Vasopressors do not influence cerebral critical closing pressure during systemic inflammation evoked by experimental endotoxemia and sepsis in humans

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Abstract

Aim

The aim of this study was to investigate the effects of different vasopressors on the cerebral vasculature during experimental human endotoxemia and sepsis. We used the critical closing pressure (CrCP) as a measure of cerebral vascular tone.

Methods

We performed a prospective pilot study, at the intensive care department (ICU) of a tertiary care university hospital in the Netherlands, in 40 healthy male subjects during experimental human endotoxemia (administration of bacterial lipopolysaccharide [LPS]) and in 10 patients with severe sepsis or septic shock.

Subjects in the endotoxemia study were randomized to receive a 5 hour infusion of either 0.05 µg/kg/min noradrenaline (n=10, “LPS-nor”), 0.5 µg/kg/min phenylephrine (n=10, “LPS-phenyl”), 0.04 IU/min vasopressin (n=10, “LPS-AVP”) or saline (n=10, “LPS-placebo”) starting 1 hour before intravenous administration of 2 ng/kg LPS. In patients with sepsis, fluid resuscitation and vasopressor use was at the discretion of the medical team, aiming at normovolemia and a mean arterial pressure (MAP) >65 mmHg, using noradrenaline.

The mean flow velocity in the middle cerebral artery (MFV_{MCA}) was measured by transcranial Doppler (TCD) with simultaneously recording of heart rate, arterial blood pressure, respiratory rate and oxygen saturation. CrCP was estimated using the cerebrovascular impedance model.

Results

The CrCP decreased in the LPS-placebo group from 52.6 [46.6-55.5] mmHg at baseline to 44.1 [41.2-51.3] mmHg at 270 min post-LPS (P=0.03). Infusion of phenylephrine increased the CrCP in the
period before LPS administration from 46.9 [38.8-53.4] to 53.8 [52.9-60.2] mmHg (P=0.02), but after
LPS administration, a similar decrease was observed compared with the LPS-placebo group.
Noradrenaline or vasopressin prior to LPS did not affect the CrCP. The decrease in CrCP after LPS
bolus was similar in all treatment groups. The CrCP in the sepsis patients equaled 35.7 [34.4-42.0]
mmHg, and was lower compared to that in the LPS-placebo subjects from baseline until 90 min after
LPS (P<0.01).

**Conclusions**

Experimental human endotoxemia results in a decreased CrCP due to a loss of vascular resistance of
the arterial bed. Vasopressors did not prevent this decrease in CrCP. Findings in patients with sepsis are
comparable to those found in subjects after LPS administration.

Patients with sepsis, despite treatment with vasopressors, have a risk for low cerebral blood flow and
ischemia.

KeyWords:- Cerebral critical closing pressure, experimental endotoxemia, sepsis, Vasopressors
Introduction

Sepsis-associated encephalopathy (SAE) is a common complication of sepsis and septic shock and associated with increased mortality and long-term cognitive impairment (1). Reduced cerebral blood flow (CBF) is one of the major features in the pathophysiology of SAE. In addition, autoregulation is frequently disturbed in patients with severe sepsis and SAE, and may further increase the risk of ischemia if blood pressure decreases below the lower limit of autoregulation (2).

The critical closing pressure (CrCP) is a method to describe and quantify characteristics of the cerebrovascular bed and is defined as the lower limit of arterial blood pressure below which vessels collapse and flow ceases (3, 4). CrCP is a valuable and clinically relevant tool in cerebrovascular research, as it allows for estimation of the minimal cerebral perfusion pressure required to prevent collapse of vessels and ischemia (5-7). The CrCP is mainly a feature of the arteriolar bed and the difference between the arterial (inflow) pressure and the CrCP is regarded as the driving pressure for cerebral perfusion (8). Because CrCP cannot be measured directly, several models have been developed to estimate CrCP indirectly from other physiological parameters or their derivatives. Varsos et al. proposed a model using cerebrovascular impedance to determine the CrCP. This model can accurately detect changes in vascular properties induced by changes in intracranial pressure (ICP), PaCO₂ and blood pressure (9).

