Effects of Different Crystalloid Solutions on Hemodynamics, Peripheral Perfusion, and the Microcirculation in Experimental Abdominal Sepsis

Diego Orbegozo, M.D., Fuhong Su, M.D., Ph.D., Carlos Santacruz, M.D., Xinrong He, M.D., Koji Hosokawa, M.D., Ph.D., Jacques Creteur, M.D., Ph.D., Daniel De Backer, M.D., Ph.D., Jean-Louis Vincent, M.D., Ph.D.

ABSTRACT

Background: Crystalloid solutions are used to restore intravascular volume in septic patients, but each solution has limitations. The authors compared the effects of three crystalloid solutions on hemodynamics, organ function, microcirculation, and survival in a sepsis model.

Methods: Peritonitis was induced by injection of autologous feces in 21 anesthetized, mechanically ventilated adult sheep. After baseline measurements, animals were randomized to lactated Ringer's (LR), normal saline (NS), or PlasmaLyte as resuscitation fluid. The sublingual microcirculation was assessed using sidestream dark field videomicroscopy and muscle tissue oxygen saturation with near-infrared spectroscopy.

Results: NS administration was associated with hyperchloremic acidosis. NS-treated animals had lower cardiac index and left ventricular stroke work index than LR-treated animals from 8 h and lower mean arterial pressure than LR-treated animals from 12 h. NS-treated animals had a lower proportion of perfused vessels than LR-treated animals after 12 h (median, 82 [71 to 83] *vs.* 85 [82 to 89], $P = 0.04$) and greater heterogeneity of proportion of perfused vessels than PlasmaLyte or LR groups at 18 h. Muscle tissue oxygen saturation was lower at 16 h in the NS group than in the other groups. The survival time of NS-treated animals was shorter than that of the LR group (17 [14 to 20] *vs.* 26 [23 to 29] h, $P < 0.01$) but similar to that of the PlasmaLyte group (20 [12 to 28] h, $P = 0.74$).

Conclusions: In this abdominal sepsis model, resuscitation with NS was associated with hyperchloremic acidosis, greater hemodynamic instability, a more altered microcirculation, and more severe organ dysfunction than with balanced fluids. Survival time was shorter than in the LR group. **(ANESTHESIOLOGY 2016; 125:744-54)**

OPTIMIZATION of intravascular volume is a fundamental component of initial resuscitation during septic shock, and crystalloids are the fluids most commonly used for this purpose.[1] Crystalloids are effective, cheap, and relatively safe, but each solution possesses a unique profile of potential adverse effects based on its specific composition.[2]

Normal saline (NS) is the most frequently used crystalloid during resuscitation in many situations.[3] Although not a physiologic solution, NS is very cheap due to the simplicity of its preparation.[4] NS has a high-chloride content that can induce hyperchloremic acidosis, depending on the amount of fluid administered.[5] Experimental studies have indicated that NS may induce coagulopathy,[6] renal vasoconstriction,[7] and even renal failure.[8] Moreover, hyperchloremic acidosis has been implicated in the release of inflammatory molecules and development of hemodynamic instability during sepsis,[9] although this study was performed with infused hydrochloric acid and not NS. Some clinical studies have reported an association between NS use and the development of renal failure,[10] and in a recent large retrospective cohort of septic patients matched by a propensity score, there was a higher

What We Already Know about This Topic

- Crystalloid solutions remain a fundamental component of resuscitation in septic shock to optimize intravascular volume
- Each crystalloid solution has specific properties and potential side effects based on its unique composition that warrant further investigation.

What This Article Tells Us That Is New

- In a comparison of normal saline, lactated Ringer's, and PlasmaLyte as resuscitation fluids in a large animal model of abdominal peritonitis and sepsis, normal saline was associated with more adverse side effects (acidosis, hemodynamic instability, altered microcirculation, and organ dysfunction) than the balanced solutions

Corresponding article on page 622. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org).

