

CRITICAL CARE

Gut microcirculatory and mitochondrial effects of hyperdynamic endotoxaemic shock and norepinephrine treatment

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Editor's key points

- In septic shock, abnormal microcirculatory and mitochondrial function occur.
- Regional blood flow and norepinephrine may have an effect.
- In this study in sheep, microcirculatory and mitochondrial dysfunction were seen, despite adequate blood flow in the gut.
- Norepinephrine did not affect the gut microcirculation.
- Altering perfusion pressure has limited impact on the gut microcirculation in sepsis.

Background. Microcirculatory and mitochondrial dysfunction are important factors in the development of septic shock. In this study, we investigated the effects of fluid resuscitated endotoxaemic shock and norepinephrine treatment on intestinal microcirculation and mitochondrial function in sheep.

Methods. Eight anaesthetized sheep received an i.v. infusion of endotoxin. After 24 h, mean arterial pressure (MAP) was restored to baseline levels with a norepinephrine infusion. Five sheep served as sham experiments. Central and regional haemodynamics were monitored, and ileal microcirculation was evaluated with laser Doppler and sidestream dark-field videomicroscopy techniques. Gut mucosal acidosis was assessed by air tonometry, and ileal wall biopsies were analysed for mitochondrial activity.

Results. After 24 h of endotoxaemia, the animals had developed hyperdynamic shock with systemic and mucosal acidosis. Although superior mesenteric artery (SMA) flow was higher than the baseline values, ileal microcirculatory perfusion and mitochondrial complex I activity decreased. After norepinephrine was started, SMA flow, ileal microcirculation, and mucosal acidosis remained unchanged. Although no statistically significant difference could be demonstrated, norepinephrine increased mitochondrial complex I activity in five of the six animals from which ileal biopsies were taken.

Conclusions. Although fluid resuscitated endotoxaemic shock increased regional blood flow, microcirculatory and mitochondrial alterations were still present. Restoring MAP with norepinephrine did not affect ileal microcirculation or mucosal acidosis, indicating that perfusion pressure manipulation is of limited importance to the intestinal microcirculation in established endotoxaemic shock.

Keywords: laser Doppler; perfusion pressure; sepsis; sheep; sidestream dark-field imaging

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Septic shock is a leading cause of mortality in intensive care units. Both microcirculatory failure¹ and mitochondrial dysfunction² are thought to play a role in the progression to multiple organ failure and death, and in septic patients with comparable systemic haemodynamics, persistent microcirculatory alterations³ as well as mitochondrial derangements⁴ have been correlated to patient outcome. Currently, septic shock therapy is mainly guided by systemic haemodynamic parameters, but to what extent microcirculatory and mitochondrial function are affected by changes in systemic blood pressure and flow remains to be clarified. Moreover, little is known about the microcirculatory and

mitochondrial effects of the drugs currently used in the therapy of septic shock.

Persistent hypotension in spite of adequate fluid resuscitation is a hallmark of septic shock. However, microcirculatory changes are often dissociated from systemic haemodynamics, and the microcirculatory response to treatment can differ from the systemic haemodynamic effects.^{5,6} Current guidelines for the treatment of septic shock include norepinephrine as a first-line vasopressor to increase perfusion pressure,⁷ but concerns have been raised that norepinephrine can induce an excessive vasoconstriction causing further microcirculatory deterioration in the splanchnic organs.⁸ In

two recent studies using sidestream dark-field (SDF) microscopy, the use of norepinephrine to increase mean arterial pressure (MAP) did not significantly alter sublingual microcirculation in septic patients.^{9, 10} However, for obvious reasons, changes in regional blood flow were not evaluated in these studies, making it hard to draw firm conclusions regarding the role of the change in perfusion pressure. Also, whether the sublingual microcirculation is representative of other vascular beds remains controversial.^{11–13}

Intestinal microcirculation is impaired in sepsis,¹⁴ and the intestinal mucosa is particularly sensitive to microcirculatory disturbances due to its counter-current blood supply and high oxygen extraction. The gut has been suggested to have an important role in the pathogenesis of septic multiple organ failure¹⁵ and restoring microcirculatory perfusion and preventing splanchnic organ dysfunction could be of major importance in the treatment of septic shock. Furthermore, the importance of alterations in mitochondrial function to gut dysfunction in septic shock has not yet been defined.

Previous studies investigating the intestinal microcirculatory effects of raising perfusion pressure with norepinephrine have yielded conflicting results,^{8, 16–21} and studies using longer, more clinically relevant models are lacking. Also, little is known about the effects of norepinephrine on intestinal mitochondrial function in septic shock.

