

CRITICAL CARE

**Arginine-vasopressin attenuates beneficial norepinephrine effect on jejunal mucosal tissue oxygenation during endotoxaemia**

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**Background.** The objective of the present study was to investigate the effects of increasing doses of norepinephrine (NE) with or without arginine-vasopressin (AVP) on intestinal oxygen supply and jejunal mucosal tissue oxygen tension in an acute endotoxic pig model.

**Methods.** In this prospective, randomized, experimental study on 24 domestic pigs, jejunal mucosal tissue  $P_{O_2}$  ( $P_{O_2muc}$ ) was measured using two Clark-type surface oxygen electrodes. Oxygen saturation of jejunal microvascular haemoglobin ( $HbO_{2j}$ ) was determined by tissue reflectance spectrophotometry. Systemic haemodynamic variables, mesenteric-venous and systemic acid–base and blood gas variables, and lactate measurements were recorded. Measurements were performed at baseline, after *Escherichia coli* lipopolysaccharide (LPS) administration, and at 20 min intervals during incremental NE infusion (0.05, 0.1, 0.5, 1.0, and 2  $\mu\text{g kg}^{-1} \text{min}^{-1}$ , respectively) with 57 mU  $\text{kg}^{-1} \text{h}^{-1}$  AVP ( $n=8$ ; NE+AVP group) or without ( $n=8$ ; NE group); or infusion of an equal amount of normal saline ( $n=8$ ; CON group).

**Results.** LPS infusion led to a significant ( $P<0.05$ ) decrease of  $P_{O_2muc}$  and  $HbO_{2j}$ . Both NE and NE+AVP increased arterial pressure, cardiac output, and mesenteric artery blood flow. Concomitant to an increase in systemic oxygen delivery, NE improved  $P_{O_2muc}$  and  $HbO_{2j}$ . NE alone was superior in restoration of  $P_{O_2muc}$  when compared with NE+AVP.

**Conclusions.** Both NE and NE+AVP improved global haemodynamics and systemic oxygen transport variables when compared with control animals in an acute endotoxic pig model. NE improved jejunal  $P_{O_2muc}$  at all dosages. NE effects were significantly blunted by simultaneous administration of AVP.

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Septic shock still has a mortality rate of 40–60%,<sup>1 2</sup> and studies have demonstrated the significance of microcirculatory alterations and dysfunction in the early stages of sepsis.<sup>3 4</sup> Alterations in the intestinal microcirculation resulting from sepsis can cause damage to the microcirculatory endothelium, significant changes in vascular permeability, and disruption of the intestinal mucosal epithelium, leading to possible bacterial translocation.<sup>5</sup>

Vasopressor therapy is required to maintain perfusion during vasoplegia in septic shock. Below a certain mean arterial pressure, autoregulation in various vascular beds may be lost. If the perfusion pressure decreases below this lower range, organ blood flow also decreases in a linear fashion.<sup>6</sup> Thus, vasopressor agents are infused in critically ill patients who are hypotensive, despite adequate fluid resuscitation, to augment systemic perfusion pressure.

The international guidelines for management of severe sepsis and septic shock 2008 recommend either norepinephrine (NE) or dopamine as the first-choice vasopressor agent to correct hypotension in septic shock.<sup>7</sup> In recent years, several investigators including our group demonstrated that an infusion of 2–4 IU h<sup>-1</sup> of arginine-vasopressin (AVP) can successfully stabilize systemic haemodynamics even in patients with advanced vasodilatory shock.<sup>8–10</sup> Thus, the surviving sepsis campaign recommends that AVP may be added to NE in patients with refractory shock, despite high-dose conventional vasopressor therapy.<sup>7</sup> But we have to keep in mind that the VASST trial demonstrated that low-dose AVP combined with NE did not reduce mortality rates in septic shock patients compared with those treated with NE alone.<sup>10</sup> The study of Micek and colleagues<sup>11</sup> even showed increased mortality in patients with septic shock receiving additional AVP to NE.

Until now, the effects of either NE or AVP on oxygen supply on the microcirculatory level, especially the gut mucosa, are under debate.<sup>12–18</sup> For example, AVP alone was reported either to decrease mucosal blood flow, has no effect, or even increases mucosal blood flow in different investigations.<sup>15 19–21</sup> In particular, the effects on mucosal microcirculation when both NE and AVP are administered together are not fully elucidated. To address these questions, we conducted a prospective, randomized, experimental animal study. In an acute endotoxaemic pig model, we measured direct effects of incremental doses of NE with or without an additional fixed rate of AVP on intestinal tissue oxygen supply and, in particular, jejunal mucosal tissue oxygen tension ( $P_{O_2muc}$ ). The novelty of this trial is the investigation of gut oxygen supply during administration of both NE and vasopressin together. This study design mirrors clinical practice more precisely than a comparison of these two drugs administered separately.

## Methods

### *Anaesthesia and animal instrumentation*

The experimental protocol was approved by the Federal Ministry of Science and Research in Vienna, Austria. Animals were managed in accordance with the American Physiological Society institutional guidelines, and the Position of the American Heart Association on Research Animal Use, as adopted on November 11, 1984. Anaesthesia was used in all surgical interventions, all unnecessary suffering was avoided, and research was terminated if unnecessary pain or distress resulted. Our animal facilities meet the standards of the American Association for Accreditation of Laboratory Animal Care.

Twenty-four domestic pigs (35–42 kg, both sexes) were fasted for 12 h, but had free access to water. After induction of anaesthesia with i.m. ketamine hydrochloride (20 mg kg<sup>-1</sup>), the tracheas of the animals were intubated and

the lungs mechanically ventilated with a PEEP of 5 mm Hg. Tidal volume and respiratory frequency were adjusted to maintain a  $P_{CO_2}$  of 4.66–6.0 kPa at baseline; fractional inspiratory oxygen concentration was primarily set at 0.3 and further adjusted to arterial oxygen tension measurements ( $P_{a_{O_2}}$  of about 17 kPa). Anaesthesia was maintained using a continuous infusion of midazolam (0.5 mg kg<sup>-1</sup> h<sup>-1</sup>) and fentanyl (10 µg kg<sup>-1</sup> h<sup>-1</sup>). If haemodynamic variables or clinical evaluation indicated an inadequate depth of anaesthesia, additional bolus doses of midazolam (5 mg) and fentanyl (100 µg) were administered. All animals were infused to an equal extent with Ringer's lactate and modified gelatin (MW 22 600) to keep central venous pressure constant at about 12 mm Hg throughout the experiment. After preparation of the right carotid artery and the internal jugular vein, an arterial line and a 7.5 Fr gauge pulmonary artery catheter (Baxter, Irvine, CA, USA) were inserted. Midline laparotomy was then performed, and a 16 gauge catheter was placed in the superior mesenteric vein for intermittent blood sampling. The arteria mesenterica superior was prepared for measurement of blood flow measuring. To expose part of the mucosa for tissue oxygenation measurements, a 20 cm antimesenteric enterotomy was performed in the mid-jejunum. The boundary of the mucosa was sutured on a cork plate with an oval opening. The intestine was re-introduced into the abdominal cavity with the exception of the exposed mucosa. The temperature of the preparation was maintained at 38.5°C (normal porcine temperature), by covering the preparation with a plastic box, including a temperature sensor and a servo-controlled heated water bath.