Submitted for publication March 26, 2015. Accepted for publication June 1, 2016. From the Department of Intensive Care, Erasme University Hospital, Unversité Libre de Bruxelles, Brussels, Belgium.

 Anesthesiology 2016; 125:744-54

<zdoi;10.1097/ALN.0000000000001273>

mortality when chloride-rich solutions were used compared with balanced solutions.[3]

In lactated Ringer's (LR), some of the excess chloride present in NS is replaced by lactate, but this solution is somewhat hypotonic,[11] as it was developed by Hartman for fluid replacement in dehydrated children. Although blood lactate levels are correlated with severity of shock states, lactate is not very toxic and can be used as a fuel by myocardial cells and neurons.[12,13]

Another commonly used balanced solution is PlasmaLyte (Baxter, Belgium), which contains gluconate and acetate. Gluconate has been extensively used by pharmaceutical and food industries and has a good safety profile,[14] although some bacteria may use it as a fuel source.[15] Acetate can be easily metabolized but may induce myocardial depression and vasodilation; hence, its use in hemodialysis baths has been abandoned.[16] A recent multicenter randomized clinical trial found no differences between PlasmaLyte and NS in terms of renal function and mortality; however, the investigators included a heterogeneous population of intensive care unit patients with low disease severity and few comorbidities.[17] Other studies comparing different fluid types in patients with sepsis are ongoing.

Experimental data comparing different crystalloid solutions during sepsis are limited and only available from mice studies.[8,18,19] We studied the effects of the three most widely used crystalloid solutions in a sheep model of bacterial septic shock to evaluate whether they would have a different impact on survival time. Data on hemodynamics, organ function, tissue perfusion, and microcirculation were collected as potential explanatory mechanisms for any differences in outcome.

Materials and Methods

Approval from the animal ethics committee of the Université Libre de Bruxelles (Brussels, Belgium) was obtained before initiation of the study. Care and handling of the animals were in accordance with National and International Guidelines.[20] Twenty-one adult female sheep between 10 and 12 months of age and weighing 24 to 34 kg were studied. Animals were fasted for 24 h before the experiments, with free access to water.

Premedication, Anesthesia, and Ventilation

Animals were premedicated with an intramuscular mixture of 25 mg of ketamine hydrochloride (Ceva Santé Animal, France) per kilogram and 0.3 mg of midazolam (Braun, Germany) per kilogram and then placed in a supine position. The cephalic vein was cannulated with a peripheral venous 18-gauge catheter (Surflo IV Catheter; Terumo Medical Corporation, Belgium), and LR (Hartmann, Baxter) was initiated as the only intravenous fluid during surgery.

Endotracheal intubation, facilitated by an intravenous bolus of 10 μg fentanyl citrate (Janssen, Belgium) per kilogram and 0.1 mg of rocuronium bromide (Esmeron; The Netherlands) per kilogram, was performed under direct laryngoscopy (8 mm, Hi-Contour; Mallinckrodt Medical, Ireland). Volume-controlled mechanical ventilation (Servo 300 Ventilator; Siemens-Elema, Sweden) was started with the following settings: tidal volume of 10 ml/kg, respiratory rate of 18 breaths/min, positive end-expiratory pressure of 10 cm H_2O, inspired oxygen fraction of 0.5, inspiratory time to expiratory time ratio of 1:2, and a square-wave flow pattern. Respiratory rate was the only parameter adjusted during the experiment in an attempt to maintain $Paco_2$ between 31 and 34 mmHg to avoid any possible effect on systemic hemodynamics.[21] Continuous intravenous anesthesia was initiated with an infusion of midazolam (0.4 to 0.8 mg kg^{-1} h^{-1}), ketamine hydrochloride (4 to 8 mg kg^{-1} h^{-1}), and morphine (0.4 to 0.8 mg kg^{-1} h^{-1}), adjusting the doses to provide a sufficient level of anesthesia (the absence of movement of the limbs or head or chewing on painful stimulation).[22] The dose was then kept constant throughout the experiment. Thereafter, a continuous infusion of rocuronium (0.2 mg kg^{-1} h^{-1}) was started to avoid possible movement artifacts during the experiment that would interfere with the videos of the sublingual microcirculation.