Vasopressors are frequently applied to counteract hypotension and subsequent decreased cerebral perfusion in sepsis and septic shock (10). The effects of different vasopressors on cerebral hemodynamics in sepsis are however unknown. Directly studying the effects of different vasopressors on cerebral perfusion in sepsis patients is hampered by the heterogeneity of the patient population, encompassing large variability in hemodynamic compromise and vasopressor requirements. In addition, current sepsis resuscitation protocols dictate a central role for noradrenaline, with vasopressin only used as an additional drug in ‘catecholamine-resistant’ shock. The experimental human
endotoxemia model is a safe and reproducible model of systemic inflammation in humans *in vivo*, capturing many (hemodynamic) hallmarks of early sepsis, including a reduced MAP and increased heart rate. As such, this model allows for a head-to-head comparison of the effects of different vasopressors on cerebral hemodynamics in sepsis-like conditions (11).

Taken together, microvascular and macrovascular alterations play a key role in the pathogenesis of SAE. The effects of vasopressors on the cerebrovascular tone in SAE are unknown, despite the widespread use these agents in daily clinical practice. The effects of vasopression on CBF may be mediated by a change in systemic MAP. Alternatively, different vasopressors may induce changes in cerebrovascular resistance through direct effects on the cerebral vasculature.

The aim of this study was to investigate the effects of different vasopressors on the cerebral vasculature during experimental human endotoxemia and to compare these to data obtained in sepsis patients. We focused on the parameter CrCP as a measure of cerebral vascular tone.

**Methods**

*Study design, setting, and subjects*

We performed a randomized controlled experimental endotoxemia pilot study in healthy subjects. Furthermore, we performed a prospective observational pilot study in patients with severe sepsis or septic shock. Both studies were performed at the intensive care department (ICU) of a tertiary care university hospital in the Netherlands.

Forty healthy, non-smoking, male volunteers, aged 18 to 35 years, were included in the human endotoxemia study that was registered at Clinicaltrials.gov under NCT02675868. All subjects provided written informed consent and experiments were in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and approved by the local ethics committee (document number 2015-2079). Subjects were screened before start of the experiment and had a normal physical
examination, electrocardiography and routine laboratory values. Subjects were excluded from participation in the endotoxemia trial in case of febrile illness in the two weeks preceding the experiment, use of any medication or nicotine, signs and symptoms of cardiovascular disease, renal or liver impairment, or in case they previously participated in an endotoxemia experiment. Subjects refrained from alcohol or caffeine intake 24 hours before the experiment and refrained from food 12 hours before the start of the endotoxemia experiment.

Ten patients with severe sepsis or septic shock were included. The local Institutional Review Board waived the need for informed consent. Severe sepsis or septic shock was defined by the international sepsis definition conference (12), requiring the use of vasopressors. Sepsis patients were treated according to international guidelines for management of severe sepsis and septic shock (13). All sepsis patients were sedated and mechanically ventilated. Sedation was performed using propofol and/or midazolam and sufentanil to achieve a target Richmond Agitation and Sedation Scale (RASS) score of -3 to 0.

General exclusion criteria for all participants were an irregular heart rhythm, insufficient transtemporal bone window, age <18 years, pregnancy, thrombolytic therapy, refractory cardiogenic shock or life expectancy less than 24 hours.