### *Haemodynamic and blood gas measurements*

Arterial-, pulmonary artery, and central venous pressure were measured using three Statham P10EZ pressure transducers (Spectramed-Statham, Bilthoven, The Netherlands). Cardiac output was determined in triplicate by the thermodilution method. Heart rate, arterial pressure, and core temperature were continuously recorded. Zero reference for all pressures was the mid-chest position. Arterial, central venous, and mesenteric venous blood gases and acid–base status were determined using an automatic blood gas analyzer (AVL 995, AVL, Graz, Austria). Haemoglobin oxygen saturation was measured with a haemo-oximeter (Cooximeter, AVL). Haemoglobin concentration was assessed using the cyanmethaemoglobin method. Arterial and mesenteric venous lactate was measured with a lactate analyser based on reflectance photometry (Accusport, Boehringer, Mannheim, Germany). A Transonic Animal Research Flowmeter (Transonic, Ithaca, NY, USA) was used for measuring mesenteric arterial blood flow.

### *Measurements of jejunal mucosal tissue oxygenation*

Measurement of  $P_{O_2muc}$  (Clark-type multiwire surface electrodes; Eschweiler, Kiel, Germany) and

microvascular haemoglobin oxygen saturation [ $\text{HbO}_2\text{j}$ ; Erlangen microlight-guide spectrophotometer (EMPHO II), BGT, Überlingen, Germany] has been described in detail in previous studies.<sup>16 22 23</sup> Briefly, mucosal tissue oxygen tension ( $P_{\text{O}_2\text{muc}}$ ) was measured by two Clark-type multiwire surface electrodes (Eschweiler). These electrodes were calibrated using pure nitrogen and room air in a water bath warmed to  $38.5^\circ\text{C}$ . One electrode consists of eight platinum wires, each of which has a diameter of  $15\ \mu\text{m}$ , representing an individual measuring point and one Ag–AgCl reference electrode. These electrodes rather measure the oxygen tension in the superficial endothelial layer of the mucosa. An EMPHO II was used for determination of jejunal microvascular haemoglobin saturation ( $\text{HbO}_2$ ). The measuring principle is based on the use of one illuminating, and six detecting, microlight guides (each  $250\ \mu\text{m}$  in diameter), and a rapidly rotating band-pass interference filter disk for the generation of monochromatic light in 2 nm steps, within the spectral range of 502–628 nm, representing 64 different wavelengths. Penetration depth of this measurement device exceeds the gut mucosa and assesses also haemoglobin saturation from deeper intestinal layers.<sup>23</sup>

### Experimental procedure

Animals were randomly assigned to one of the three study groups. NE animals ( $n=8$ ) were given NE by infusion at incremental rates (0.05, 0.1, 0.5, 1.0, and  $2.0\ \mu\text{g}\ \text{kg}^{-1}\ \text{min}^{-1}$ , respectively). NE-AVP pigs ( $n=8$ ) received NE in same dosages (0.05, 0.1, 0.5, 1.0, and  $2.0\ \mu\text{g}\ \text{kg}^{-1}\ \text{min}^{-1}$ )

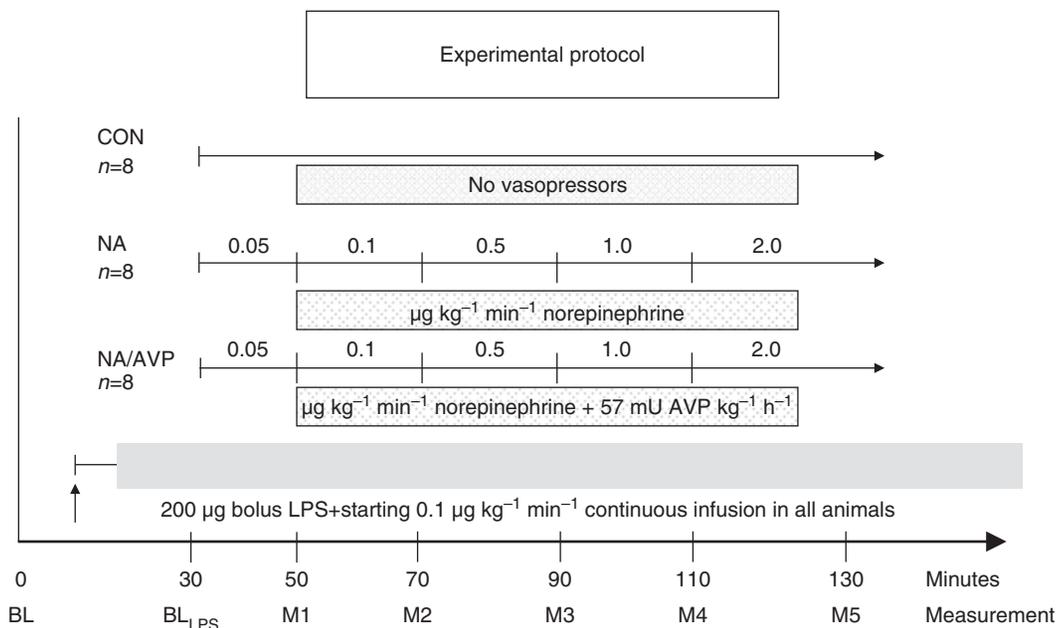
plus concomitant administration of AVP at a constant rate ( $57\ \text{mU}\ \text{kg}^{-1}\ \text{h}^{-1}$ ). NE doses were chosen according to a previous trial investigating NE effects on jejunal oxygenation in healthy pigs.<sup>14</sup> This AVP dosage reflects a clinically used dosing regimen of  $4\ \text{IU}\ \text{h}^{-1}$  ( $=0.067\ \text{IU}\ \text{min}^{-1}$ ) in a 70 kg patient.<sup>9</sup> CON animals ( $n=8$ ) served as controls.