A 60-cm plastic tube (inner diameter, 1.8 cm) was inserted into the stomach *via* the esophagus to drain its content and to prevent gastric distension. A 14-French Foley catheter (Beiersdorf AG, Germany) was placed in the urinary bladder to collect urine.

Surgical Preparation and Hemodynamic Monitoring

The right external carotid artery was surgically exposed, and a 6-French arterial catheter (Vygon, UK) was introduced and connected to a pressure transducer (Edwards Lifesciences, USA) zeroed at mid-chest level and connected to a Siemens SC 9000 Monitor (Siemens-Elema) for continuous monitoring of systemic systolic, diastolic, and mean arterial pressures (MAPs). The right external jugular vein was surgically exposed, and a 7.5-French introducer (Edwards Lifesciences) was placed and used to insert a 7-French pulmonary artery catheter (Edwards Lifesciences) into the pulmonary artery guided by pressure curves until adequate pulmonary artery occlusion pressure (PAOP) was obtained. Proximal and distal lumens were connected to different pressure transducers (Edwards Lifesciences) and zeroed at the mid-chest level for continuous monitoring of central venous pressure and pulmonary pressures. The pulmonary artery catheter was connected to a Vigilance I Monitor (Edwards Lifesciences) for measurement of cardiac output (CO) by continuous thermodilution. The right femoral artery and vein were then surgically exposed. A perivascular flowmeter probe (Transonic, USA) was gently placed around the artery in a proximal portion and connected to a TS420 perivascular flowmeter module (Transonic). A 6-French catheter (Vygon) was placed into the vein for intermittent venous blood sampling.

We performed a small cecotomy through a midline laparotomy to collect feces. A pursestring suture was used to

close the incision, and the area around was disinfected with iodine solution. The laparotomy wound was closed in two layers, leaving a plastic tube with a large diameter through the abdominal wall for later injection of feces.

After abdominal surgery, the animals were turned to the prone position, and a recruitment maneuver consisting of increasing the positive end-expiratory pressure to 25 cm H_2O for 20 s was performed to avoid possible atelectasis induced by the supine position. The left kidney was then surgically exposed through the retroperitoneum. The left renal artery was identified, and a perivascular flowmeter probe (Transonic) was placed to continuously monitor left renal blood flow. The incision was closed, and the probe was connected to a TS420 perivascular flowmeter module (Transonic) for continuous monitoring.

Hemodynamic monitoring variables were collected before sepsis induction (baseline) and exactly every hour thereafter.

Peripheral and Microcirculatory Monitoring

The right posterior leg was shaved, a small incision made in the skin, the biceps femoris muscle identified, and a microdialysis probe (CMA 20; CMA Microdialysis AB, Sweden) placed *in situ*. The probe was connected to a CMA 402 microdialysis syringe pump (CMA Microdialysis AB) infusing T1 peripheral liquid (CMA Microdialysis AB) at a rate of 0.3 μl/min. Samples were collected thereafter every hour and processed immediately in a clinical microdialysis analyzer (ISCUS; M Dialysis AB, Sweden) to measure lactate, pyruvate, and lactate/pyruvate ratio concentrations in the muscle.

A 15-mm InSpectra probe (Hutchinson Technology, USA) for near-infrared spectroscopy (NIRS) was sutured to the skin of the external face of the rear leg, respecting muscle integrity. This probe was connected to a 650 InSpectra tissue spectrometer (Hutchinson Technology) for continuous monitoring of muscle tissue oxygen saturation (StO_2) and the total hemoglobin index.

