

Bolus hypertonic or normal saline resuscitation in gram-negative sepsis: systemic and regional haemodynamic effects in sheep

Li Wan, Rinaldo Bellomo and Clive N May

Severe sepsis remains the leading cause of intensive care unit mortality.¹⁻³ Its haemodynamic hallmark⁴⁻¹² is the presence of hypotension, inadequate tissue perfusion and hypoxia.¹³⁻¹⁵ Bolus fluid resuscitation is recommended as a primary therapeutic intervention to restore organ perfusion, and thereby preserve organ function.^{12,16-18} In addition, there is particular concern over decreased renal blood flow during sepsis, especially when the systemic venous pressure is low and urine output decreased. Bolus fluid resuscitation to restore renal blood flow in this setting is thus considered a fundamental therapeutic intervention.¹⁸ Unfortunately, it is unknown whether such fluid resuscitation improves perfusion of vital organs. It is also unknown whether such improvement is substantial and long lasting. Moreover, there is uncertainty regarding the best type of fluid to be used for such bolus resuscitation.

Normal (0.9%) saline (NS) is perhaps the most widely available and most commonly used agent for fluid resuscitation in severe sepsis.¹²⁻¹⁹ Hypertonic (3%) saline (HTS) has also been used for treatment of sepsis, mainly in experimental studies, with evidence of transiently improved systemic haemodynamics.²⁰⁻²³ However, the magnitude and duration of its effect is poorly understood, and the regional circulation effects and renal functional impact of HTS in sepsis remain unknown.

Abbreviations

ANOVA	Analysis of variance
CFU	Colony-forming units
HTS	Hypertonic saline
CO	Cardiac output
CVP	Central venous pressure
DO ₂	Oxygen delivery
HR	Heart rate
MAP	Mean arterial pressure
NS	Normal saline
SV	Stroke volume

ABSTRACT

Background: Fluid resuscitation with saline in severe sepsis is controversial. Hypertonic (3%) saline (HTS) may be superior to normal (0.9%) saline (NS).

Objective: To compare the effects of HTS and NS on regional blood flow in sepsis.

Design: Randomised controlled crossover large animal study.

Setting: University physiology laboratory.

Subjects: Seven merino cross ewes.

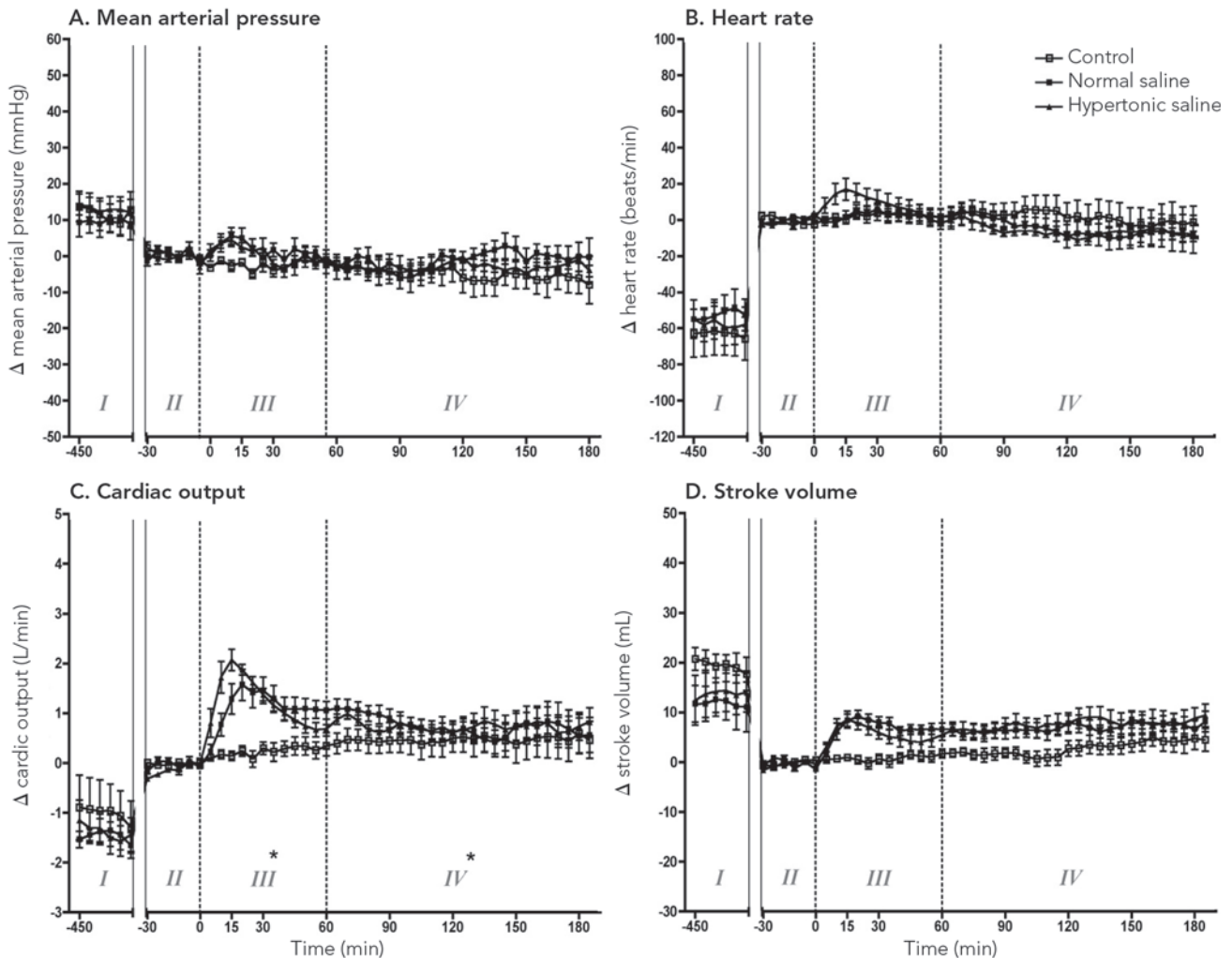
Interventions: We implanted chronic flow probes around aorta, mesenteric, coronary and renal arteries. Sepsis was induced by the intravenous injection of 3×10^9 colony-forming units of live *Escherichia coli*. We randomised animals to three groups after onset of sepsis: observation (control), NS (1000 mL over 15 minutes) and HTS (300 mL over 15 minutes).