After animal preparation and a resting period of 60 min, baseline measurements were performed (BL;  $t=0$  min): systemic haemodynamics, mesenteric artery blood flow,  $P_{\text{O}_2\text{muc}}$ ,  $\text{HbO}_2\text{j}$ , arterial and mesenteric-venous blood gas and acid–base parameters, and serum lactate concentrations (Fig. 1). Subsequently, a  $200\ \mu\text{g}$  i.v. bolus of *Escherichia coli* lipopolysaccharide (LPS; serotype O111:b4, Difco Laboratories, Detroit, MI, USA) was given, followed by a continuous infusion of  $0.1\ \mu\text{g}\ \text{kg}^{-1}\ \text{min}^{-1}$  LPS throughout the experiment. Thirty minutes after starting LPS, a further measurement was performed (BL-LPS;  $t=30$  min). Afterwards, infusion of study drugs (NE and NE-AVP), respectively, and solvent (CON) was started. Further measurements were conducted at 20 min intervals (M1–M5;  $t=50, 70, 90, 110,$  and 130 min).

At the end of each experiment, the animals were killed by a bolus injection of midazolam, fentanyl, and potassium chloride 40 mM.

### Statistical methods

Systemic oxygen delivery, oxygen consumption, and systemic and mesenteric oxygen extraction ratio were calculated according to the standard formulae.  $P_{\text{O}_2\text{muc}}$  and



**Fig 1** Experimental protocol. After baseline measurements at time point 0 min (BL), a bolus injection of  $200\ \mu\text{g}$  of *E. coli* LPS followed by a continuous infusion of  $0.1\ \mu\text{g}\ \text{kg}^{-1}\ \text{min}^{-1}$  throughout the experiment was administered. After another measurement in endotoxaemia at time point 30 min ( $\text{BL}_{\text{LPS}}$ ), continuous infusion of increasing dosages of NE (NE group), NE plus a constant dosage of arginine vasopressin (AVP; NE/AVP group), or saline (CON group) was started. Further measurements were made in 20 min intervals (M1–M5).

HbO<sub>2j</sub> were recorded for a period of at least 100 s. Mean values of these variables were used for statistical comparison.

The Shapiro–Wilk test was used to test normality and Levene’s test to control the homogeneity of the variance. Analysis of variance for repeated measurements was performed to analyse differences in means between and within the groups (time×group effect and time effect, respectively). The global hypothesis was tested two-tailed at the *P*<0.05 significance level. Multiple comparisons between the vasopressor groups and the control group were performed by the two-tailed Dunnett’s *t*-test. A Bonferroni correction was used for comparison of multiple measurements within the groups vs baseline. Data in text, tables, and figures are presented as mean values (sd), if not indicated otherwise.

### Results

At baseline (*t*=0) and after beginning of LPS administration (*t*=30 min), no significant differences between the groups were detected. Arterial normoxaemia and normocapnia were maintained in all groups (Table 1). Central

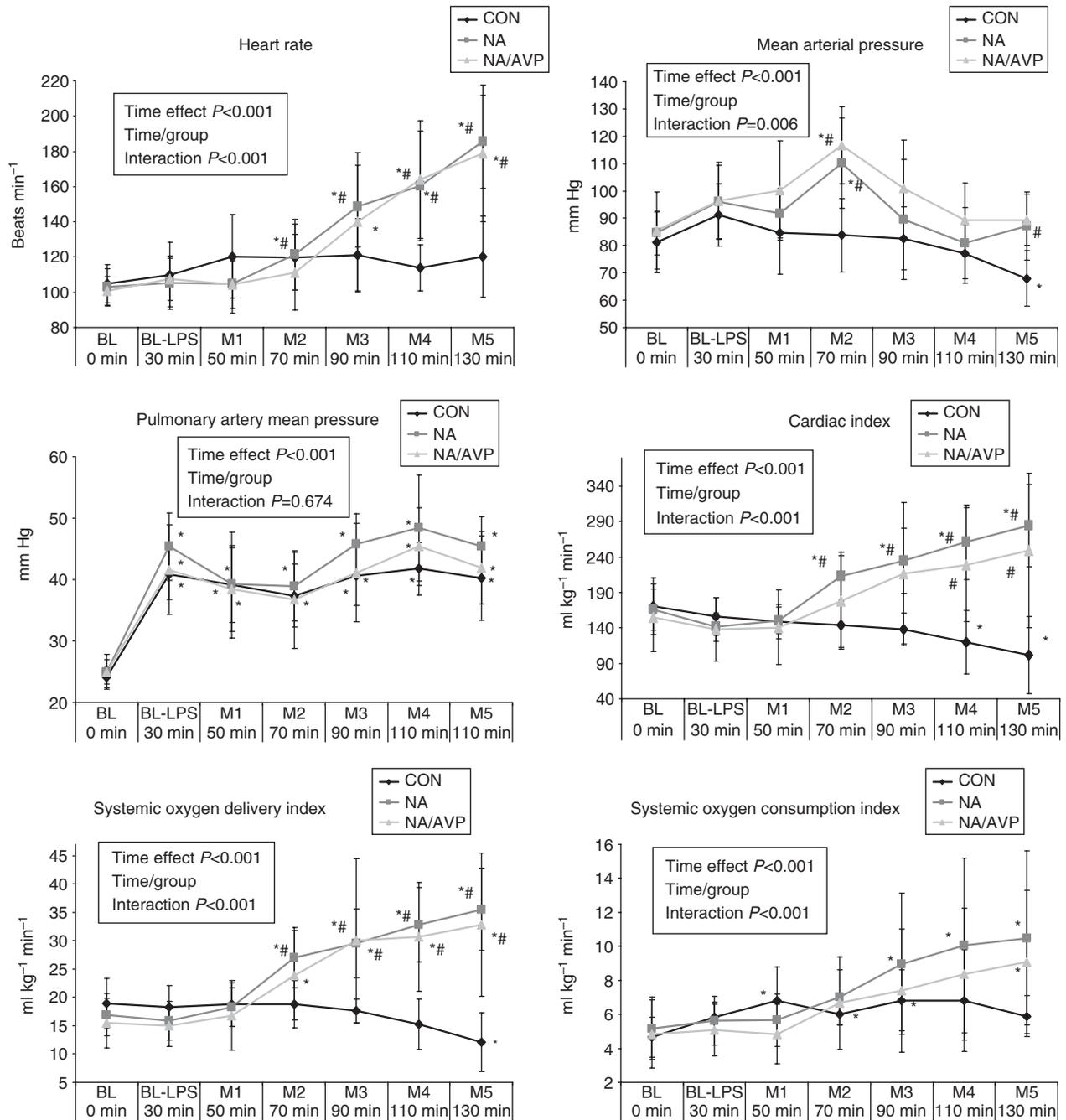
venous pressure and pulmonary capillary wedge pressure did not differ between the groups throughout the experiments. For spontaneous changes of measured variables over the time in a control group without any intervention, we would like to refer to previous studies.<sup>14 16 24</sup>

### Systemic and intestinal variables after endotoxin administration

Infusion of *E. coli* LPS was characterized by an increase in mean pulmonary artery pressure and a decrease of mean arterial pressure over time (Fig. 2). LPS administration led to a decrease in systemic oxygen delivery index due to a decrease in cardiac output at time points 110 and 130 min, which was accompanied by a decrease in arterial and mesenteric venous pH, and an increase in arterial and mesenteric venous lactate (Figs 2 and 3, Table 1). Furthermore, mesenteric artery blood flow decreased significantly after endotoxin administration, leading to an increase in intestinal oxygen extraction ratio (Fig. 3). This decrease in mesenteric artery blood flow was more pronounced compared with the decline in systemic blood flow (54% vs 34% from baseline).