**Experimental human endotoxemia**

Purified lipopolysaccharide (LPS, US Standard Reference Endotoxin *Escherichia coli* O:113) obtained from the Pharmaceutical Development Section of the National Institutes of Health, supplied as a lyophilized powder, was reconstituted in 5 mL saline 0.9% for injection and vortex mixed for at least 20 minutes. The LPS solution was administered as an intravenous bolus injection at a dose of 2 ng/kg body weight in 1 min at $T=0$ min as described previously (14). All subjects received 1.5 L of 2.5% glucose/0.45% saline solution in the hour before the administration of LPS, followed by 150 mL/h...
2.5% glucose/0.45% saline solution during the first 6 hours after the LPS administration and 75 mL/h until the end of the experiment. LPS-induced symptoms, including headache, nausea, shivering, muscle and back pain, were scored every 30 min on a six-point Likert scale (0 = no symptoms, 5 = worst ever experienced), resulting in a total score of 0–25.

_Vasopressors_

Subjects in the LPS trial were randomized using the sealed envelope method to receive either a 5 hour infusion of 0.05µg/kg/min noradrenaline (n=10, “LPS-nor”), 0.5 µg/kg/min phenylephrine (n=10, “LPS-phenyl”), 0.04 IU/min vasopressin (n=10, “LPS-AVP”) or saline (NaCl 0.9%) (n=10, “LPS-placebo”). Continuous infusion of the vasopressors started at one hour before LPS administration until 4 hours afterwards. A visual outline of the study is depicted in Figure 1.

In sepsis patients, fluid resuscitation and vasopressor use was at the discretion of the medical team, aiming at normovolemia and a mean arterial pressure (MAP) >65mmHg, using noradrenaline.

_Data collection_

In subjects participating in the endotoxemia study, the radial artery was cannulated using a 20 gauge arterial catheter (Angiocath; Becton Dickinson) which was connected to an arterial pressure monitoring set (Edwards Lifesciences). Heart rate, blood pressure, respiratory rate and oxygen saturation were recorded continuously, starting 2 hours before administration of LPS until discharge from the ICU 8 hours after LPS administration. In sepsis patients the radial artery was cannulated using a 20 gauge arterial catheter (Angiocath; Becton Dickinson) which was connected to an arterial pressure monitoring set (Edwards Lifesciences). Heart rate, blood pressure, respiratory rate and oxygen saturation were recorded continuously during 30 minutes.
The mean flow velocity in the middle cerebral artery (MFV\textsubscript{MCA}) in both healthy volunteers and patients was measured using transcranial Doppler (TCD) through the temporal window with a 2-Mhz probe (Multi-Dop T Digital, Compumedics DWL, Singen, Germany) according to the method developed by Aaslid et al (15). The probe was positioned over the temporal bone window above the zygomatic arch and fixed. This procedure ensured that the angle and the individual depth of insonation remained constant during the investigation. The TCD was measured on both sides. For the recordings, the bone window with the most optimal signal was chosen. All recordings were made with subjects/patients in the supine position with the head elevated to 30°.

A minimum of 10-12 minute windows of MFV, heart rate (ECG) and arterial blood pressure (ABP) were simultaneously recorded on a laptop computer and stored on a hard disk with a sample rate of 200 Hz by an A/D converter (NI USB-6211, National Instrument, Austin, TX, USA). In subjects participating in the endotoxemia study, recordings were performed at 90 and 30 minutes before, and at 90, 210 and 270 minutes after LPS administration. Sepsis patients were measured once, under stable hemodynamic (normotensive) and respiratory conditions (normocapnic).

Data analysis

ABP and MFV data were analysed using custom-written MATLAB scripts (Matlab R2014b, The MathWorks Inc. Massachusetts, USA). First, the time series were filtered with an 5th-order low-pass Butterworth filter (25 Hz), to ascertain signal stationarity. Second, periods of 5 minutes of artefact- and calibration-free data were selected by visual inspection for subsequent analysis. Finally, mean blood pressure and cerebral blood flow velocity were obtained simultaneously using a 4th order low-pass Butterworth filter.
Critical Closing Pressure (CrCP)

CrCP was determined according to the method suggested by Varsos et al. (9, 16).

\[
CrCP = ABP - \frac{CPP}{\sqrt{(CVR \cdot Ca \cdot HR \cdot 2\pi)^2 + 1}}
\]