Main outcome measures: Continuously measured systemic haemodynamics, organ blood flows and markers of renal function for 210 minutes.

Results: In septic sheep, bolus resuscitation with HTS had similar systemic haemodynamic effects as NS and both increased cardiac output and mesenteric blood flow during the first hour compared with control ($P < 0.05$). However, this effect dissipated after 60 minutes. These effects were mirrored by effects on mesenteric and coronary blood flow. In contrast, renal blood flow was not changed by either HTS or NS. HTS transiently increased total and mesenteric oxygen delivery ($P < 0.05$), while NS transiently decreased total and renal oxygen delivery. Urine output and creatinine clearance decreased with sepsis and only transiently increased with NS ($P < 0.05$) but not HTS.

Conclusions: In gram-negative sepsis, bolus resuscitation with HTS and NS have similar and transient systemic and regional haemodynamic effects, but no effects on renal perfusion and only short-lived effects on renal function. These findings challenge the physiological rationale for fluid bolus resuscitation in sepsis.

Crit Care Resusc 2011; 13: 262–270

Figure 1. Systemic haemodynamic changes in the control, normal saline and hypertonic saline groups

All changes expressed in relation to the zero value representative of the mean for the sepsis control period. Treatments were given over 15 minutes. I = pre-sepsis period. II = sepsis control period. III = initial 60-minute period. IV = delayed period from 60 to 210 minutes. * Statistically significant difference between hypertonic saline, normal saline and control groups in a particular period.

We conducted a randomised crossover controlled animal experiment to investigate the effect of a bolus infusion >20 mL/kg (a commonly administered amount in humans and equivalent to 1000 mL of NS and 300 mL of HTS in 40 kg sheep) on vital organ blood flow and renal function in the setting of gram-negative sepsis induced by intravenous infusion of live *Escherichia coli*.

Methods

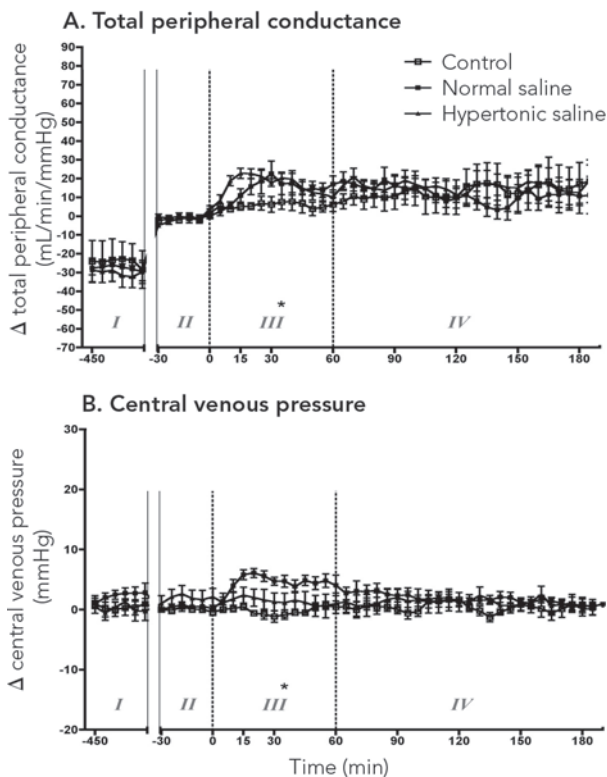
Experimental procedures were approved by the Animal Experimental Ethics Committee of the Howard Florey Institute in accordance with the *Prevention of Cruelty to Animals Act 1986* (Vic), under the guidelines of the

National Health and Medical Research Council *Australian code of practice for the care and use of animals for scientific purposes*,²⁴ which conforms with the United States National Institutes of Health *Guide for the care and use of laboratory animals*.²⁵

Merino ewes weighing between 35 and 45 kg were procured for chronic instrumentation. The animals underwent three separate operations for the placement of chronically indwelling flow probes.