**Table 1.** Arterial and intestinal venous blood gases and pH in control (CON), norepinephrine (NE), and norepinephrine/arginine-vasopressin (NE/AVP) treated animals. \*Significant vs baseline. BL, baseline; BL-LPS, baseline-lipopolysaccharide administration; M, measurement; Art pH, arterial pH; Art *P*O<sub>2</sub>, arterial oxygen tension; Art *P*CO<sub>2</sub>, arterial carbon dioxide tension; Int ven pH, intestinal venous pH; Int ven *P*O<sub>2</sub>, intestinal venous oxygen tension; Int ven *P*CO<sub>2</sub>, intestinal venous carbon dioxide tension. Values are mean (sd)

	0 min BL	30 min BL-LPS	50 min M1	70 min M2	90 min M3	110 min M4	130 min M5	Time effect	Time-group interaction
Drugs (μg kg <sup>-1</sup> min <sup>-1</sup> )									
(μg kg <sup>-1</sup> min <sup>-1</sup> )									
(mU kg <sup>-1</sup> h <sup>-1</sup> )									
CON	0	0	0	0	0	0	0		
NE	0	0	0.05	0.1	0.5	1.0	2.0		
NE/AVP	0	0	0.05/57	0.1/57	0.5/57	1.0/57	2.0/57		
Art pH									
CON	7.45 (0.01)	7.41 (0.04)*	7.42 (0.05)	7.41 (0.07)	7.39 (0.08)	7.38 (0.08)	7.37 (0.09)*	<b>P&lt;0.001</b>	<i>P</i> =0.421
NE	7.44 (0.03)	7.43 (0.02)	7.43 (0.03)	7.40 (0.05)	7.38 (0.07)*	7.36 (0.07)*	7.34 (0.12)*		
NE/AVP	7.43 (0.04)	7.42 (0.03)	7.40 (0.04)	7.40 (0.07)	7.38 (0.05)	7.36 (0.03)*	7.36 (0.07)*		
Art <i>P</i> O <sub>2</sub> (kPa)									
CON	15.3 (1.3)	15.3 (1.6)	14.8 (2.4)	14.8 (1.6)	15.0 (2.1)	14.8 (1.2)	14.4 (1.7)	<i>P</i> =0.235	<i>P</i> =0.994
NE	15.0 (1.7)	15.2 (1.6)	14.0 (1.2)	14.2 (1.5)	14.0 (1.7)	14.0 (1.2)	14.2 (1.5)		
NE/AVP	15.3 (1.5)	14.8 (0.9)	14.2 (1.6)	14.4 (1.9)	14.2 (1.9)	14.0 (1.7)	14.1 (1.7)		
Art <i>P</i> CO <sub>2</sub> (kPa)									
CON	5.1 (0.1)	5.1 (0.3)	5.2 (0.4)	5.5 (0.5)	5.5 (0.7)	5.3 (0.4)	5.3 (0.5)	<b>P=0.002</b>	<i>P</i> =0.621
NE	4.9 (0.3)	5.1 (0.3)	5.3 (0.4)	5.5 (0.5)	5.5 (0.5)*	5.6 (0.3)*	5.6 (0.5)*		
NE/AVP	5.1 (0.4)	4.9 (0.3)	5.1 (0.7)	5.5 (0.5)	5.2 (0.3)	5.3 (0.4)	5.5 (0.4)*		
Int ven pH									
CON	7.38 (0.02)	7.34 (0.04)*	7.36 (0.05)	7.33 (0.06)*	7.31 (0.06)*	7.30 (0.07)*	7.26 (0.12)*	<b>P&lt;0.001</b>	<i>P</i> =0.765
NE	7.38 (0.05)	7.36 (0.04)	7.37 (0.07)	7.35 (0.06)	7.33 (0.06)	7.30 (0.07)*	7.27 (0.11)*		
NE/AVP	7.39 (0.04)	7.37 (0.03)	7.35 (0.04)	7.34 (0.05)	7.30 (0.03)*	7.30 (0.03)*	7.28 (0.04)*		
Int ven <i>P</i> O <sub>2</sub> (kPa)									
CON	6.1 (0.7)	5.7 (0.5)	5.2 (0.7)*	5.1 (0.5)*	5.3 (0.8)	4.8 (0.5)*	4.5 (1.3)*	<b>P&lt;0.001</b>	<i>P</i> =0.910
NE	5.3 (0.5)	5.3 (0.8)	5.3 (0.7)	5.6 (0.9)	5.7 (1.1)	5.5 (1.2)	5.5 (1.1)		
NE/AVP	5.6 (1.1)	5.6 (1.1)	5.6 (0.9)	5.1 (0.9)	5.6 (0.8)	5.5 (0.8)	5.5 (0.9)		
Int ven <i>P</i> CO <sub>2</sub> (kPa)									
CON	6.0 (0.3)	6.0 (0.4)	6.1 (0.5)	6.8 (1.1)	6.8 (1.1)	6.8 (1.1)*	7.0 (1.5)	<b>P&lt;0.001</b>	<i>P</i> =0.933
NE	6.0 (0.3)	6.1 (0.5)	6.4 (0.7)	6.7 (0.8)*	6.7 (0.9)	6.9 (0.8)*	7.0 (1.1)*		
NE/AVP	5.9 (0.4)	5.9 (0.4)	6.3 (0.5)	6.7 (0.4)*	6.7 (0.4)*	6.8 (0.5)*	7.0 (0.7)*		