With CVR the cerebrovascular resistance, Ca the compliance of the vascular bed of the brain and HR the heart rate. The multiplication of CVR and Ca is also called the timeconstant Tau (\(\tau\)). Cerebral perfusion pressure (CPP) is defined as ABP – ICP, however in this study the ICP was not measured. Therefore the mean ABP was used as an approach of CPP, as described by Varsos et al. (9). CVR was calculated by dividing ABPmean by MFVmean. To determine the Ca, the cerebral arterial blood volume (CABV) was calculated by integrating the MFV signal over time. Then Ca was calculated by dividing the amplitude of the first harmonic of the CABV by the amplitude of the first harmonic of the ABP. Heart rate (HR) was defined as the first harmonic frequency of ABP.

Calculations and statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA). All baseline characteristics data are presented as means +/- SD, as they were normally distributed (determined by Shapiro-Wilk tests).

All other results are presented as median with 25th and 75th percentile (interquartile range [IQR]), as they were not normally distributed (determined by Shapiro-Wilk tests). Changes over time were analyzed with Friedman tests.

A P-value of <0.05 was considered to indicate significance.
Results

Population

Baseline characteristics of the healthy volunteers participating in the experimental human endotoxemia study are presented in Table 1. There were no differences in baseline characteristics between the 4 treatment groups. All subjects developed the typical flulike symptoms, starting approximately 75 minutes after LPS administration. As expected, symptoms completely subsided after 5-6 hours.

The 10 sepsis patients had a SAPS 2 score of 55.0 [48.5-76.8] and an APACHE 2 score of 24.5 [18.3-28.8]; 6 sepsis patients died in the ICU due to refractory shock and multi-organ failure. All patients in the sepsis population received noradrenaline (0.30 [0.24-0.45] µg/kg/min). Baseline characteristics of sepsis patients are presented in Table 2.

Hemodynamic and clinical data

The MAP in the LPS-placebo group was 91.0 [78.8-92.4] mmHg at the start of the experiment and decreased to 78.9 [71.3-88.5] mmHg after LPS administration (P<0.01) (Figure 2). Infusion of noradrenaline and phenylephrine significantly increased MAP by 12.6 [11.5-15.2] and 22.2 [9.4-37.1] % in the period before LPS administration (P<0.01), whereas vasopressin had no effects on MAP. LPS administration decreased MAP in all treatment groups, with no significant differences between groups. MAP in sepsis patients was lower than in healthy volunteers at all time-points (69.7 [66.4-73.5] mmHg, P<0.01).

The temperature increased from 36.8 [36.6-37.2] °C at baseline to 38.4 [37.5-38.6] °C after LPS administration in the LPS-placebo group (P<0.01) and a similar temperature response was observed in endotoxemic subjects receiving vasopressors (data not shown).
The respiratory rate in the LPS-placebo group increased from 16.8 [14.0-20.0] resp/min to 19.4 [18.0-21.9] resp/min at 210 min post-LPS and 20.4 [19.7-21.5] resp/min at 270 min post-LPS (P<0.01). Administration of vasopressors neither affected respiratory rates before LPS administration, nor the LPS-induced increase in respiratory rate. Septic patients were ventilated aiming at normocarbia.

**MFV and CrCP**

The MFV\textsubscript{MCA} in the LPS-placebo group decreased significantly during human endotoxemia from 69.9 [64.2-85.3] cm/sec at baseline to 59.0 [54.3-63.1] cm/sec at 270 min post-LPS (P=0.01) (Figure 3). None of the vasopressors increased the MFV\textsubscript{MCA} in the period before LPS administration. Despite infusion of the vasopressors, the MFV\textsubscript{MCA} decreased upon LPS injection to values similar to that observed in the LPS-placebo group. The MFV\textsubscript{MCA} in sepsis patients was 69.8 [54.6-83.3 cm/sec].