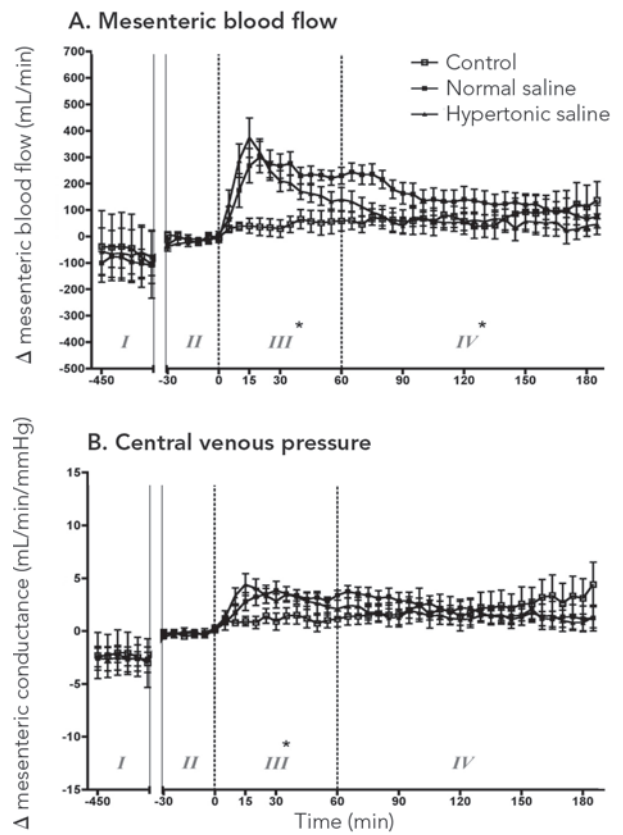
The first procedure included oophorectomy and carotid loop creation. Two to 3 weeks later, we placed transit time-flow probes (Transonic Systems, Ithaca, NY, USA) around the circumflex coronary artery (3 mm) and the ascending aorta (20 mm). About 2 weeks after this, we

Figure 2. Changes in total peripheral conductance and central venous pressure in the control, normal saline and hypertonic saline groups



All changes expressed in relation to the zero value representative of the mean for the sepsis control period. Treatments were given over 15 minutes. I = pre-sepsis period. II = sepsis control period. III = initial 60-minute period. IV = delayed period from 60 to 210 minutes. * Statistically significant difference between hypertonic saline, normal saline and control groups in a particular period.

Figure 3. Changes in mesenteric blood flow and mesenteric conductance in the control, normal saline and hypertonic saline groups



All changes expressed in relation to the zero value representative of the mean for the sepsis control period. Treatments were given over 15 minutes. I = pre-sepsis period. II = sepsis control period. III = initial 60-minute period. IV = delayed period from 60 to 210 minutes. * Statistically significant difference between hypertonic saline, normal saline and control groups in a particular period.

placed two transit-time flow probes (6 mm and 4 mm, respectively) around the superior mesenteric artery and left renal artery. The animals were allowed to recover for 3 weeks. The accuracy of chronically implanted transit-time flow probes for the measurement of regional blood vessels has been validated previously.^{26,27} We connected the transit-time flow probes to a T201 CDS flowmeter (Transonic Systems) via a TM04 four-channel sequential scanner (Transonic Systems).

The day before experimentation, we implanted a carotid loop arterial Tygon catheter (inner diameter, 1.0 mm; outer diameter, 1.7 mm [Cole-Parmer, Melbourne, Vic, Australia]) and two internal jugular venous polythene catheters (inner diameter, 1.2 mm; outer diameter, 1.7 mm [Cole-Parmer]) for the measurement of mean arterial pressure (MAP), central venous pressure (CVP) and for fluid infusion, respectively.