**Fig 2** Systemic haemodynamic and oxygen transport variables. \*Significant vs baseline. #Significant vs control.

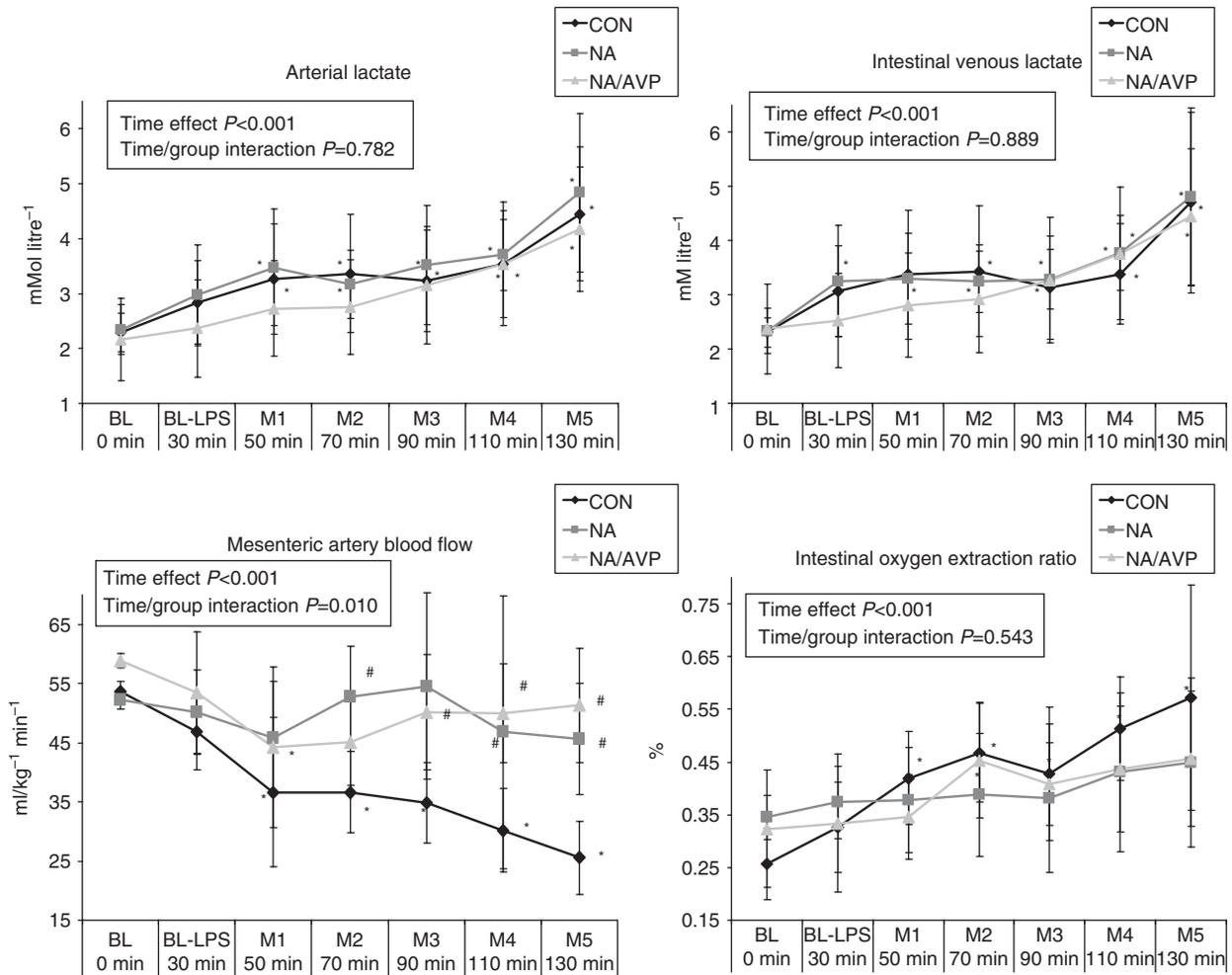
*Endotoxaemia and small intestinal mucosal tissue oxygenation*

HbO<sub>2j</sub> and P<sub>O<sub>2</sub>muc</sub> decreased significantly after LPS administration in control animals (Fig. 4).

*Systemic and intestinal variables after vasopressor administration*

Both NE and NE+AVP infusion increased heart rate and arterial pressure (Fig. 2). Pulmonary artery hypertension persisted throughout the experiment and was not

significantly influenced by NE alone or in combination with AVP. Parallel to an increase in cardiac output, mesenteric artery blood flow increased significantly after vasopressor administration when compared with CON (Figs 2 and 3). Systemic oxygen delivery increased in both the NE and the NE+AVP groups, in a dose-dependent manner, accompanied with a decreased systemic oxygen extraction compared with CON. Vasopressor administration did not alter metabolic, blood gases, or acid-base balance between the groups (Table 1, Fig. 3).



**Fig 3** Arterial and intestinal venous lactate, intestinal oxygen extraction ratio, and mesenteric artery blood flow. \*Significant vs baseline. #Significant vs control.

*Vasopressor and intestinal tissue oxygenation*

Vasopressor administration increased  $PO_{2muc}$  and  $HbO_{2j}$  (Fig. 4). Whereas the combination of AVP with NE increased  $PO_{2muc}$  only in the highest concentration, NE alone improved  $PO_{2muc}$  at any dosage administered. NE alone was superior in restoration of  $PO_{2muc}$  and  $HbO_{2j}$  when compared with NE+AVP.

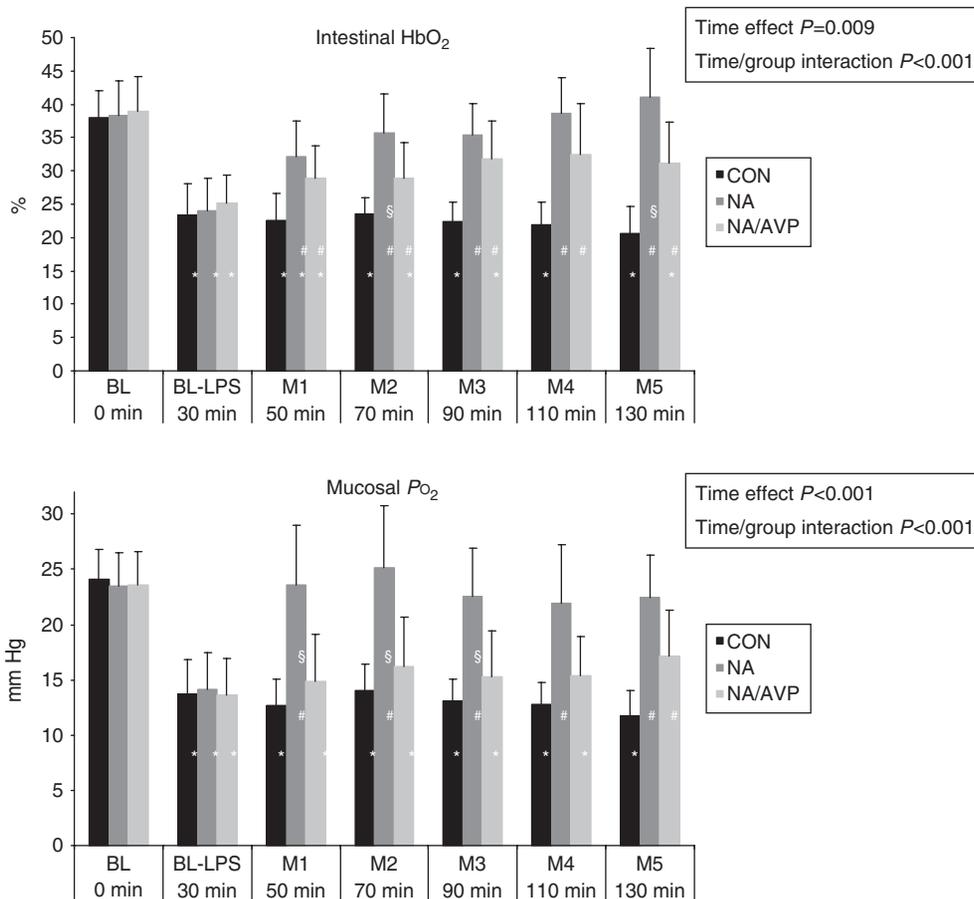
**Discussion**

Administration of NE increases jejunal  $PO_{2muc}$  and  $HbO_{2j}$  in an acute endotoxaemic pig model at all dosages administered. This favourable effect was significantly attenuated by concomitant administration of AVP at a fixed dose.