In this study, we tested the hypothesis that, in hyperdynamic endotoxaemic shock, intestinal microcirculatory and mitochondrial dysfunction will be present in spite of an increase in regional blood flow. Furthermore, we evaluated the intestinal microcirculatory and mitochondrial effects of increasing perfusion pressure with norepinephrine.

Methods

An expanded Methods section is available in the online Supplementary material.

The experimental protocol was approved by the Ethics Committee for Experiments in Animals, Stockholm, Sweden. The experiments were conducted in accordance with the current guidelines for the care of laboratory animals (see online Supplementary material for details).

Anaesthesia and surgical preparation

Thirteen ewes weighing 47 (SD 15) kg were fasted for 12 h before surgery with free access to water. Anaesthesia was induced by i.v. sodium thiopental (10 mg kg⁻¹), and maintained until the end of the experiment with a combination of isoflurane (2.0% endtidal concentration during surgical procedures followed by 1.2–1.6 endtidal% throughout the experiment) and an i.v. infusion of midazolam 0.1 mg kg⁻¹ h⁻¹. During surgical procedures, bolus doses of fentanyl 50–100 µg were administered when needed. No neuromuscular blocking agents were administered, and the adequacy of anaesthesia was assessed using vital signs. After induction of anaesthesia, the animals were intubated and ventilated with oxygen in air ($F_{I_{O_2}} = 0.3$) and positive end-expiratory pressure (PEEP) of 4 cm H₂O. The tidal volume was set to

10 ml kg⁻¹ and the respiratory rate was adjusted to reach an arterial P_{CO_2} (Pa_{CO_2}) of 4.8–5.7 kPa. All animals received an infusion of Ringer's acetate 3 ml kg⁻¹ h⁻¹ throughout the experiment. The animals were surgically instrumented with an arterial line and a pulmonary artery catheter. After a midline laparotomy, a tonometry catheter was inserted into the lumen of the ileum and used for measurements of mucosal P_{CO_2} . Laser Doppler probes (Perimed AB, Järfälla, Sweden) were used for monitoring microcirculatory blood flow in the mucosa (MCQ_{muc}) and muscularis layer (MCQ_{musc}) of the ileum. Laser Doppler flowmetry is a method of monitoring microcirculatory perfusion based on the continuous measurement of the Doppler shift of laser light caused by moving red blood cells. Values are obtained as arbitrary perfusion units and not as absolute blood flow, and are presented as changes relative to baseline in percentage. An ultrasonic flow probe (Transonic Systems Inc., Ithaca, NY, USA) was used for measuring superior mesenteric artery (SMA) flow. Finally, a loop-ileostomy was constructed. After the surgical preparation, the animals were allowed 2 h of recovery before the experimental protocol was started.

SDF imaging and analysis

An SDF imaging device (MicroVision Medical, Amsterdam, Netherlands) with a ×5 lens was used to obtain images of the microcirculation in the ileal mucosa through the ileostomy. Due to the heterogeneity in microvascular flow seen in sepsis, steady SDF videos of 10–20 sec duration were acquired at three to five sites in the ileum at each time point. All videos were analysed blindly and in random order. The microvascular flow index (MFI), the percentage of perfused villi (PV%), and the heterogeneity index (HI) were determined as previously described.²² MFI is a semiquantitative score, where the image is divided into quadrants and the type of flow in each quadrant is determined using an ordinal scale; 0, no flow; 1, intermittent flow; 2, sluggish flow; 3, normal flow. The MFI value was calculated as the average score of all quadrants at each time point. The HI was calculated at each time point as the highest flow velocity minus the lowest flow velocity divided by the mean MFI across all mucosal sites at that time point, as previously described by Trzeciak and colleagues.²³ In each video, the number of villi was counted and all villi were semiquantitatively classified as perfused, heterogeneously perfused or unperfused. The PV% was calculated at each time point as: [number of PV/total number of villi visible].

Experimental protocol

Using sealed, opaque envelopes, the animals were randomized 2:1 to receive endotoxin ($n=8$; *Escherichia coli* lipopolysaccharide, serotype 0111:B4; Sigma-Aldrich Sweden AB, Stockholm, Sweden) or serve as sham animals ($n=5$), intended to rule out any non-endotoxaemic effects over time. Initially, the study was designed for four sham animals. However, due to technical difficulties with the SDF imaging in two sham animals, an extra animal was added

to the sham group, and this animal did not undergo randomization.