Microcirculation videomicroscopy recordings were performed every 6 h using a sidestream dark field (SDF) imaging device (Microvision Medical BV, The Netherlands) with a probe on the sublingual area. At each time point, five different videos of at least 10 s were recorded from different places. For the subsequent blinded data analysis, all videos were put into a randomization table. A semiquantitative analysis of the microcirculation was performed as previously reported, calculating the proportion of perfused vessels (PPVs), proportion of perfused small vessels, perfused vessel density, microvascular flow index, and heterogeneity index of PPV.[23]

Randomization and Interventional Protocol

At the end of surgery and instrumentation, we waited 2 h before sepsis induction for stabilization of muscle microdialysis effluents. During this period, we verified that MAP, CO, Pao_2, and arterial lactate levels were within normal ranges; if not, the animals were excluded. After baseline measurements, peritonitis was induced by injection of 1.5 g autologous feces per kilogram into the abdominal cavity. The abdominal tube was then emptied by injecting 180 ml of air. Animals were then randomized using a computer-generated random number list into three groups of seven animals each. After randomization, each group received exclusively one of the three types of crystalloid solutions for fluid maintenance and volume expansion: LR, PlasmaLyte, or NS (0.9% NaCl; Baxter).

Animals were followed up until spontaneous death or for a maximum of 30 h. If they remained alive after 30 h, euthanasia was performed by injecting a high dose of potassium chloride during deep anesthesia.

Fluid Administration Protocol

After sepsis induction, a bolus of 10 ml of the allocated crystalloid solution per kilogram was given over 15 min, followed by a continuous infusion started at a rate of 3 ml kg^{-1} h^{-1}, titrated to maintain PAOP at baseline values. Fluid challenges with 10 ml of the crystalloid solution per kilogram given over 15 min were performed whenever PAOP decreased, MAP decreased below 60 mmHg, there was an hourly decrease of more than 10% in MAP, arterial lactate increased more than 2 mEq/l, or urinary output decreased less than 0.5 ml kg^{-1} h^{-1}. If the fluid administration was associated with an increase in CO of at least 10%, the rate of infusion of the crystalloid was increased by 3 ml kg^{-1} h^{-1}, up to a maximum infusion rate of 12 ml kg^{-1} h^{-1}; otherwise, the crystalloid infusion was maintained at the same rate and the animal was reevaluated 1 h later. When MAP remained less than 60 mmHg for more than 2 h with an arterial lactate more than 2 mEq/l and there was no response to fluid challenges, the rate of fluid infusion was decreased to 4 ml kg^{-1} h^{-1} until the end of the experiment.

No vasoactive agents, antibiotics, or antipyretic agents were administered at any time. Hypokalemia was corrected whenever serum potassium was less than 3.5 mEq/l by a slow 30-min infusion of 40 mEq KCl (KCl 7.45%, B. Braun Melsungen AG, Germany). Hypocalcemia was corrected whenever serum calcium was less than 0.9 mEq/l by slow 10-ml boluses of CaCl (Calciclo Sterop 11 mEq/10 ml, Sterop, Belgium). Sodium bicarbonate was never administered.

Blood Gas Analysis and Laboratory Measurements

Hemodynamic parameters were calculated using standard formulas and standardized to body surface area.[24] Arterial, mixed venous, and femoral vein blood gas analyses were performed hourly (Cobas b 123 point of care system, Roche Diagnostics, Germany) to measure arterial hemoglobin and ionized calcium concentrations; arterial, mixed venous, and femoral vein hemoglobin oxygen saturations; and the arterial, mixed venous, and femoral vein carbon dioxide pressures. Arterial blood samples were collected every 4 h to measure sodium, potassium, chloride, magnesium, phosphorus,

albumin, lactate, creatinine, international normalized ratio, and activated partial thromboplastin time in the central laboratory of Erasme Hospital (Brussels, Belgium). Urine samples were collected every 5 h for measurement of creatinine and electrolytes. We calculated the creatinine clearance and fractional excretion of different electrolytes for every 5-h period using standard formulas as reported in other human and animal studies.[25,26] All blood samples were immediately centrifuged, and plasma or serum was separated and frozen at −80°C before analysis. The strong ion difference was calculated as $(Na^+ + K^+ + Ca^+ + Mg^+) (Cl^- + \text{lactate})$. We also calculated the femoral venoarterial P_{CO_2} difference (f_{CO_2} gap) as femoral vein carbon dioxide pressure minus Pa_{CO_2}. To evaluate the effects of acute plasma expansion with different crystalloids, we calculated the relative change in MAP (delta%MAP) and cardiac index (delta%CI) before and after each fluid challenge.

