease?. *Int. J. Intensive Care,* **1,** 113.

HIRAI, F., AOYAMA, H. OHTOSHI, M., KAWASHIMA, S., OZAWA, K. & TOBE, T. (1984). Significance of mitochondrial enhancement in hepatic energy metabolism in relation to alterations in hemodynamics in septic pigs with severe peritonitis. *Eur. Surg. Res.,* **16,** 148–155.

HOHORST, H.J. (1970). L-(+)-Lactat, In: *Methoden der Enzymatischen Analyse Band II*, edited by Bergmeyer, H.U., Weinheim, Verlag Chemie, p. 1425–1429.

HOTCHKISS, R.S. & KARL, I.E. (1992). Reevaluation of the role of cellular hypoxia and bioenergetic failure in sepsis. *JAMA,* **267,** 1503–1510.

IMAMURA, M. & CLOWES G.H.A. (1975). Hepatic blood flow and oxygen consumption in starvation, sepsis and septic shock. *Surg. Gynecol. Obstet.,* **141,** 27–34.

IMAMURA, M., CLOWES, G.H., BLACKBURN, G.L., O'DONNELL, T.F., TRERICE, M., BHIMJEE, Y. & RYAN, N.T. (1975). Liver metabolism and glucogenesis in trauma and sepsis. *Surgery* **77,** 868–880.

JUNGAS, R.L., HALPERIN, M.L. & BROSNAN, J.T. (1992). Quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. *Physiol. Rev.,* **72,** 419–448.

KOBER, P.M., THOMAS, J.X. & FILKINS, J.P. (1985). Glucose dyshomeostasis and cardiovascular failure in endotoxic dogs. *Am. J. Physiol.,* **249,** R570–R577.

LAM, C., TYML, K., MARTIN, C. & SIBBALD, W. (1994). Microvascular perfusion is impaired in a rat model of normotensive sepsis. *J. Clin. Invest.,* **94,** 2077–2083.

LANG, C.H., BAGBY, G.J. & SPITZER, J.J. (1984). Carbohydrate dynamics in the hypermetabolic septic rat. *Metabolism,* **33,** 959–963.

LANG, C.H. & SPITZER, J.A. (1987). Glucose kinetics and development of endotoxin tolerance during long-term continuous endotoxin infusion. *Metabolism,* **36,** 469–474.

PHANG, T.P., CUNNINGHAM, K.F., RONCO, J.J., WIGGS, B.R. & RUSSELL, J.A. (1994). Mathematical coupling explains dependence of oxygen consumption on oxygen delivery in ARDS. *Am. J. Respir. Crit. Care Med.,* **150,** 318–323.

PASTOR, C.M., BILLIAR, T.R., LOSSER, M.R. & PAYEN, D.M. (1995). Liver injury during sepsis. *J. Crit. Care,* **10,** 183–197.

RASMUSSEN, I., ARVIDSSON, D., ZAK, A. & HAGLUND, U. (1992). Splanchnic and total body oxygen consumption in experimental fecal peritonitis in pigs: effects of dextran and iloprost. *Circ. Shock,* **36,** 299–306.

REINELT, H., RADERMACHER, P., FISCHER, G., GEISSER, W., WACHTER, U., WIEDECK, H., GEORGIEFF, M. & VOGT, J. (1997). Effects of a dobutamine-induced increase in splanchnic blood flow on hepatic metabolic activity in patients with septic shock. *Anesthesiology,* **86,** 818–824.

RUOKONEN, E., TAKALA, J., KARI, A., SAXÉN, H., MERTSOLA, J. & HANSEN, E.J. (1993). Regional blood flow and oxygen transport in septic shock. *Crit. Care Med.,* **21,** 1296–1303.

ŠANTAK, B., RADERMACHER, P., IBER, T., ADLER, J., WACHTER, U., VASSILEV, D., GEORGIEFF, M. & VOGT, J. (1997). In vivo quantification of endotoxin-induced nitric oxide production in pigs from $Na^{15}NO_3$-infusion. *Br. J. Pharmacol.,* **122,** 1605–1610.

SHAW, J.H.F. & WOLFE, R.R. (1984). A conscious septic dog model with hemodynamic and metabolic responses similar to responses of humans. *Surgery,* **95,** 553–561.