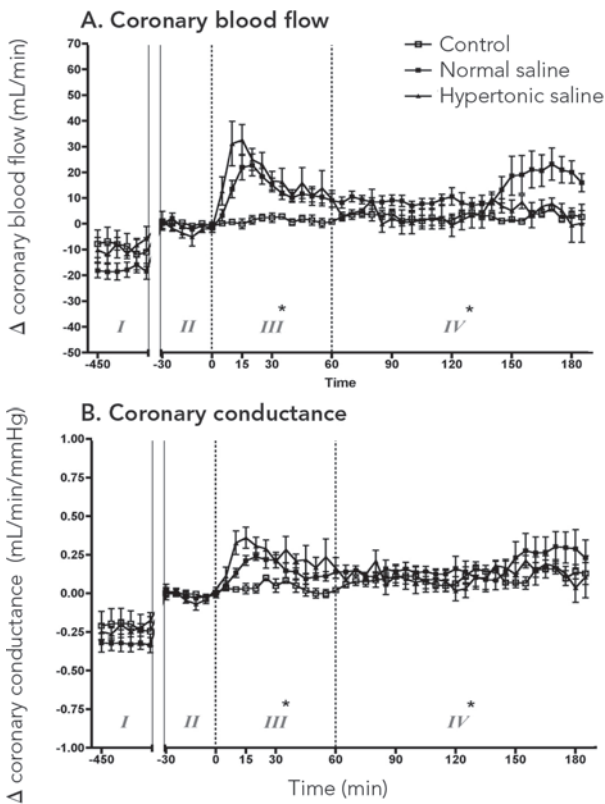
We tied TDXIII pressure transducers (Cobe Cardiovascular, Lakewood, Colo, USA) for the measurement of MAP and CVP to the back of the sheep and calibrated them against a mercury and a water manometer, respectively. We used the second venous catheter as an infusion line. We inserted a urinary catheter for urine flow measurement and sample analysis.

We collected analogue signals (MAP, CVP, cardiac output [CO], change in regional blood flows) using a personal computer data acquisition system with custom software written at the Howard Florey Institute. We collected data at 100 Hz for 10 seconds at 1-minute intervals throughout the experiment protocol.

Induction of sepsis

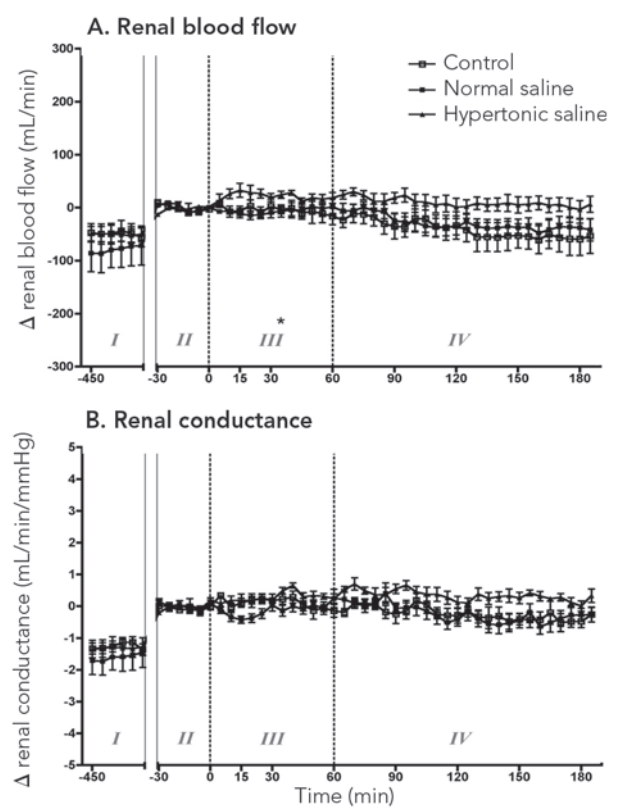
On the day of the study, food and fluid were removed 3–4 hours before the experiment and baseline measurements were performed over 30 minutes before the induction of

Figure 4. Changes to coronary blood flow and coronary conductance in the control, normal saline and hypertonic saline groups



All changes expressed in relation to the zero value representative of the mean for the sepsis control period. Treatments were given over 15 minutes. I = pre-sepsis period. II = sepsis control period. III = initial 60-minute period. IV = delayed period from 60 to 210 minutes. * Statistically significant difference between hypertonic saline, normal saline and control groups in a particular period.

Figure 5. Changes in renal blood flow and renal conductance in the control, normal saline and hypertonic saline groups



All changes expressed in relation to the zero value representative of the mean for the sepsis control period. Treatments were given over 15 minutes. I = pre-sepsis period. II = sepsis control period. III = initial 60-minute period. IV = delayed period from 60 to 210 minutes. * Statistically significant difference between hypertonic saline, normal saline and control groups in a particular period.

sepsis. Then, a bolus of 3×10^9 colony-forming units (CFU) live *E. coli* was injected intravenously over 5 minutes. This bolus was followed by 1-minutely sampling of all the systemic haemodynamic parameters and vital organ blood flows.