Statistics

Statistical analysis was performed using SPSS 22.0 (IBM, USA) software. We did not perform an *a priori* calculation of the sample size but selected the number of animals based on our previous experience with this animal model.[21,27,28] Data are presented as medians (interquartile range, 25th to 75th) or n (%) unless otherwise specified. Comparison of proportions between groups was performed using a Fisher exact test. Differences between groups in the main outcome (survival time) were evaluated by constructing Kaplan–Meier survival curves and comparing them with a log-rank test. Because we had three groups and due to the inherent presence of multiple comparisons, Bonferroni corrections were applied for all analyses. Baseline data (before sepsis induction) were compared with a Kruskal-Wallis test to verify equivalence between groups at the beginning of the experiment. To take into account missing data secondary to animals dying spontaneously during the last hours of the experiment and the presence of differences in the variance for most of the variables, we decided to model the data with the generalized estimation equations (GEE) methodology. The GEE model estimates the absolute differences in the time course and between groups for each of the repeated measurements within an autoregressive structure. When the GEE model identified significant differences for the group or for the time × group effect, we performed a pairwise comparison of estimated marginal means at each time point correcting them with a Bonferroni test. All statistics were two tailed, and $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics, infused volume until baseline, urinary output until baseline, and the total cumulative infused volume of crystalloids were similar in the three groups (Supplemental Digital Content, table S1, http://links.lww.com/ALN/B303). The changes in MAP and CI induced by each fluid challenge were similar in the three groups (Supplemental Digital Content, table S2, http://links.lww.com/ALN/B303).

Acid–Base and Electrolytes

The time course of acid–base status is shown in figure 1 and figure S1 in the Supplemental Digital Content (http://links.lww.com/ALN/B303). The NS group had a lower pH than the LR group from 4 h and than the PlasmaLyte group from 4 to 16 h, lower bicarbonate, and strong ion difference than in the LR group from 4 h and than in the PlasmaLyte group from 8 h; the arterial P_{CO_2} was similar in the three groups. Arterial lactate levels were transiently higher in the LR group than in the PlasmaLyte group at 4 h but, thereafter, were similar in the three groups.

Plasma sodium levels were higher in the NS group than in the LR and PlasmaLyte groups from 8 h (Supplemental Digital Content, table S3, http://links.lww.com/ALN/B303). The NS group received a greater amount of sodium, and, as the three groups had similar urinary sodium excretion, the NS group had a higher cumulative sodium balance than the other two groups from 15 h (Supplemental Digital Content, fig. S2, http://links.lww.com/ALN/B303).

Blood chloride levels were higher in the NS group than in the LR and PlasmaLyte groups from 4 h (Supplemental Digital Content, table S3, http://links.lww.com/ALN/B303). The NS group received a greater cumulative