Administration of *E. coli* LPS led to pulmonary hypertension, a progressive decline in cardiac index, paralleled by a decrease in mean arterial pressure. In this model, the reduction of mesenteric arterial blood flow was out of proportion to the decline in whole systemic blood flow (54%

vs 34%). The pronounced decrease in splanchnic blood flow obviously must have occurred in favour to other organs, most likely reflecting particular blood flow hierarchy among different organs and tissues similar to observations in various other shock states.<sup>25</sup> Heterogeneity in vessel receptor types, receptor density, the magnitude of regional sympathoadrenergic activity, and the importance of local blood flow autoregulation have been used as explanations for disparity in organ blood flow under pathophysiological conditions.<sup>23</sup> Administration of LPS not only decreased mesenteric arterial blood flow and increased intestinal oxygen extraction ratio, but also resulted in a decrease in mesenteric venous pH and an increase in arterial and mesenteric venous lactate concentration without an apparent increase in splanchnic lactate production.

On the microcirculatory level,  $HbO_{2j}$  and  $PO_{2muc}$  decreased significantly after LPS administration. LPS effects on jejunal mucosal tissue oxygen supply have been reported to result from regional vasoconstriction and probably tissue damage mediated by some direct or indirect endotoxin effects.<sup>26</sup> In support of this concept, LPS has



**Fig 4** Jejunal microcirculatory parameters. \*Significant vs baseline. #Significant vs control. \$Significant between combination of NE/vasopressin and NE alone.

been shown to reduce microvascular mucosal villus blood flow and to reduce the density of perfused capillaries in small intestinal villi.<sup>27 28</sup>

Although  $P_{O_2muc}$  and  $HbO_{2j}$  decreased, this was not accompanied by an apparent increase in splanchnic lactate production. Nonetheless, one should be very cautious in stating that this finding indicates no oxygen debt in the mucosal tissue, as no regional tissue metabolic measurements were assessed and only the acute phase of the endotoxaemia was examined. The mucosal endothelial tissue is very vulnerable to attenuation in oxygen supply leading to a breakdown of the barrier between the intestinal lumen and the blood stream.

Both NE and the combination of NE with AVP were equally effective in increasing perfusion pressure and systemic oxygen delivery due to an increase in cardiac output mainly generated by an increase in heart rate. The development and degree of acute pulmonary hypertension was not modified by the infusion of increasing dosages of NE with or without AVP. Interestingly, despite increasing dosages of NE in both animal groups, the drug effect on mean arterial pressure attenuated during the experiment regardless of a concomitant infusion of AVP. In face of the progressive increase in cardiac index, this result can

only be interpreted by an increase in systemic vascular conductance. We can only speculate on the organ most likely involved in arterial vasodilatation at higher dosages of NE. In a previous investigation in healthy pigs, we compared the effects of NE and phenylephrine on systemic haemodynamics, oxygen delivery, and tissue oxygen supply to the jejunal mucosa.<sup>14</sup> NE administration exhibited a similar haemodynamic response, that is, significant increase in cardiac index mediated by progressive tachycardia and a transient increase in mean arterial pressure at moderate dosages. In contrast, phenylephrine, which is predominantly an  $\alpha_1$ -adrenergic agonist, increased mean arterial pressure in a dose-dependent relationship with only small effects on systemic blood flow.<sup>14</sup> Therefore, one might speculate that some  $\beta_2$ -agonistic activity of NE might mediate vasodilatation, presumably in the vasculature of skeletal muscle, accounting for the observed attenuation of pressor response at higher dosages in the pig.

Of course, major differences do exist in the haemodynamic response of septic shock patients to an infusion of NE or a combination of NE and AVP when compared with our animal experiments. In human septic shock, the effects of NE on heart rate and cardiac index after adequate volume resuscitation may be quite variable. In

patients with normal cardiac pump function, infusion of moderate dosages of NE typically results in reversal of hypotension with a clear dose–response relationship without major changes in heart rate and cardiac index. In contrast, patients with compromised systolic pump function, that is, an ejection fraction of <45%, may exhibit a significant decrease in cardiac index in response to an increase in left ventricular afterload necessitating additional infusion with an inotropic agent.<sup>29</sup> If high dosages of NE are infused, catecholamine toxicity manifesting as tachycardia, tachyarrhythmia, and probably deterioration of pulmonary hypertension and a decrease in cardiac index may develop. In patients with advanced vasodilatory shock, we have demonstrated that administration of high dosages of NE before introducing AVP into the therapeutic regimen was associated with significantly increased mortality.<sup>30,31</sup> In addition, in patients with vasodilatory shock, co-administration of AVP at a fixed rate of 4 IU h<sup>-1</sup> resulted in a significant reduction in heart rate and a decrease in the development of new-onset tachyarrhythmias. Both findings are most likely explained by the ‘NE-sparing effects’ of AVP administration with significant reduction of  $\beta_1$ -adrenoreceptor excitation.<sup>9</sup> In this acute, short-term endotoxin model, pulmonary hypertension was not modified by additional administration of AVP. Prolonged infusion of AVP in septic shock has been shown to result in a significant decrease of pulmonary artery pressure probably related to some vasodilatory effects of AVP mediated by V<sub>2</sub>-receptors on pulmonary artery smooth muscle cells.<sup>31</sup>

Infusion of NE alone significantly improved jejunal  $P_{O_2\text{muc}}$  and  $\text{HbO}_{2\text{j}}$  after endotoxaemia. This improvement was accompanied by an increase in systemic blood flow and a normalization of mesenteric artery blood flow when compared with control animals. In a previous study in normal pigs, increasing dosages of NE similar to the dosages applied in this experiment significantly increased cardiac output and systemic oxygen delivery, but had no effect on mesenteric oxygen extraction ratio, jejunal  $P_{O_2\text{muc}}$ , or  $\text{HbO}_{2\text{j}}$ .<sup>14</sup> During acute endotoxaemia, infusion of NE increased mesenteric arterial blood flow to baseline values already at moderate dosages. A normalization of  $P_{O_2\text{muc}}$  and  $\text{HbO}_{2\text{j}}$  occurred in parallel to the improvement in mesenteric arterial blood flow strongly suggesting a cause to effect relationship. Since the pressure response to NE disappeared at higher dosages, this improvement cannot be simply explained by an increase in systemic perfusion pressure. The natural agonist NE activates, with variable affinity and intrinsic efficacy, all adrenoreceptors in mammals. However, the effect responsible for the amelioration of mucosal tissue oxygenation by NE administration was probably not due to  $\beta_2$ -adrenoreceptor activation. In all likelihood, a redistribution of blood flow occurs away from the submucosal and muscularis layer in favour of the mucosal tissue layer after NE administration, an effect attenuated by the additional AVP infusion.