After the surgical preparation, sham animals were followed for 24 h without any intervention, and subsequently killed using a lethal dose of sodium pentobarbital and potassium chloride injected into a central vein. In the endotoxin group, an i.v. endotoxin infusion was started at the beginning of the experiment (T0) at a rate of $0.3 \mu\text{g kg}^{-1} \text{h}^{-1}$. The rate was subsequently adjusted throughout the experiment to achieve a MAP of ~ 60 mm Hg after 24 h. Bolus doses of hydroxyethyl starch 130/0.4 were administered when MAP remained < 60 mm Hg for > 30 min, or when arterial lactate was ≥ 2.5 mmol litre $^{-1}$. After 24 h of endotoxaemia (T24), MAP in the endotoxin group was restored to baseline levels with an i.v. infusion of norepinephrine, and the animals were followed for one additional hour, making the total experimental period in the endotoxin group 25 h. After T24 the endotoxin infusion was left unchanged, and no additional fluid bolus was given. After 25 h of endotoxaemia (T25), the animals were killed. Ileal wall biopsies, SDF images, and tonometry values were collected at T0, T24, and T25 (endotoxin group only).

Mitochondrial enzymes

Tissue samples were homogenized in a KCl-based buffer using a Potter-Elvehjem homogenizer and analysed for activity of citrate synthase, mitochondrial complex I and IV as previously described.^{24 25}

Statistics

Data are presented as mean (SD) or median [interquartile range]. In the endotoxin group, changes over time and effects of norepinephrine were analysed with one-way ANOVA, Wilcoxon matched pair test or Student's *t*-test as appropriate. The significance level was set as $P \leq 0.05$.

Results

None of the animals in the endotoxin group or in the sham group died during the experiment. The mean rate of the endotoxin infusion was 0.28 (SD 0.06) $\mu\text{g kg}^{-1} \text{h}^{-1}$ after 12 h and 0.26 (SD 0.03) $\mu\text{g kg}^{-1} \text{h}^{-1}$ after 24 h of the experimental protocol. The mean volume of hydroxyethyl starch infused in the animals in the endotoxin group was 519 (SD 342) ml, and the mean rate of norepinephrine from T24 to T25 was 318 (SD 119) $\text{ng kg}^{-1} \text{min}^{-1}$.

Effects of endotoxaemia on haemodynamic and metabolic parameters (T0 to T24)

All animals receiving endotoxin developed a hyperdynamic shock with increased cardiac index (cardiac output/body weight; CI; Table 1) and systemic hypotension (Fig. 1). Pulmonary capillary wedge pressure (PCWP) increased over time, and urine output remained at baseline levels ($P=0.25$, Table 1). Although mixed venous oxygen saturation (Sv_{O_2}) increased, base excess decreased, and arterial lactate increased during the first 24 h (Table 1). No significant differences from baseline were found in pulmonary gas exchange

parameters (Table 1). In the endotoxin group, haematocrit decreased over time (Table 1). All animals developed a febrile response to endotoxin (Table 1).

Sham animals did not display any changes in central haemodynamics with CI (Table 1), MAP (Fig. 1), and heart rate (Table 1) remaining unchanged. Acid–base parameters, lactate and urine output were stable from T0 to T24 (Table 1). Body temperature was maintained at near baseline levels (Table 1).

Effects of endotoxaemia on intestinal perfusion, mitochondria, and mucosal P_{CO_2} (T0 to T24)

At baseline, PV%, MFI, and HI were within normal limits in the endotoxin group (Fig. 2), and sham animals (PV%=100, MFI=3 and HI=0 for all animals, $n=3$). Even though regional intestinal blood flow [SMA flow/body weight; SMA_{FI}] increased in the endotoxin group (Fig. 1), PV% and MFI decreased, and HI increased at T24 (Fig. 2). MCQ_{muc} and MCQ_{musc} also decreased from T0 to T24 (Fig. 1). In parallel with the decrease seen in microcirculatory perfusion, the gap between mucosal and arterial P_{CO_2} levels ($P_{CO_{2muc-art}}$) increased from baseline values in the endotoxin group (Fig. 2). Endotoxaemia also significantly decreased citrate synthase and complex I activity (Table 2), but complex IV activity remained unchanged ($P=0.78$; Table 2). In order to correct for mitochondrial density, the ratio of complexes I and IV to citrate synthase was calculated (complex I/CS and IV/CS, respectively). No significant differences were found for complex I/CS ($P=0.09$; Table 2) or complex IV/CS ($P=0.21$; Table 2).