The CrCP decreased significantly in the LPS-placebo group from 52.6 [46.6-55.5] mmHg at baseline to 44.1 [41.2-51.3] mmHg at 270 min after LPS administration (P=0.03) (Figure 4). Infusion of phenylephrine before LPS injection increased the CrCP significantly from 46.9 [38.8-53.4] to 53.8 [52.9-60.2] mmHg (P=0.02). Noradrenaline or vasopressin had no effect on the CrCP prior to LPS administration. The decrease in CrCP after LPS bolus was similar in all treatment groups. The CrCP in the sepsis patients was 35.7 [34.4-42.0] mmHg, which was significantly lower compared to values in the LPS-placebo subjects from baseline until 90 min post-LPS (P<0.01).

The cerebrovascular arterial compliance (Ca) represents the change of arterial blood volume in response to change in arterial pressure and is estimated as a ratio of pulse amplitude of cerebral arterial blood volume and pulse amplitude of the arterial blood pressure. In the LPS-placebo group, Ca decreased from 0.16 [0.12-0.26] mmHg/cm\textsuperscript{3} before LPS admission to 0.11 [0.10-0.14] mmHg/cm\textsuperscript{3} at 270 min post-LPS (P=0.03) (Figure 5a). None of the vasopressors changed the Ca significantly before
LPS administration. Furthermore, no significant changes compared to the placebo group were observed in subjects treated with vasopressors after LPS administration. Ca values in sepsis patients were lower compared to values in healthy volunteers before LPS administration (0.10 [0.07-0.13] mmHg/cm³, P<0.01).

The CVR represents the resistance of small cerebral arteries and arterioles, which was estimated from the MAP and MFV. The initial CVR in endotoxemia subjects was 1.37 [1.01-1.45] mmHg*sec/cm and did not change significantly after LPS administration (P=0.63) (Figure 5b). Phenylephrine increased the CVR from 1.32[1.07-1.54] to 1.71[1.52-1.87] mmHg*sec/cm (P=0.02). No changes in CVR occurred in any of the other treatment groups. The CVR in sepsis patients was 1.11 [0.87-1.56] mmHg*sec/cm, and did not differ from the LPS subjects (P=0.50).

Tau (τ) is the time constant of the cerebral arterial bed and is the product of brain arterial compliance Ca and resistance CVR. It provides an estimation as to how quickly cerebral blood arrives in the cerebral arterial bed during each cardiac cycle. Tau decreased from 0.21 [0.17-0.25] sec to 0.15 [0.15-0.18] sec in the LPS-placebo group (P<0.01) (Figure 5c). None of the vasopressors changed Tau significantly in the period before LPS injection. Furthermore, no significant changes compared to the placebo group were found in the subjects treated with vasopressors after LPS administration. Tau in the sepsis group was 0.12 [0.08-0.15] sec, and significantly lower compared to values before LPS administration (P<0.01).

Discussion

Endothelial dysfunction and disturbed NO production play a key role in the pathogenesis of SAE (17, 18). After LPS administration, cerebral blood flow velocity decreases (19) most likely as a result of decreased outflow from the circle of Willis to the middle and anterior cerebral arteries (20). The
decrease in cerebral blood flow velocities was accompanied by a decrease in CrCP. This decrease in CrCP probably maintains adequate cerebral driving pressure and thereby serves as a mechanism to protect the brain against ischemia. The decrease in cerebrovascular compliance in our study is consistent with previous work indicating that systemic vasoparalysis does not affect the brain circulation in sepsis (21). In contrast to the systemic vasodilation in sepsis, decreased blood flow velocities together with higher pulsatility indices in patients with SAE strongly suggest vasoconstriction of the resistance arterioles in the brain (22).