However, when discussing catecholamine effects on regional haemodynamics, we have to keep in mind that significant species differences in the response of the splanchnic vascular bed to NE have been reported in the literature.<sup>32–35</sup> Therefore, extrapolation of experimental results obtained from animal models to the clinical situation can only be made with great caution. In patients suffering from septic shock, NE has been reported to increase splanchnic blood flow and tonometrically derived gastric mucosal pH and to decrease splanchnic lactate production.<sup>36–38</sup> In contrast, in one study, an unpredictable effect of NE on splanchnic blood flow was reported.<sup>39</sup> In addition, the study period is limited to the very acute phase of endotoxaemia. No conclusions can be made on long-term effects of these vasopressors in the present investigation.

In previous studies in pigs, we have already demonstrated that administration of AVP in increasing dosages up to a maximal dose of 0.229 U kg<sup>-1</sup> h<sup>-1</sup> did not additionally compromise jejunal mucosal oxygenation in comparison with untreated control animals during endotoxaemia.<sup>15</sup> In contrast, in healthy animals, the same dosages significantly decreased jejunal microvascular blood flow and mucosal oxygen supply in a dose-dependent manner.<sup>16</sup> In the present study, administration of AVP at a fixed dose comparable with 4 IU h<sup>-1</sup> in a 70 kg patient reversed the beneficial regional effect of NE on mucosal oxygen supply, despite a comparable increase in mesenteric arterial blood flow in NE+AVP animals. Therefore, the improvement in regional splanchnic oxygen supply in particular to the mucosal layer brought about by the administration of NE was entirely blunted by concomitant administration of AVP. AVP and NE exhibit distinctive haemodynamic effects on the splanchnic vasculature which may account for the observed decrease in mucosal tissue oxygen supply. *In vitro* experiments using rat and human mesenteric arteries have demonstrated that AVP even at very low dosages causes a concentration-dependent potentiation of the contractions elicited by NE, that is, a shift of the dose–response contraction curve to NE to the left.<sup>40,41</sup> This effect could be impeded by pre-treatment with a specific V<sub>1a</sub>-receptor antagonist. Therefore, it is conceivable that the addition of AVP greatly enhances the vasoconstrictor response to NE explaining the observed decrease in  $P_{O_2\text{muc}}$  and  $\text{HbO}_{2\text{j}}$  in our experiments. Unfortunately, little is known on specific receptor density and in particular receptor distribution within the vasculature of the different layers of the small intestine. In our experiment, we observed a significant reduction in  $P_{O_2\text{muc}}$  and  $\text{HbO}_{2\text{j}}$ , despite similar mesenteric blood flow when AVP was additionally infused to NE. This finding strongly suggests that redistribution of blood flow at the expense of the submucosal and mucosal tissue layers must have occurred in the presence of AVP implying significant differences in V<sub>1a</sub>-receptor density, distribution within the microvasculature of the different intestinal layers, or both.

Administration of this amount of *E. coli* LPS does not cause a vasoplegic shock in this pig model of endotoxaemia. For that reason, we are not able to say if we would see the same effects on haemodynamic alterations in pigs with manifest vasoplegic shock, although we do see a significant and pronounced decrease in mucosal tissue oxygenation. This impairment in mucosal tissue oxygenation due to LPS in the blood was shown to be independent of systemic haemodynamics,<sup>24</sup> such that interpretation of the results is unlikely.

## Conclusion

This is the first study investigating the combined effects of NE and AVP vs sole administration of NE on jejunal tissue oxygen supply in an endotoxin model in the pig. The addition of AVP to NE attenuated any beneficial effect of NE on tissue oxygen supply, in particular to the jejunal mucosa, without affecting the effects of NE on systemic haemodynamics and mesenteric arterial blood flow. Our findings strongly suggest that administration of AVP with NE at any dosage results in a significant redistribution of jejunal blood flow at the expense of the mucosa and probably the submucosal tissue compartment. These results are of potential clinical importance and underline the necessity for a caution when AVP treatment is given in addition to patients receiving an NE infusion.