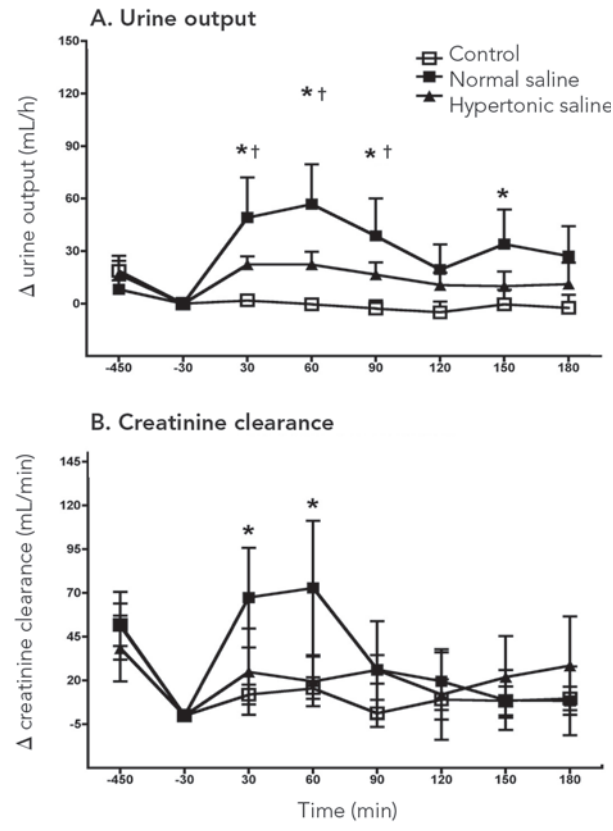
About 6–8 hours after the initial bolus, the animal typically reached predefined criteria for randomisation. These criteria had to be simultaneously fulfilled for the experiment to be conducted: > 10% decrease of MAP and > 50% increase of heart rate (HR) compared with pre-*E. coli* values. The aim of waiting for this period and for these physiological changes to occur was to simulate the clinical situation, where any intervention would be delayed and take place in response to clinical changes.

Experimental protocol

Before randomisation, all animals were monitored for 30 minutes (control period) and then randomised to: (1) control group; (2) NS group; or (3) HTS group. Accordingly, each animal received one of the following: observation (control), a bolus of NS (1 L over 15 min) or a bolus of 3% saline (300 mL over 15 min). Monitoring was continued for 3 hours after the start of the infusion. At the end of the experiment, all catheters were removed and the animals were allowed to recover for 10–14 days before being crossed over to the other arm of the study.

Blood samples were collected at –45, –30, 0, 15, 30, 60, 90, 120, 150 and 180 minutes. Urine samples were collected every 30 minutes throughout the experiment.

Figure 6. Change in urine output and creatinine clearance over time in the control, normal saline and hypertonic saline groups



All changes expressed in relation to the zero value representative of the mean for the sepsis control period. Treatments were given over 15 minutes. * Statistically significant difference between normal saline and control groups at particular time point. † Statistically significant difference between hypertonic saline and normal saline groups at particular time point.

Oxygen delivery (Do_2) was calculated using the following equations:

$$\text{Arterial } O_2 \text{ content} = 1.34 \times \text{haemoglobin (g/L)} \times SaO_2 + 0.0031 \times PaO_2$$

$$Do_2 \text{ (mL } O_2/\text{min)} = \text{arterial } O_2 \text{ content} \times CO \text{ (L/min)}$$

$$\text{Organ } Do_2 = \text{arterial } O_2 \text{ content} \times \text{organ blood flow (L/min)}$$

Statistical analysis

We used repeated measures analysis of variance (ANOVA) to compare the three groups. Statistical analysis was performed using SAS, version 9.1 (SAS Institute Inc, Cary, NC, USA). Variables were initially assessed for normality and log-transformed where appropriate. Group comparisons over time were determined using generalised linear

modelling (PROC MIXED), with each sheep treated as a random effect. Whether groups behaved differently over time was determined using an interaction between group and time.

Data are presented as mean (SD), and $P \leq 0.05$ considered statistically significant.

Results

Effects of sepsis

Intravenous injection of 3×10^9 CFU of live *E. coli* induced a severe septic state. The animals became ill, sat quietly in the cage and were tachypnoeic (respiratory rate, > 50 breaths/min), febrile (temperature $> 42.0^\circ\text{C}$) and tachycardic (HR, > 140 beats/min). Their HR increased by $> 50\%$, their MAP decreased by 10%–30%, and their stroke volume (SV) decreased by 25%–50% ($P < 0.05$) (Figure 1). The CO was sustained by means of marked tachycardia. All animals developed oliguria and decreased creatinine clearance (organ failure).

Systemic haemodynamics

Immediately after bolus infusion, compared with control, both NS (about 25 mL/kg) and HTS (about 8 mL/kg) caused similar and significant increases in MAP, CO, SV and total peripheral conductance ($P < 0.05$; Figure 1 and Figure 2). These effects, however, were short-lived and had essentially dissipated after 60 minutes, with no difference from controls for the rest of the observation period ($P > 0.05$; Figure 1 and Figure 2).

HTS transiently increased HR (15 beats/min; $P < 0.05$; Figure 1), while NS did not. HTS caused a greater increase in CO than NS, with a difference of about 0.5 L/min lasting about 60 minutes ($P > 0.05$; Figure 1). CVP increased significantly (but transiently) only in the NS group ($P < 0.05$; Figure 2).