In sham animals, values remained stable over time for both regional blood flow and the ileal microcirculation assessed with laser Doppler (Fig. 1) and SDF (PV%=100, MFI=3 and HI=0 for all animals, $n=3$). $P_{CO_{2muc-art}}$ values also remained at baseline levels (online Supplementary material, Fig. SE1). The citrate synthase activity, complex IV activity, and complex IV/CS in sham animals showed a similar pattern as in the endotoxaemic animals (Table 2). However, in contrast to the endotoxin animals, there was no apparent decrease in complex I activity in sham animals from T0 to T24 (Table 2).

Effects of norepinephrine (T24 to T25)

Norepinephrine restored MAP to baseline levels (Fig. 1). CI and Sv_{O_2} increased from T24 to T25 (Table 1), but PCWP was unchanged ($P=0.78$; Table 1). In spite of the increase seen in CI, SMA_{FI} remained unchanged ($P=0.66$; Fig. 1). There were no significant differences in urine output ($P=0.07$), acid–base parameters or pulmonary gas exchange parameters from T24 to T25 (Table 1), but norepinephrine significantly increased haematocrit (Table 1).

Norepinephrine did not significantly affect PV% ($P=0.60$; Fig. 2), MFI ($P=0.35$; Fig. 2), HI ($P=0.80$; Fig. 2), MCQ_{muc} ($P=0.85$; Fig. 1), or MCQ_{musc} ($P=0.75$; Fig. 1). $P_{CO_{2muc-art}}$ also remained unchanged from T24 to T25 ($P=0.75$; Fig. 2). Although norepinephrine increased complex I activity and complex I/CS in five out of six animals, this did not reach

Table 1 Descriptive data. SvO_2 , mixed venous oxygen saturation; PCWP, pulmonary capillary wedge pressure; SaO_2 , arterial oxygen saturation. ^aSignificant ($P < 0.05$) difference T0–T24 in the endotoxin group (ANOVA RM or Wilcoxon matched pairs test). ^bSignificant ($P < 0.05$) difference T24–T25 (Student's paired *t*-test or Wilcoxon matched pairs test). Data are presented as mean (sd) or median (interquartile range). $n = 6–8$ in the endotoxin group, $n = 4–5$ in the sham group

	Group	T0	T6	T12	T18	T24	T25
Cardiac index, $ml\ kg^{-1}\ min^{-1}$	Endotoxin	96 (23)	107 (33)	152 (44)	156 (51)	160 (59)	206 (72) ^{a,b}
	Sham	76 (12)	80 (14)	81 (12)	87 (11)	86 (13)	
Heart rate, bpm	Endotoxin	88 (15)	88 (14)	98 (19)	92 (15)	89 (9)	106 (13) ^b
	Sham	89 (10)	91 (15)	82 (15)	81 (21)	86 (24)	
SvO_2 , %	Endotoxin	80 (5)	79 (3)	83 (6)	84 (6)	85 (7)	88 (6) ^{a,b}
	Sham	80 (7)	78 (3)	78 (8)	82 (7)	82 (5)	
PCWP, mm Hg	Endotoxin	8.1 (3.3)	8.5 (2.4)	10.7 (4.7)	10.5 (3.5)	11.3 (3.4)	11.1 (2.4) ^a
	Sham	8.1 (1.6)	8.4 (3.7)	8.8 (2.7)	8.1 (2.6)	8.4 (1.6)	
pH	Endotoxin	7.48 (0.03)	7.43 (0.04)	7.36 (0.09)	7.40 (0.07)	7.42 (0.07)	7.39 (0.07) ^a
	Sham	7.48 (0.04)	7.47 (0.03)	7.51 (0.04)	7.49 (0.06)	7.51 (0.08)	
Base excess, $mmol\ litre^{-1}$	Endotoxin	6.0 (1.9)	3.7 (4.3)	0.1 (4.3)	−1.4 (3.3)	−1.3 (2.6)	−2.6 (3.4) ^a
	Sham	8.7 (2.6)	8.3 (1.7)	6.6 (3.4)	7.8 (4.8)	8.4 (6.9)	
$PaCO_2$, kPa	Endotoxin	5.4 (0.3)	5.5 (0.5)	6.1 (1.5)	5.0 (0.9)	4.7 (0.6)	4.9 (0.5)
	Sham	6.0 (0.4)	6.5 (0.4)	5.7 (0.8)	6.1 (0.8)	5.7 (0.6)	
Arterial lactate, $mmol\ litre^{-1}$	Endotoxin	1.2 (1.1–1.2)	1.3 (1.1–2.2)	2.0 (2–2.8)	2.2 (2.0–3.8)	3.0 (2.6–3.4)	3.0 (2.9–3.6) ^a
	Sham	1.1 (0.8–1.3)	0.8 (0.5–1.2)	1.1 (0.8–1.2)	0.8 (0.5–1.1)	1.1 (1–1.2)	
SaO_2 , %	Endotoxin	99.6 (0.5)	99.2 (0.6)	99.1 (0.9)	99.7 (0.3)	99.2 (1.0)	98.6 (1.6)
	Sham	99.7 (0.3)	99.2 (0.6)	99.6 (0.1)	99.7 (0.3)	99.5 (0.6)	
Urine output, $ml\ kg^{-1}\ h^{-1}$	Endotoxin	0.9 (0.4)	0.6 (0.1)	0.5 (0.4)	0.7 (0.3)	0.7 (0.4)	1.3 (1.0)
	Sham	0.4 (0.1)	0.4 (0.3)	0.4 (0.3)	0.6 (0.6)	0.5 (0.5)	
Body temperature, °C	Endotoxin	39.2 (0.5)	40.5 (0.8)	40.9 (0.8)	40.9 (0.7)	40.7 (0.6)	40.5 (0.7) ^a
	Sham	38.5 (0.6)	39.0 (0.5)	39.3 (0.3)	39.3 (0.4)	39.2 (0.4)	
Haematocrit, %	Endotoxin	25.4 (2.3)				21.7 (2.7)	23.3 (3.4) ^{a,b}
	Sham	21.4 (3.8)				22.6 (6.0)	