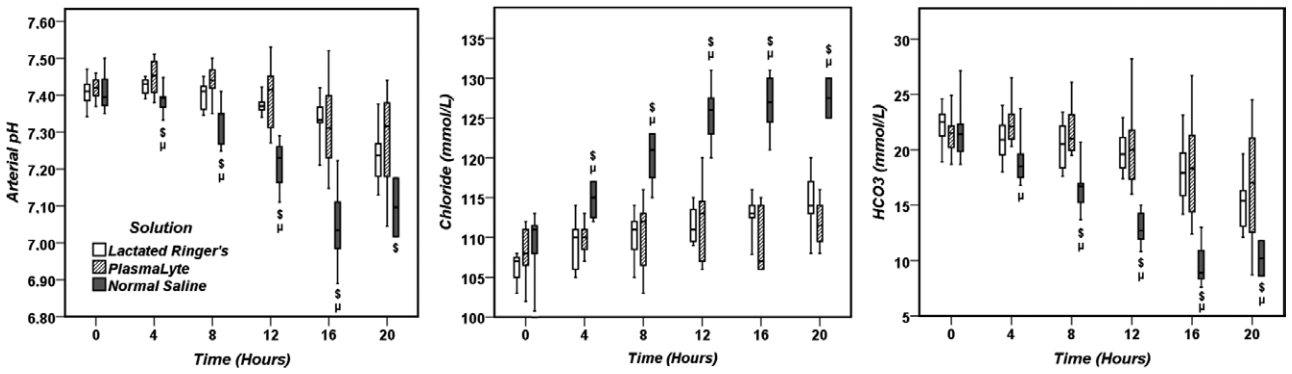

Fig. 1. Time course of main acid–base status variables. $\$P < 0.05$ *versus* lactated Ringer's group, $\mu P < 0.05$ *versus* PlasmaLyte group.

amount of chloride, and as urinary excretion was the same in the three groups, there was a higher cumulative chloride balance in the NS group than in the other groups from 10 h (Supplemental Digital Content, fig. S2, http://links.lww.com/ALN/B303).

Blood potassium levels were lower in the PlasmaLyte group than in the LR group at 8 and 12 h and higher in the NS group than in the PlasmaLyte group at 12 h (Supplemental Digital Content, table S3, http://links.lww.com/ALN/B303). The PlasmaLyte group had greater urinary potassium excretion and had more hypokalemic episodes, resulting in a higher cumulative administered dose of potassium chloride and thus a higher total administered cumulative dose of potassium (Supplemental Digital Content, table S4, http://links.lww.com/ALN/B303).

Blood magnesium levels were higher in the PlasmaLyte group than in the other groups from 4 h, although the differences compared to the NS group were no longer significant after 12 h (Supplemental Digital Content, table S3, http://links.lww.com/ALN/B303). Blood calcium levels were lower in the PlasmaLyte group than in the LR group from 4 to 20 h and than in the NS group from 8 to 12 h (Supplemental Digital Content, table S3, http://links.lww.com/ALN/B303); only two animals, both in the PlasmaLyte group, needed calcium supplementation (P = 0.11). Blood phosphorus levels were similar in the three groups throughout the experiment.

Global Hemodynamics and Perfusion Indexes

CI, stroke volume index (SVI), and left ventricular stroke work index were lower in the NS group than in the LR group from 8 h (table 1). MAP and oxygen delivery index were lower in the NS group than in the LR group from 12 h. This resulted in lower mixed venous oxygen saturation in the NS group than in the LR group from 12 h. A detailed comparison of all hemodynamic variables in the three groups is shown in table 1.

Peripheral Muscle and Sublingual Microcirculation Indexes

Muscle lactate levels were higher in the NS and PlasmaLyte groups than in the LR group from 12 h, but there were no significant differences in muscle pyruvate and lactate/pyruvate ratios (table 2). NIRS StO_2 values were lower in the NS group than in the LR and PlasmaLyte groups at 16 h. The fco_2 gap was higher in the NS group than in the LR group at 12 and 16 h (table 2).

The time course of the main SDF microcirculatory parameters is shown in figure 2. The PPV was lower in the NS group than in the LR group at 12 h and than in the LR and PlasmaLyte groups at 18 h. The perfused vessel density was lower in the NS group than in the LR group at 18 h. The heterogeneity index of PPV was higher in the NS group than in the other groups at 18 h.

Renal and Coagulation Function

The NS group developed oliguria earlier (at 12 h) than the LR and PlasmaLyte groups. Renal blood flow was transiently (at 4 h) lower in the PlasmaLyte group than in the LR or NS groups, but from 16 h, it was lower in the NS group than in the LR group (table 3). Creatinine levels were similar in the three groups (table 3), but creatinine clearance was lower in the NS group than in the LR and PlasmaLyte groups at 15 h (Supplemental Digital Content, table S4, http://links.lww.com/ALN/B303).