Remarkably, CVR was unmodified during endotoxemia. Ideally, CVR is calculated by dividing pressure in the resistance arterioles by MFVmean. In this study, the CVR was estimated from the MAP and MFV in the MCA and thus not measured in the cerebral resistance arterioles. This may explain the fact that CVR did not change in endotoxemic subjects. Ca decreased in our study, reflecting a reduced ability of arteries to expand and contract with changes in blood pressure. Generally, Ca and CVR are inversely correlated, but the correlation between these parameters may change in pathological states, such as SAE (23).

Infusion of noradrenaline and phenylephrine before LPS bolus injection increased MAP, whereas vasopressin had no effect on blood pressure. The latter finding was not unexpected, because the hemodynamic effect of vasopressin in septic shock is based on the relative vasopressin deficiency in these patients (24, 25). Therefore, exogenous vasopressin infusion will not change the vascular tone in healthy subjects with adequate endogenous vasopressin levels. The increased MAP after initiation of noradrenaline and phenylephrine infusion did not result in a change of the MFV<sub>MCA</sub>. Probably, the simultaneous increase in CrCP ensured a constant cerebral driving pressure despite the increase in MAP, suggestive of cerebrovascular adaptation in these subjects.
None of the vasopressors used in this study prevented the decrease in MFV\textsubscript{MCA} after LPS administration. In addition, the decrease in CrCP in the subjects receiving vasopressors was similar to that in the placebo group. So far, few studies have compared the effectiveness of commonly used agents to augment cerebral perfusion, and the available literature is restricted patients suffering from traumatic brain injury (26-30). None of these studies found significant differences in cerebral hemodynamic responses or oxygenation between different vasopressors. However, several animal studies have examined the effects of different vasopressors on cerebral perfusion. For instance, augmentation of cerebral perfusion with noradrenaline resulted in a more pronounced increase in brain tissue oxygenation compared to phenylephrine in a large animal model of pediatric closed head injury (31). The lack of effect of the $\alpha$-agonist phenylephrine and the combined $\alpha$-$\beta$-agonist noradrenaline in our study may reflect the low density of $\alpha$ and $\beta$ receptors in the human cerebral vasculature. Alternatively, the dose of phenylephrine (0.5 $\mu$g/kg/min) and noradrenaline (0.05$\mu$g/kg/min) may have been too low to induce an effect on CBF, either directly by influencing cerebrovascular tone or indirectly by augmentation of MAP. The fact that CrCP decreased simultaneously with decreasing MAP, indicates that cerebrovascular adaptation was not affected by the use of phenylephrine and noradrenaline.

The reported effects of vasopressin on the cerebral vasculature are heterogeneous, with cerebral vasoconstriction or dilation depending on the species, artery size and vasopressin dose (32-35). The relevance of these studies for the treatment of patients with septic shock remains to be established. In our population of healthy subjects during endotoxemia, no effects of vasopressin (administered in a clinically relevant dose) on CBF were observed.

The cerebrovascular effects in the human endotoxemia model strongly resembled the findings in septic patients. In both endotoxemia and sepsis the MFV\textsubscript{MCA} decreased, together with a lowered CrCP. Previously, dynamic cerebral autoregulation was believed to be enhanced in human endotoxemia and
impaired in sepsis (19, 36). More recently it was suggested that dynamic cerebral autoregulation is also enhanced in the earlier stages of sepsis, indicating that in early sepsis cerebrovascular adaptation to changes in cerebral perfusion are similar to those observed in human endotoxemia (37). This suggests that the human endotoxemia model is a relevant model to study the efficiency of therapeutic approaches in SAE.

This study has a number of limitations. First, CrCP cannot be measured directly but is estimated using a mathematical model, with its inherent risks of bias. Most importantly, ICP is required for the most accurate calculation of CrCP, but this parameter was not obtained in the current study. Nevertheless, since ICP is low under septic conditions, it is unlikely that the absence of ICP data significantly influenced the estimation of CrCP (38). Second, we measured MAP through a catheter in the radial artery, at heart level instead of brain level. Measurement of the MAP in the MCA would have resulted in a more accurate estimation of cerebral perfusion pressure but is not feasible. Thirdly, cerebral perfusion changes after a LPS bolus or in sepsis may be heterogeneously distributed through the brain, with some areas more affected than others. As CrCP is derived from the MFV_{MCA}, this heterogeneity in flow cannot be assessed using this technique.