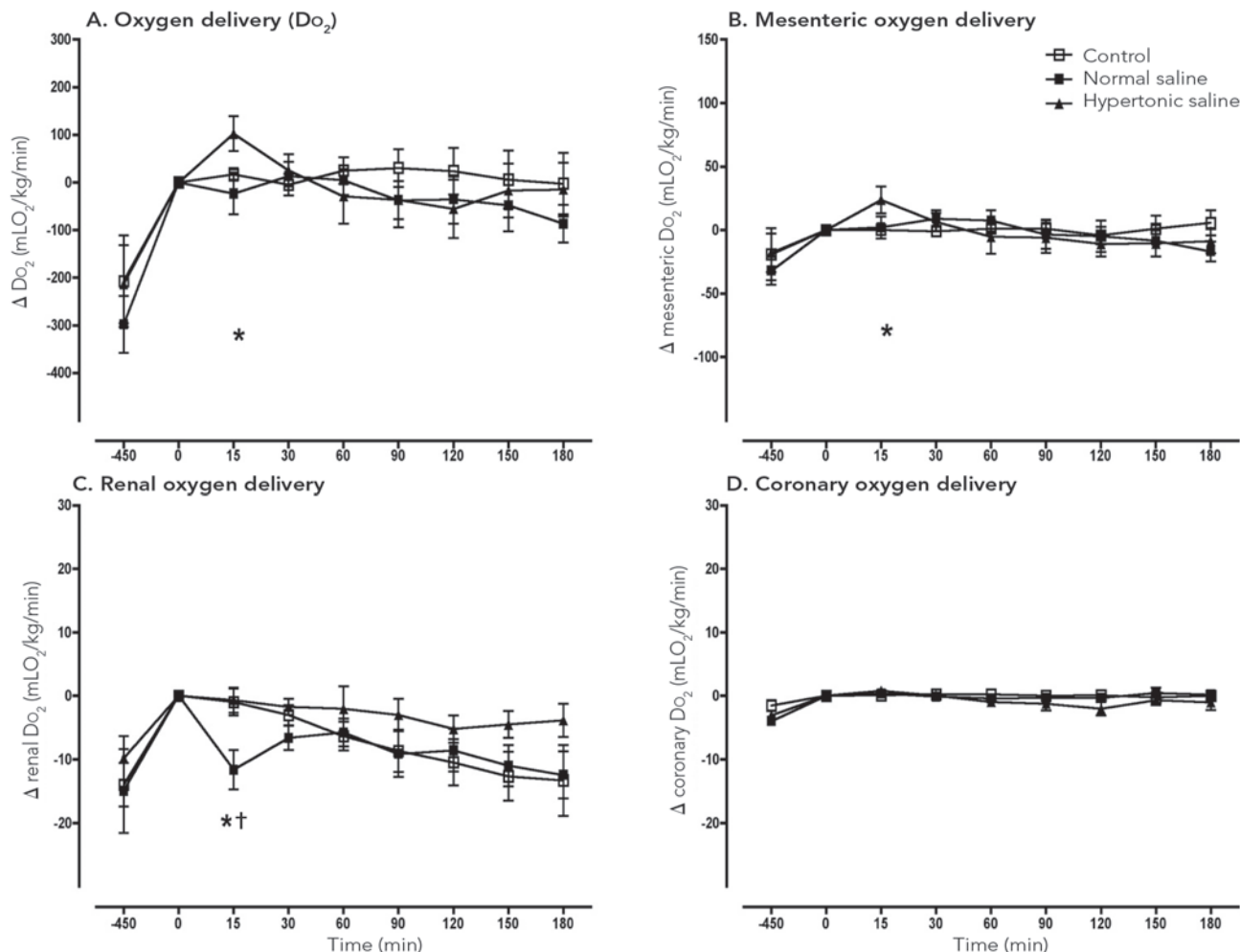
Regional haemodynamics

Compared with controls, both NS and HTS significantly but transiently increased mesenteric blood flow, mesenteric conductance, coronary blood flow and coronary conductance immediately after infusion ($P < 0.05$; Figure 3 and Figure 4).

HTS had greater but somewhat earlier and shorter effects on mesenteric blood flow and mesenteric conductance than NS ($P < 0.05$; Figure 3). A similar pattern applied to coronary blood flow and coronary conductance.

HTS and NS did not significantly change renal blood flow or renal conductance compared with controls ($P > 0.05$; Figure 5).

Figure 7. The change of total oxygen delivery changes, mesentery oxygen delivery, renal oxygen delivery and coronary oxygen delivery in the control, normal saline and hypertonic saline groups



All changes expressed in relation to the zero value representative of the mean for the sepsis control period. * Statistically significant difference between hypertonic saline and normal saline groups at particular time point. † Statistically significant difference between normal saline and control groups at particular time point.

Renal function

Urine output significantly increased with NS infusion ($P < 0.05$), but only mildly and not significantly with HTS ($P > 0.05$; Figure 6). Only the NS group significantly but transiently increased creatinine clearance at 30 and 60 minutes ($P < 0.05$; Figure 6). There were no differences in creatinine clearance between the HTS and control groups ($P > 0.05$; Figure 6).