statistical significance ($P = 0.12$ and 0.18 , respectively; Fig. 3). Norepinephrine did not affect citrate synthase activity ($P = 0.29$; Table 2), complex IV activity ($P = 0.90$; Table 2), or complex IV/CS ($P = 0.21$; Table 2).

Discussion

In this model of hyperdynamic endotoxaemic shock, intestinal microcirculatory and mitochondrial changes developed independently of regional blood flow. Restoring MAP with norepinephrine did not significantly affect microcirculatory perfusion or mucosal acidosis. Although five out of six animals displayed improved complex I and complex I/CS activity, we could not demonstrate any statistically significant increase in mitochondrial enzyme activity with norepinephrine treatment.

Septic shock is best described as a distributive shock, with an abnormal distribution of blood flow and signs of metabolic distress although cardiac output is normal or even increased. Microcirculatory dysfunction, with disturbed local vasomotor control and regional mismatch of oxygen supply and demand, seems to be the main factor behind the maldistribution of blood flow seen in septic shock. The introduction of the orthogonal polarization spectral imaging technique and its technical successor SDF has contributed to a new understanding of the microcirculatory dysfunction seen in sepsis,²⁶ but the factors behind this microcirculatory failure are not completely understood. Previously, Dubin and

colleagues¹² and Verdant and colleagues¹³ demonstrated that microcirculatory derangements in the intestinal mucosa appear in spite of a maintained SMA_{FI} in endotoxaemic shock. The decrease in microcirculatory perfusion seen after 24 h of endotoxaemia in the current study occurred in spite of a substantial increase in regional blood flow, strengthening the hypothesis that changes in regional blood flow is not of major importance in the pathogenesis of intestinal microcirculatory failure. Two recent studies using SDF indicated that perfusion pressure is of limited importance in mediating the disturbances seen in the sublingual microcirculation in septic shock.^{9 10} However, for obvious reasons, regional blood flow was not measured in these studies, and the fact that MAP could have increased at the expense of regional blood flow makes it hard to draw firm conclusions regarding the effects of the increase in perfusion pressure. In the current study, microcirculatory perfusion was reduced at T24 in parallel with a decrease in MAP from 90 to 60 mm Hg, indicating that the reduction in perfusion pressure could be an important factor contributing to microcirculatory dysfunction. After administration of norepinephrine, MAP was restored, whilst regional blood flow remained constant, allowing us to selectively evaluate the change in perfusion pressure. Increasing perfusion pressure did not improve any of the measured parameters characterizing the microcirculation, and this indicates that the role of perfusion pressure as a mediator of the microcirculatory