Fourth, as mentioned before, because healthy subjects were studied in the endotoxemia study, only low-dose vasopressors were administered. We cannot exclude a possible effect on cerebrovascular adaptation with higher dosing.

A fifth limitation of this study is the small number of subjects and that the population in the endotoxemia group differed from the sepsis group in terms of sex and age. We included only men in the endotoxemia study to limit subject variability, because we know from earlier experimental human endotoxemia studies that females show a more pronounced proinflammatory innate immune response associated with less attenuation of norepinephrine sensitivity (39). We included only young volunteers in the endotoxemia study to limit subject variability and for reasons of safety (comorbidity). Injection
of E. coli endotoxin in elderly patients (median age 66yrs) is associated with a more pronounced reduction in blood pressure compared to younger control subjects (40). Aging is associated with decreasing cerebral blood flow velocities. However this occurs mainly in the posterior cerebral circulation and at significantly older ages than those of our sepsis population. A study measuring cerebral blood flow velocity and pulsatility index by transcranial Doppler in 303 healthy elderly subjects indicated a slight but non-significant decrease in cerebral blood flow velocities in the middle cerebral artery (33.8±0.9 to 32.2±1.1 and 34.8±0.9 to 32.8±1.1 cm/s) between patient groups 70-74 yrs to > 85 yrs. The pulsatility index (reflecting cerebrovascular resistance) increased from 1.55±0.04 to 1.66±0.04 and from 1.59±0.04 to 1.64±0.04 in these age groups (41). In our study, the CrCP in the (older) septic population was significantly lower compared to the (younger) endotoxemia group. In addition, the CVR was similar in both populations. This strongly suggests that in our population, changes in CrCP and CVR were mainly influenced by sepsis, rather than the age per se.

A sixth limitation of the study is that data obtained in healthy volunteers were not controlled for carbon dioxide tension: as the respiratory rate and body temperature increased after LPS administration, we cannot exclude an influence of altered levels of CO₂ on reduced MFV and cerebrovascular adaptation.

Conclusions
Cerebral blood flow velocity decreases during human endotoxemia and vasopressors do not prevent this decrease. In addition, vasopressors do not influence the change in CrCP during human endotoxemia. These results have important clinical implications as they indicate that titration of the systemic circulation to a specific minimal MAP with vasopressors will not automatically restore cerebral perfusion in patients with septic shock. Monitoring of cerebral perfusion parameters such as CrCP may assist in titration of the optimal systemic MAP to prevent cerebral hypoperfusion and brain ischemia.
**List of abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABP</td>
<td>Arterial blood pressure</td>
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<td>$C_a$</td>
<td>Compliance of the vascular bed of the brain</td>
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<td>CABV</td>
<td>Cerebral arterial blood volume</td>
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<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
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<tr>
<td>CPP</td>
<td>Cerebral perfusion pressure</td>
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<td>CrCP</td>
<td>Critical Closing Pressure</td>
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<tr>
<td>CVR</td>
<td>Cerebrovascular resistance</td>
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<td>HR</td>
<td>Heart rate</td>
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<tr>
<td>ICP</td>
<td>Intracranial pressure</td>
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<tr>
<td>ICU</td>
<td>Intensive Care Unit</td>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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<td>MAP</td>
<td>Mean arterial pressure</td>
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<tr>
<td>MFV$_{MCA}$</td>
<td>Mean flow velocity in the middle cerebral artery</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<tr>
<td>SAE</td>
<td>Sepsis-associated encephalopathy</td>
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<td>TCD</td>
<td>Transcranial Doppler</td>
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Declarations

Ethics approval and consent to participate

The human endotoxemia study was approved by the local Institutional Review Board (document number 2015-2079, NCT02675868) and written informed consent was obtained from all participants prior to the study.