Less than 20% of the administered fluid was eliminated in the urine 180 minutes after administration.

Oxygen delivery

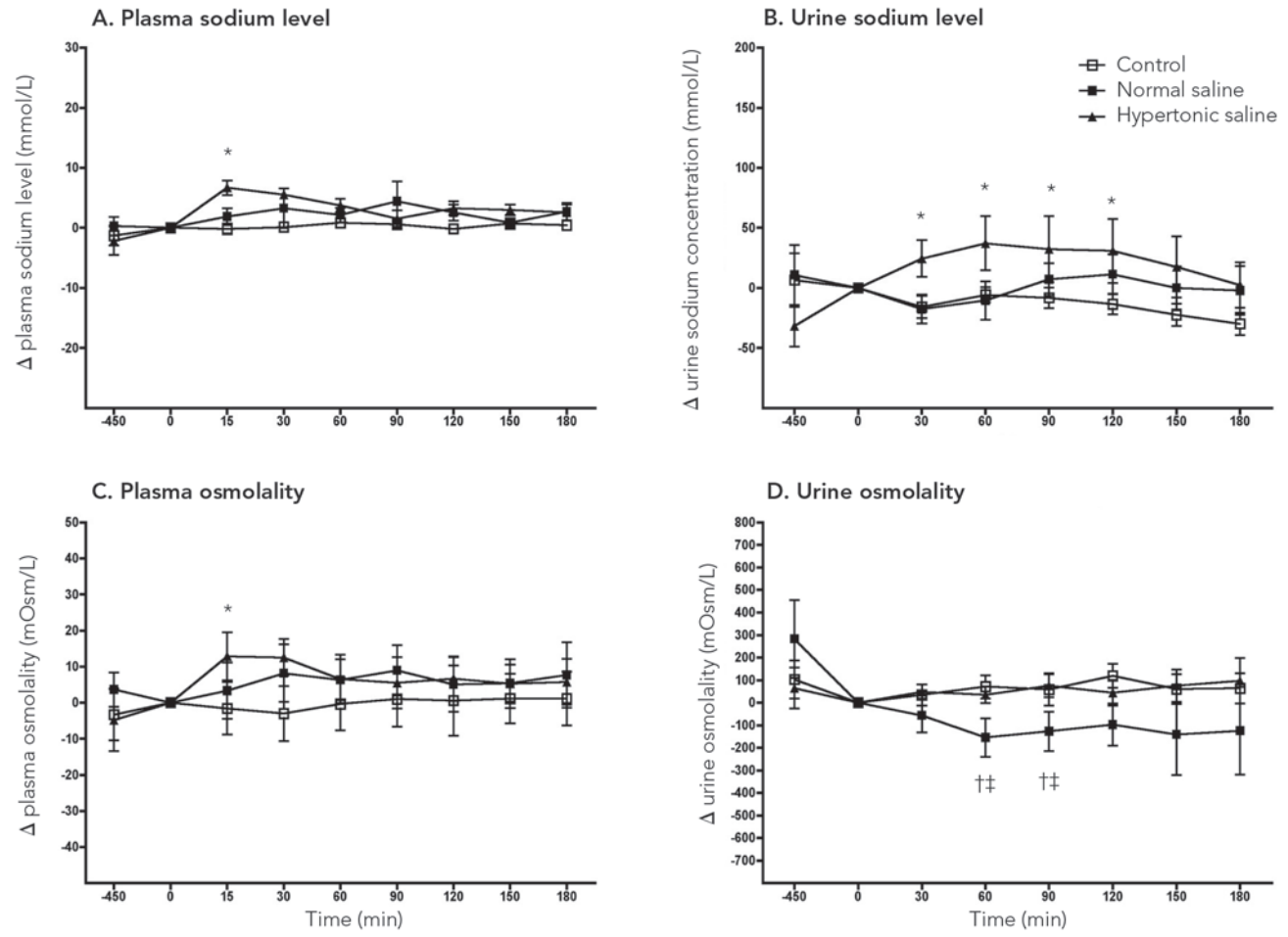
For the first 15 minutes after infusion, HTS, but not NS, significantly increased systemic Do_2 and mesenteric Do_2

($P < 0.05$; Figure 7). Neither solution affected coronary Do_2 ($P > 0.05$; Figure 7). However, while HTS did not alter renal Do_2 at any time ($P > 0.05$; Figure 7), renal Do_2 was significantly lower than controls for the first 15 minutes after infusion in the NS group ($P < 0.05$; Figure 7).

Osmolality

Plasma osmolality and plasma sodium level increased significantly in the HTS group for the first hour ($P < 0.05$; Figure 8). Similarly, the urine sodium level increased in the HTS group for the first 2 hours after infusion ($P < 0.05$; Figure 8). NS significantly decreased urine osmolality 60 and 90 minutes after infusion ($P < 0.05$; Figure 8), while HTS did not have measurable effects.