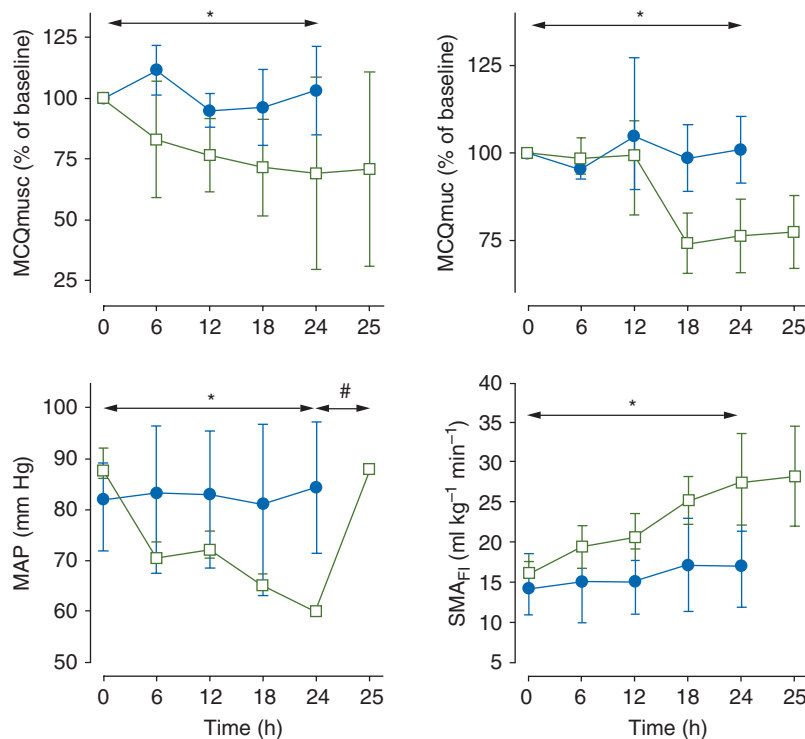


Fig 1 Microcirculatory blood flow in the muscularis layer (MCQ_{musc}), microcirculatory blood flow in the mucosa (MCQ_{muc}), mean arterial pressure (MAP), and superior mesenteric artery flow indexed to bodyweight (SMA_{FI}) in the endotoxin group (open squares, $n=6-8$) and sham animals (filled circles, $n=4-5$). Data are presented as mean (sd). * $P<0.05$ significant difference for change over time from 0 to 24 h in the endotoxin group. # $P<0.05$ significant difference for change over time from 24 to 25 h in the endotoxin group.

failure seen in endotoxaemic shock is limited, although we cannot rule out that the microcirculation over time for some reason was rendered unresponsive to changes in perfusion pressure.

Previous studies on septic shock patients have shown mitochondrial dysfunction in skeletal muscle,^{27, 28} and studies using animal models have found compromised mitochondrial function in various organ systems due to sepsis.²⁹⁻³² In this study, we demonstrated a decline in gut complex I activity in a fluid resuscitated model of hyperdynamic endotoxaemic shock, indicating that mitochondrial derangements could be a contributing factor to gut dysfunction in sepsis and further strengthening the hypothesis that mitochondrial dysfunction might be an important player in the development of organ failure.

A cardinal symptom of septic shock is severe systemic hypotension, and current guidelines for the treatment of septic shock include norepinephrine as a first-line vasopressor to increase perfusion pressure.⁷ However, concerns have been raised that norepinephrine can induce an excessive vasoconstriction causing further microcirculatory deterioration in the splanchnic organs.⁸ Previously, a large number of different animal models and monitoring techniques have been used to investigate the effects of norepinephrine on microcirculation in the intestinal mucosa in septic shock. The results from these studies are conflicting, yielding beneficial,¹⁶

detrimental,⁸ or no¹⁷⁻¹⁹ effects of norepinephrine in short-term models of endotoxaemic or septic shock. In a 12 h porcine model of bacteraemia, norepinephrine did not improve microcirculatory perfusion in the ileal mucosa measured with laser Doppler flowmetry,²⁰ but in this model there was only moderate hypotension (MAP 74 at treatment), and the achieved increase with norepinephrine was small (Δ MAP 9 mm Hg). Data from previous studies using short-term models of sepsis also indicate that norepinephrine could have detrimental effects on microcirculatory perfusion in the muscular layer of the small intestine.^{8, 21}

With the aforementioned disparity in results, we aimed to reach a better understanding of the intestinal microcirculatory effects of raising perfusion pressure with norepinephrine in septic shock. In order to mimic the clinical picture of vasoplegic septic shock, we used a fluid-resuscitated model of endotoxaemic shock where hyperdynamic hypotensive shock developed progressively over 24 h. The use of long-term models to study the effects of norepinephrine is important, since adrenergic receptor function and the response to catecholamines are modulated over time in septic shock.^{33, 34} The doses of norepinephrine used in the current study were similar to the doses used in clinical practice [318 (119) ng kg⁻¹ min⁻¹], and increased MAP from 60 to 90 mm Hg. However, we could not find any significant microcirculatory effects of norepinephrine administration. This was consistent