SHAW, J.H.F., KLEIN, S. & WOLFE, R.R. (1985). Assessment of alanine, urea, and glucose interrelationships in normal subjects and in patients with sepsis with stable isotopic tracers. *Surgery,* **97,** 557–568.

STADLER, J., CURRAN, R.D., OCHOA, J.B., HARBRECHT, B.G., HOFFMAN, R.A., SIMMONS, R.L. & BILLAR, T.R. (1991). Effect of endogenous nitric oxide on mitochondrial respiration of rat hepatocytes in vitro and in vivo. *Arch. Surg.,* **126,** 186–191.

STEFFES, C.P., DAHN, M.S. & LANGE, M.P. (1994). Oxygen transport-dependent splanchnic metabolism in the sepsis syndrome. *Arch. Surg.,* **129,** 46–52.

STUMVOLL, M., CHINTALAPUDI, U., PERRIELLO, G., WELLE, S., GUTIERREZ, O. & GERICH, J. (1995). Uptake and release of glucose by the human kidney. Postabsorptive rates and responses to epinephrine. *J. Clin. Invest.,* **96,** 2528–2533.

TAKALA, J., KEINÄNEN, O., VÄISÄNEN, P. & KARI, A. (1989). Measurement of gas exchange in intensive care: Laboratory and clinical validation of a new device. *Crit. Care Med.,* **17,** 1041–1047.

THIEMERMANN, C., RUETTEN, H., WU, C.C. & VANE, J.R. (1995). The multiple organ dysfunction caused by endotoxin in the rat. attenuation of liver dysfunction by inhibitors of nitric oxide synthase. *Br. J. Pharmacol.,* **116,** 2845–2851.

TIGHE, D., MOSS, R., HEYWOOD, G., AL-SAADY, N., WEBB, A. & BENNETT, D. (1995). Goal-directed therapy with dopexamine, dobutamine, and volume expansion: effects of systemic oxygen transport on hepatic ultrastructure in porcine sepsis. *Crit. Care Med.,* **23,** 1997–2007.

TUCHSCHMIDT, J., FRIED, J., ASTIZ, M. & RACKOW, E. (1992). Elevation of cardiac output and oxygen delivery improves outcome in septic shock. *Chest,* **102,** 216–220.

VANDERMEER, T.J., WANG, H. & FINK, M.P. (1995). Endotoxemia causes ileal mucosal acidosis in the absence of mucosal hypoxia in a normodynamic porcine model of septic shock. *Crit. Care Med.,* **23,** 1217–1226.

VAN LAMBALGEN, A.A., RUNGE, H.C., VAN DEN BOS, G.C. & THIJS, L.G. (1988). Regional lactate production in early canine endotoxin shock. *Am. J. Physiol.,* **254,** E45–E51.

VINCENT, J.L. (1996). Prevention and therapy of multiple organ failure. *World J. Surg.,* **20,** 465–470.

VINCENT, J.L., MORENO, R., TAKALA, J., WILLATTS, S., DE MENDONÇA, A., BRUINING, H., REINHART, C.K., SUTER, P.M. & THIJS L.G. (1996). The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med.,* **22,** 707–710.

WICHTERMAN, K.A., BAUE, A.E. & CHAUDRY, I.H. (1980). Sepis and septic shock - a review of laboratory models and a proposal. *J. Surg. Res.,* **29,** 189–201.

WILMORE, D.W., GOODWIN, C.W., AULICK, L.H., POWANDA, M.C., MASON, A.D. & PRUITT, B.A. (1980). Effect of injury and infection on visceral metabolism and circulation. *Ann. Surg.,* **192,** 491–504.

WOLFE, R.R., ELAHI, D. & SPITZER, J.J. (1977). Glucose and lactate kinetics after endotoxin administration in dogs. *Am J. Physiol.,* **232,** E180–E185.

WOLFE, R.R. & BURKE, J.F. (1978). Glucose and lactate metabolism in experimental septic shock. *Am. J. Physiol.,* **235,** R219–R227.

WOLFE, R.R. (1982), Stable isotope approaches for study of energy substrate metabolism. *Fed. Proc.,* **41,** 2692–2697.

(Received January 26, 1998
Revised April 28, 1998
Accepted May 21, 1998)