Figure 8. Plasma and urine sodium and osmolality level changes over 180 minutes of observation in the control, normal saline and hypertonic saline groups



All changes expressed in relation to the zero value representative of the mean for the sepsis control period. * Statistically significant difference between hypertonic saline and control groups at particular time point. † Statistically significant difference between normal saline and control groups at particular time point. ‡ Statistically significant difference between hypertonic saline and normal saline groups at particular time point.

Discussion

In this randomised controlled crossover large animal study, we found that bolus resuscitation with HTS and NS achieved broadly similar transient effects on systemic and regional haemodynamics. These findings dissipated within about an hour of administration, such that the systemic and regional circulations were indistinguishable from control animals thereafter. Additionally, NS caused a significant but transient decrease in renal DO_2 . Finally, less than 20% of the administered fluid was excreted in the urine during the 180 minutes of the study.

Our findings are consistent with previous observations on the effects of NS on regional blood flows and function.^{28,29} They indicate that, in gram-negative sepsis, bolus resuscitation with NS only induces a short-lived increase in CVP and

CO, and that these changes have modest and transient effects on regional circulations and no effect on renal perfusion.

Previous studies of HTS in sepsis have often combined HTS with a colloid (eg, dextran) solution, making it difficult to understand the specific effects of HTS. Even in such models, measurement of the regional flow effects of HTS has been uncommon. In a model of porcine sepsis using microspheres, Kreimeier and colleagues found that HTS increased CO, but not CVP or MAP, and did not affect regional blood flow.³⁰ In a similar canine model, Rahal and colleagues administered HTS followed by NS to maintain a mixed venous oxygen saturation greater than 70% and reported a partial, transient increase in CO with HTS, which was accompanied by a similar effect on portal blood

flow.³¹ In a sepsis model in rats, Batista and colleagues combined HTS with 6% hydroxyethyl starch and found a significant increase in MAP.³² This increase was associated with increase vasopressin levels and was blocked by a V1 receptor antagonist, suggesting vasopressin release triggered by increased plasma tonicity, an effect confirmed in another study.³³ However, they did not assess regional flows or renal function. In another canine model of sepsis, HTS transiently improved mesenteric blood flow and decreased mesenteric oxygen extraction.²⁰ Finally, Hanne-man and colleagues measured the systemic haemodynamic effects of HTS in 21 patients with hyperdynamic sepsis.³⁴ They found that HTS increased CO and filling pressures, and that this effect had dissipated by 60 minutes after infusion. These effects in septic humans are essentially identical to those we report in septic animals. They are also similar to those seen in normal animals, with the major difference being that the effect of HTS on systemic haemodynamics and regional flows took longer to dissipate in normal sheep. Importantly, in both septic and normal experimental animals, renal blood flow was not affected by HTS or NS.

Our findings provide experimental evidence in support of the notion that, in mammalian gram-negative sepsis, fluid bolus resuscitation with crystalloids (NS or HTS) has only transient, modest effects on systemic and regional circulations, and no discernible effect on renal circulation. The similarly transient effect on urine output and creatinine clearance likely reflects the effect of salt loading on diuresis and of haemodilution on oncotic pressure. These findings can be explained by the increased tonicity-mediated release of vasopressin induced by HTS and by the effect of hypernatraemia on natriuresis.³² Our findings are in accordance with evidence and argument that challenge the view that renal protection can be achieved by intravenous fluid loading.^{35,36} Finally, these experimental findings are in accordance with recent clinical findings also questioning the role of fluid bolus-based resuscitation in sepsis.³⁷

Our study has several strengths. First, to our knowledge, this is the first study to compare the systemic and regional haemodynamic effects of HTS and NS is fluid bolus resuscitation in sepsis. Second, in our hands, this septic model has a mortality rate of about 20%–30%, which is similar to the mortality rate in human septic shock. Third, unlike methodologies that use microspheres, we were able to measure flows over time and provide an integrated picture of regional perfusion. Fourth, we are the first to provide data on renal blood flow and renal conductance in association with such resuscitation.

Our study also has limitations. Unfortunately, although we estimated systemic and regional Do₂, we did not

estimate oxygen extraction. This makes it difficult to assess the consequences of changes in Do₂. However, we wished to study conscious animals to avoid the confounding effects of anaesthesia on systemic and regional flows. We have found that this approach makes it technically extraordinarily difficult to maintain cannulation of regional veins, and promotes thrombosis. We could not provide information on gut function. However, in a ruminant animal such as the sheep, such information is of uncertain relevance to humans.

Conclusions

Bolus resuscitation with HTS and NS achieved only modest and transient effects on systemic and regional haemodynamics. These effects dissipated within an hour in association with a haemodilutional decrease in Do₂. Additionally, more than 80% of the administered fluid was retained 3 hours after administration. These findings challenge the physiological rationale for fluid bolus resuscitation in sepsis.

Acknowledgements

This study was supported by a grant from the Austin Hospital Intensive Care Trust Fund.

Competing interests

None declared.

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