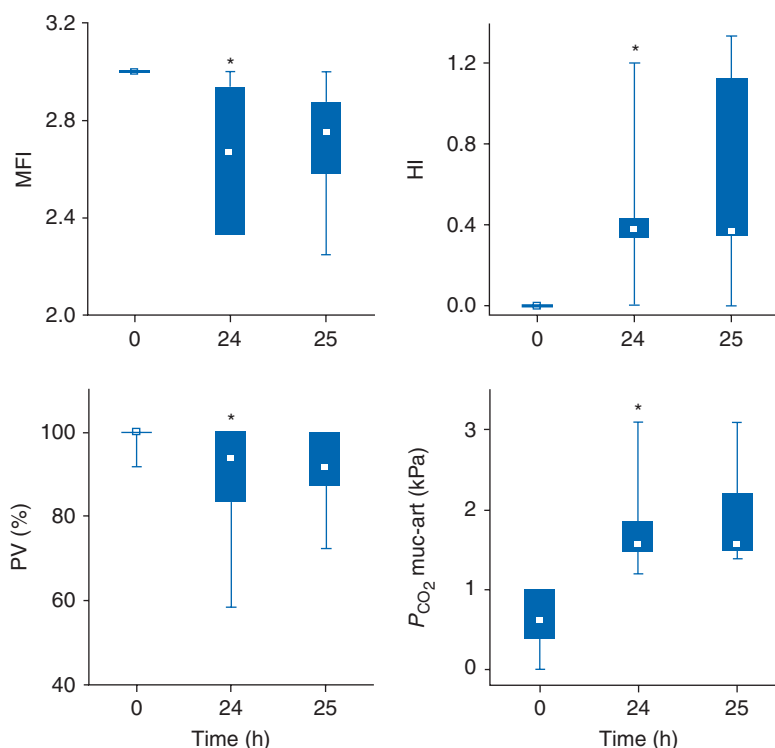


Fig 2 SDF-derived variables and mucosal-arterial P_{CO_2} -gap in the endotoxin group. MFI, microvascular flow index; HI, heterogeneity index; PV%, percentage of perfused villi; P_{CO_2} muc-art, mucosal-arterial P_{CO_2} -gap. Data are presented as median [interquartile range]. * $P < 0.05$ significant difference for change over time from 0 to 24 h. $n = 7$. In sham animals, these parameters remained stable over time, and for the sake of clarity this group was not included in the figure.

Table 2 Activities of mitochondrial enzymes. ^aSignificant ($P < 0.05$) difference T0–T24 in the endotoxin group (Student’s paired t -test or Wilcoxon matched pairs test). Data are presented as mean (sd) or median (interquartile range). [†]Expressed as $\mu\text{mol min}^{-1} \text{g wet weight}^{-1}$. [‡]Expressed as the ratio of enzyme activity to citrate synthase activity. $n = 6–7$ in the endotoxin group, $n = 3$ in the sham group

	Group	T0	T24	T25
Citrate synthase [†]	Endotoxin	15.7 (2.4)	13.6 (2.3)	12.1 (2.2) ^a
	Sham	15.7 (2.5)	12.7 (2.7)	
Complex I [†]	Endotoxin	3.2 (1.1)	2.0 (0.8)	2.6 (0.6) ^a
	Sham	3.5 (1.0)	3.5 (1.5)	
Complex I/CS [‡]	Endotoxin	0.22 (0.17–0.24)	0.16 (0.12–0.16)	0.21 (0.21–0.24)
	Sham	0.20 (0.19–0.25)	0.25 (0.24–0.29)	
Complex IV [†]	Endotoxin	6.3 (0.4)	6.2 (1.2)	6.1 (0.9)
	Sham	6.0 (1.3)	6.0 (1.3)	
Complex IV/CS [‡]	Endotoxin	0.41 (0.06)	0.46 (0.06)	0.51 (0.03)
	Sham	0.38 (0.05)	0.45 (0.10)	

for MFI, PV%, HI, and microcirculatory perfusion in the mucosal and muscular layer measured with laser Doppler flowmetry, providing a strong indication that norepinephrine had neither beneficial nor detrimental microcirculatory

effects. Furthermore, tonometry-derived changes in tissue P_{CO_2} levels have previously been shown to have a good correlation with microcirculatory changes in sepsis,³⁵ indicating that the development of acidosis in the intestinal mucosa is linked to microcirculatory disturbances. In line with this, P_{CO_2} muc-art increased over the first 24 h, but we could not demonstrate any change in P_{CO_2} muc-art after the administration of norepinephrine. Also, administration of norepinephrine did not affect the distribution of microcirculatory blood flow between the mucosa and muscularis layer as measured with laser Doppler flowmetry. A possible explanation to the absence of further microcirculatory vasoconstriction with norepinephrine is the ability of the intestinal microcirculation to recover flow from an initial norepinephrine-induced vasoconstriction despite continued vasoconstrictor stimuli, a phenomenon called the autoregulatory escape.³⁶