The local Institutional Review Board waived the need for informed consent in the sepsis patients.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analysed during the study are available from the corresponding author on reasonable request.

Competing interests

The authors have no financial or non-financial competing interests

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Authors' contributions

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Not applicable
REFERENCES


### Legends to tables and figures

#### Table 1  Demographic and clinical data at baseline of LPS subjects

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n=40)</th>
<th>LPS-nor (n=10)</th>
<th>LPS-phenyl (n=10)</th>
<th>LPS-AVP (n=10)</th>
<th>LPS-placebo (n=10)</th>
<th>P value</th>
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<tr>
<td>Age (years)</td>
<td>22.4 ±1.6</td>
<td>20.9 ±1.6</td>
<td>22.4 ±2.8</td>
<td>22.9 ±2.2</td>
<td>23.8 ±1.2</td>
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<td>BMI (kg/m²)</td>
<td>22.6 ±2.7</td>
<td>20.90 ±2.2</td>
<td>22.61 ±2.7</td>
<td>23.5 ±2.3</td>
<td>20.9 ±2.9</td>
<td>P = 0.34</td>
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<td>HR (BPM)</td>
<td>86.6 ±10.5</td>
<td>64.5 ±8.5</td>
<td>67.8 ±10.8</td>
<td>71.8 ±11.8</td>
<td>62.50 ±10.0</td>
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<td>MAP (mmHg)</td>
<td>89.0 ±7.4</td>
<td>86.44 ±6.2</td>
<td>87.6 ±10.5</td>
<td>92.9 ±10.0</td>
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<td>Temperature (°C)</td>
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<td>36.9 ±0.4</td>
<td>36.9 ±0.3</td>
<td>36.9 ±0.4</td>
<td>36.8 ±0.3</td>
<td>P = 0.98</td>
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Table 2  
Demographic and clinical data at baseline of sepsis patients

<table>
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<tr>
<th>Measurement</th>
<th>Sepsis (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.1 ±12.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2 ±13.3</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>105.2 ±13.8</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>68.1 ±10.2</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.4 ±1.3</td>
</tr>
<tr>
<td>Hb (mmol/L)</td>
<td>6.0 ±1.1</td>
</tr>
</tbody>
</table>
**Figure 1** Outline of the human endotoxemia trial

- **HD**: Hemodynamic
- **LPS**: Lipopolysaccharide
- **TCD**: Transcranial Doppler
- **NIRS**: Near Infrared Spectroscopy
Figure 2  MAP after LPS injection in LPS-placebo, LPS-nor, LPS-phenyl and LPS-AVP,

Time 0 = time of LPS bolus

LPS  Lipopolysaccharide

MAP  Mean arterial pressure
**Figure 3**  \( MFV_{MCA} \) in sepsis patients and after LPS injection in LPS-placebo, LPS-nor, LPS-phenyl and LPS-AVP, Time 0 = time of LPS bolus

LPS  Lipopolysaccharide

\( MFV_{MCA} \)  Mean flow velocity in the middle cerebral artery
Figure 4  CrCP in sepsis patients after LPS injection in LPS-placebo, LPS-nor, LPS-phenyl and LPS-AVP, Time 0 = time of LPS bolus

CrCP  Critical closing pressure

LPS  Lipopolysaccharide
**Figure 5a, b, c**  Ca, CVR and Tau in sepsis patients after LPS injection in LPS-placebo, LPS-nor, LPS-phenyl and LPS-AVP, Time 0 = time of LPS bolus

- **Ca**: Compliance of the vascular bed of the brain
- **CVR**: Cerebrovascular resistance
- **LPS**: Lipopolysaccharide
- **Tau**: Timeconstant