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## References

- Friedman G, Silva E, Vincent JL. Has the mortality of septic shock changed with time. *Crit Care Med* 1998; **26**: 2078–86
- Annane D, Aegerter P, Jars-Guincestre MC, Guidet B. Current epidemiology of septic shock: the CUB-Rea Network. *Am J Respir Crit Care Med* 2003; **168**: 165–72
- Ince C, Sinaasappel M. Microcirculatory oxygenation and shunting in sepsis and shock. *Crit Care Med* 1999; **27**: 1369–77
- Hinshaw LB. Sepsis/septic shock: participation of the microcirculation: an abbreviated review. *Crit Care Med* 1996; **24**: 1072–8
- Fink MP. Gastrointestinal mucosal injury in experimental models of shock, trauma, and sepsis. *Crit Care Med* 1991; **19**: 627–41
- Johnson PC. Autoregulation of blood flow. *Circ Res* 1986; **59**: 483–95
- Dellinger RP, Levy MM, Carlet JM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med* 2008; **36**: 296–327
- Morales DL, Gregg D, Helman DN, et al. Arginine vasopressin in the treatment of 50 patients with postcardiotomy vasodilatory shock. *Ann Thorac Surg* 2000; **69**: 102–6
- Dunser MW, Mayr AJ, Ulmer H, et al. Arginine vasopressin in advanced vasodilatory shock: a prospective, randomized, controlled study. *Circulation* 2003; **107**: 2313–9
- Russell JA, Walley KR, Singer J, et al. Vasopressin versus norepinephrine infusion in patients with septic shock. *N Engl J Med* 2008; **358**: 877–87
- Micek ST, Shah P, Hollands JM, et al. Addition of vasopressin to norepinephrine as independent predictor of mortality in patients with refractory septic shock: an observational study. *Surg Infect (Larchmt)* 2007; **8**: 189–200
- Nakajima Y, Baudry N, Duranteau J, Vicaut E. Effects of vasopressin, norepinephrine, and L-arginine on intestinal microcirculation in endotoxemia. *Crit Care Med* 2006; **34**: 1752–7
- Levy B, Vallee C, Lauzier F, et al. Comparative effects of vasopressin, norepinephrine, and L-canavanine, a selective inhibitor of inducible nitric oxide synthase, in endotoxic shock. *Am J Physiol Heart Circ Physiol* 2004; **287**: H209–15
- Schwarz B, Hofstotter H, Salak N, et al. Effects of norepinephrine and phenylephrine on intestinal oxygen supply and mucosal tissue oxygen tension. *Intensive Care Med* 2001; **27**: 593–601
- Knotzer H, Maier S, Dunser MW, et al. Arginine vasopressin does not alter mucosal tissue oxygen tension and oxygen supply in an acute endotoxemic pig model. *Intensive Care Med* 2006; **32**: 170–4
- Knotzer H, Pajk W, Maier S, et al. Arginine vasopressin reduces intestinal oxygen supply and mucosal tissue oxygen tension. *Am J Physiol Heart Circ Physiol* 2005; **289**: H168–73
- Treggiari MM, Romand JA, Burgener D, Suter PM, Aneman A. Effect of increasing norepinephrine dosage on regional blood flow in a porcine model of endotoxin shock. *Crit Care Med* 2002; **30**: 1334–9
- Sennoun N, Montemont C, Gibot S, Lacolley P, Levy B. Comparative effects of early versus delayed use of norepinephrine in resuscitated endotoxic shock. *Crit Care Med* 2007; **35**: 1736–40
- Morelli A, Rocco M, Conti G, et al. Effects of terlipressin on systemic and regional haemodynamics in catecholamine-treated hyperkinetic septic shock. *Intensive Care Med* 2004; **30**: 597–604
- Asfar P, Pierrot M, Veal N, et al. Low-dose terlipressin improves systemic and splanchnic hemodynamics in fluid-challenged endotoxic rats. *Crit Care Med* 2003; **31**: 215–20
- Hiltebrand LB, Krejci V, Jakob SM, Takala J, Sigurdsson GH. Effects of vasopressin on microcirculatory blood flow in the gastrointestinal tract in anesthetized pigs in septic shock. *Anesthesiology* 2007; **106**: 1156–67
- Hasibeder W, Germann R, Sparr H, et al. Vasomotion induces regular major oscillations in jejunal mucosal tissue oxygenation. *Am J Physiol* 1994; **266**: G978–86
- Knotzer H, Hasibeder W. Microcirculatory function monitoring at the bedside—a view from the intensive care. *Physiol Meas* 2007; **28**: R65–86
- Hasibeder W, Germann R, Wolf HJ, et al. Effects of short-term endotoxemia and dopamine on mucosal oxygenation in porcine jejunum. *Am J Physiol* 1996; **270**: G667–75
- Chittock DR, Russell JA. Oxygen delivery and consumption during sepsis. *Clin Chest Med* 1996; **17**: 263–78
- Maier S, Pajk W, Ulmer H, et al. Epoprostenol improves mucosal tissue oxygen tension in an acute endotoxemic pig model. *Shock* 2009; **31**: 104–10
- Schmidt H, Secchi A, Wellmann R, et al. Effect of endotoxemia on intestinal villus microcirculation in rats. *J Surg Res* 1996; **61**: 521–6

- 28 Birnbaum J, Lehmann C, Stauss HM, *et al.* Sympathetic modulation of intestinal microvascular blood flow oscillations in experimental endotoxemia. *Clin Hemorheol Microcirc* 2003; **28**: 209–20
- 29 Vieillard-Baron A, Caille V, Charron C, *et al.* Actual incidence of global left ventricular hypokinesia in adult septic shock. *Crit Care Med* 2008; **36**: 1701–6
- 30 Dunser MW, Mayr AJ, Ulmer H, *et al.* The effects of vasopressin on systemic hemodynamics in catecholamine-resistant septic and postcardiotomy shock: a retrospective analysis. *Anesth Analg* 2001; **93**: 7–13
- 31 Luckner G, Dunser MW, Jochberger S, *et al.* Arginine vasopressin in 316 patients with advanced vasodilatory shock. *Crit Care Med* 2005; **33**: 2659–66
- 32 Immink WF, Beijer HJ, Charbon GA. Hemodynamic effects of norepinephrine and isoprenaline in various regions of the canine splanchnic area. *Pflugers Arch* 1976; **365**: 107–18
- 33 Marshall I, Nasmyth PA, Sheppherson NB. The influence of pre-synaptic alpha-adrenoceptors on the overflow of norepinephrine in the stimulated mouse vas deferens [proceedings]. *Br J Pharmacol* 1977; **60**: 304P–5P
- 34 Priebe HJ, Noldge GF, Armbruster K, Geiger K. Differential effects of dobutamine, dopamine, and norepinephrine on splanchnic haemodynamics and oxygenation in the pig. *Acta Anaesthesiol Scand* 1995; **39**: 1088–96
- 35 Schiffer ER, Schwieger IM, Gosteli P, Poinot O, Mentha G, Morel DR. Systemic and splanchnic oxygen supply–demand relationship with fenoldopam, dopamine and norepinephrine in sheep. *Eur J Pharmacol* 1995; **286**: 49–60
- 36 Marik PE, Mohedin M. The contrasting effects of dopamine and norepinephrine on systemic and splanchnic oxygen utilization in hyperdynamic sepsis. *J Am Med Assoc* 1994; **272**: 1354–7
- 37 Meier-Hellmann A, Specht M, Hannemann L, Hassel H, Bredle DL, Reinhart K. Splanchnic blood flow is greater in septic shock treated with norepinephrine than in severe sepsis. *Intensive Care Med* 1996; **22**: 1354–9
- 38 Levy B, Bollaert PE, Charpentier C, *et al.* Comparison of norepinephrine and dobutamine to epinephrine for hemodynamics, lactate metabolism, and gastric tonometric variables in septic shock: a prospective, randomized study. *Intensive Care Med* 1997; **23**: 282–7
- 39 Ruokonen E, Takala J, Kari A. Regional blood flow and oxygen transport in patients with the low cardiac output syndrome after cardiac surgery. *Crit Care Med* 1993; **21**: 1304–11
- 40 Noguera I, Medina P, Segarra G, *et al.* Potentiation by vasopressin of adrenergic vasoconstriction in the rat isolated mesenteric artery. *Br J Pharmacol* 1997; **122**: 431–8
- 41 Medina P, Noguera I, Aldasoro M, Vila JM, Flor B, Lluch S. Enhancement by vasopressin of adrenergic responses in human mesenteric arteries. *Am J Physiol* 1997; **272**: H1087–93