Interestingly, norepinephrine induced an increase in complex I activity and complex I/CS in five out of six animals, suggesting positive mitochondrial effects, and implying a possible difference in the effect of norepinephrine on the mitochondrial and microcirculatory level. The possibility of a beneficial effect of norepinephrine on intestinal mitochondrial function is supported by a recent study by Regueira and colleagues,³⁷ where norepinephrine improved complex I activity in liver mitochondria in a model of porcine endotoxaemia. Further studies are warranted to gain more

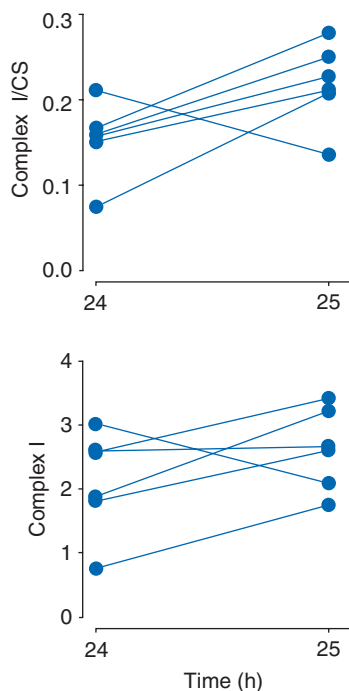


Fig 3 Individual values for change in complex I (mitochondrial complex I activity, $\mu\text{mol min}^{-1} \text{g wet weight}^{-1}$) and complex I/CS (the ratio of complex I activity to citrate synthase activity) between 24 and 25 h in the endotoxin group.

knowledge about the effects of norepinephrine on mitochondrial function, and what clinical importance any such effects may have.

Citrate synthase activity decreased to a similar extent in the endotoxin and sham groups, indicating depletion of mitochondria in both groups. This could be explained by the fact that the sheep were unable to ruminate, and this may have caused a progressive loss of enterocytes leading to lower mitochondrial count over time. Another possible explanation is the 12 h fasting period preceding the experiments.

Limitations

In this study, we aimed to mimic the clinical situation seen in septic shock patients using a model of fluid-resuscitated hyperdynamic, hypotensive shock developing over 24 h. Still, results from animal models of endotoxaemia are not directly applicable to the clinical situation of bacterial sepsis in humans, and this should be kept in mind when interpreting the results. We did not investigate the effects of titrating norepinephrine to different levels of MAP, and although a previous study showed identical effects of norepinephrine on sublingual microcirculation at MAP levels of 70, 80, and 90 mm Hg in septic shock,⁹ we cannot exclude that our results would have been different if we had targeted another MAP. The direct microcirculatory and mitochondrial effects of norepinephrine were evaluated, and we cannot draw any conclusions on the long-term effects of using

norepinephrine in septic shock. Mitochondrial enzyme activity was analysed in ileal wall biopsies; hence, it was not possible to separately determine changes in mitochondrial function in the muscular and mucosal layer. In this study, we focused on the intestinal microcirculation. However, in sepsis there seems to be a poor correlation between microvascular perfusion in different organs,³⁸ and the effects of norepinephrine on the microcirculation could have been different in other vascular beds. Laser Doppler flowmetry values are influenced by both red blood cell velocity and tissue haematocrit, and accordingly laser Doppler-derived perfusion indices are influenced by changes in both of these parameters. Also, although our results indicate that an increase in perfusion pressure is of limited importance for the intestinal microcirculation in endotoxaemic shock, we acknowledge that there are obviously levels of hypotension that will strongly influence the microcirculatory flow.

Conclusions

In conclusion, this model of fluid-resuscitated hyperdynamic endotoxaemic shock caused both microcirculatory and mitochondrial derangements in the ileum, indicating that both are likely to be important factors in the development of organ failure. Intestinal microcirculatory alterations developed in spite of an increase in regional blood flow. Restoring MAP with norepinephrine did not significantly alter the intestinal microcirculation as evaluated with laser Doppler flowmetry and SDF microscopy, indicating that perfusion pressure manipulation is of limited importance to the intestinal microcirculation in hyperdynamic endotoxaemic shock. However, norepinephrine improved mitochondrial complex I function in five of six animals, suggesting a potential divergence in the microcirculatory and mitochondrial effect of norepinephrine, and further studies investigating possible positive mitochondrial effects of norepinephrine are warranted.

Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

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Declaration of interest

None declared.

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