The NS group had a higher international normalized ratio than the LR group from 12 h and a higher activated partial thromboplastin time at 20 h (table 3).

Survival Time

The survival time of NS-treated animals was shorter than that of the LR group (17 [14 to 20] *vs.* 26 [23 to 29] h, P < 0.01) but not different from that of the PlasmaLyte group (20 [12 to 28], P = 0.74). The survival times in the LR and PlasmaLyte groups were similar (P = 0.38; fig. 3).

Discussion

We have demonstrated that NS administration during sepsis was associated not only with hyperchloremic acidosis but also with more severe systemic alterations, worse peripheral perfusion, greater impairment of the microcirculation, and more severe organ dysfunction, particularly compared to LR. NS administration was also associated with a shorter survival time compared to LR administration.

It is well established that NS infusion induces hyperchloremic acidosis. A bolus of 50 ml of NS per kilogram resulted in a decrease in pH by a mean of 0.04 in healthy volunteers[11] and an infusion of 60 ml/kg over 2 h decreased pH to 7.28 in patients undergoing gynecologic surgery.[5] In our study, the rapid increase in blood chloride levels and subsequent metabolic acidosis seen with NS were related not only to the high chloride load but also to the lack of increase in renal chloride excretion. The same was true for sodium. These observations likely reflect the compensatory mechanisms (overstimulation of the adrenergic system and the renin–angiotensin–aldosterone axis) that occur during the initial phase of severe sepsis to maintain an appropriate effective intravascular volume[29]; during the late phases of sepsis, some degree of renal failure may also be involved.[8]

The high chloride load may be responsible for the observed differences in renal function between groups. In a classic experiment, Wilcox[30] showed that hyperchloremia induced renal vasoconstriction in a denervated and autotransplanted kidney in dogs. A recent study in human volunteers using magnetic resonance imaging showed that NS infusion, but not PlasmaLyte, decreased mean renal artery flow velocity and renal cortical tissue perfusion.[7] However, we did not observe any decrease in renal blood flow with NS, except when shock was already established, perhaps because the sepsis-induced vasodilation masked

Table 1. Time Course of Measured Global Hemodynamic Variables

		Time, h					
Variable	Group	0	4	8	12	16	20
MAP, mmHg	LR	103 (97–111)	90 (77–94)	82 (72–90)	68 (60–85)	59 (55–79)	55 (54–57)
	PlasmaLyte	107 (104–109)	81 (72–89)	88 (71–91)	72 (44–92)	52 (49–67)	51 (41–54)
	NS	97 (93–109)	88 (79–93)	67 (63–81)	52 (41–63)*	37 (33–42)*	46 (36–57)*
CI, L min^{-1} m^{-2}	LR	5.1 (4.0–5.6)	4.4 (3.9–6.6)	4.7 (3.9–5.1)	4.6 (3.4–6.0)	4.3 (3.5–5.4)	4.9 (3.5–5.9)
	PlasmaLyte	4.2 (3.9–5.1)	3.8 (3.0–4.4)	4.1 (3.8–4.2)	3.8 (3.0–4.4)	4.1 (3.2–4.4)	3.5 (2.2–4.0)*
	NS	4.9 (4.0–5.5)	4.8 (3.8–5.5)	3.2 (2.6–4.2)*	2.9 (2.7–3.3)*	2.1 (1.5–2.8)*†	3.1 (2.9–3.3)*
SVI, ml $beat^{-1}$ m^{-2}	LR	39 (37–40)	37 (25–52)	36 (26–48)	30 (28–44)	33 (24–41)	29 (24–44)
	PlasmaLyte	38 (34–42)	33 (20–48)	33 (24–44)	30 (25–37)	33 (23–35)	27 (24–30)
	NS	35 (33–37)	30 (26–40)	27 (22–32)*	19 (17–23)*	12 (11–17)*†	26 (24–28)
SVRI, dynes sec cm^{-5} m^{-2}	LR	1,746 (1,372–1,995)	1,464 (1,098–1,752)	1,632 (1,213–1,642)	1,121 (1,001–1,743)	965 (938–1,170)	920 (701–1,051)
	PlasmaLyte	1,987 (1,609–2,256)	1,786 (1,407–2,202)	1,526 (1,341–1,827)	1,416 (815–2,144)	1,056 (876–1,268)	1,159 (984–1,598)
	NS	1,680 (1,497–2,031)	1,392 (1,189–1,738)	1,830 (1,420–1,921)	1,213 (1,086–2,037)	1,347 (1,208–1,575)	1,157 (967–1,347)
PAOP, mmHg	LR	6 (5–7)	5 (5–6)	6 (4–7)	6 (5–7)	6 (6–8)	7 (6–8)
	PlasmaLyte	6 (5–7)	5 (5–7)	6 (5–7)	7 (5–7)	7 (7–8)	7 (6–8)
	NS	6 (4–6)	6 (5–6)	6 (5–7)	6 (5–7)	6 (6–7)	7 (7–7)
LVSWI, g m^{-2} $beat^{-1}$	LR	52 (49–58)	36 (27–61)	37 (27–50)	29 (23–33)	22 (16–34)	19 (15–23)
	PlasmaLyte	51 (46–57)	25 (21–50)	37 (28–43)	30 (15–37)	21 (8–31)	15 (12–17)*
	NS	43 (40–58)	31 (22–53)	26 (17–28)*†	10 (8–21)*	5 (3–8)*†	14 (11–16)*
Svo_2, %	LR	71 (69–86)	70 (69–75)	73 (70–74)	73 (66–77)	60 (53–78)	67 (50–76)
	PlasmaLyte	75 (71–78)	70 (59–77)	74 (62–81)	63 (50–76)	66 (30–79)	55 (53–67)
	NS	78 (73–83)	73 (72–80)	71 (66–74)	61 (55–68)*	42 (35–53)*	59 (50–68)*
DO_2I, ml min^{-1} m^{-2}	LR	749 (632–918)	773 (624–883)	703 (654–773)	769 (566–837)	681 (623–832)	684 (591–789)
	PlasmaLyte	582 (548–611)	486 (473–635)*	667 (553–711)	491 (442–752)	616 (465–805)	497 (330–698)*
	NS	671 (555–748)	745 (632–835)†	533 (464–681)	529 (504–582)*	339 (271–453)*	532 (417–646)*
VO_2I, ml min^{-1} m^{-2}	LR	203 (158–247)	227 (200–266)	205 (164–255)	201 (153–258)	201 (176–296)	216 (195–249)
	PlasmaLyte	161 (144–184)	168 (147–194)*	189 (170–192)	154 (135–185)	175 (156–197)	192 (151–199)
	NS	144 (134–174)	195 (155–224)	185 (139–203)	192 (156–240)	188 (157–194)*	202 (192–212)

All values are given as median (25th to 75th percentiles).

*$P < 0.05$ *vs.* LR group. †$P < 0.05$ *vs.* PlasmaLyte group.

CI = cardiac index; DO_2I = oxygen delivery index; LR = lactated Ringer's; LVSWI = left ventricular stroke work index; MAP = mean arterial pressure; NS = normal saline; PAOP = pulmonary artery occlusion pressure; SVI = stroke volume index; Svo_2 = mixed venous oxygen saturation; SVRI = systemic vascular resistance index; VO_2I = oxygen consumption index.

Table 2. Time Course of Measured Peripheral Perfusion Variables

Variable	Group	Time, h					
		0	4	8	12	16	20
$SvfO_2$,%	LR	79 (63–88)	70 (67–76)	66 (59–70)	63 (55–68)	51 (48–74)	58 (41–71)
	PlasmaLyte	84 (73–91)	67 (52–73)	72 (43–84)	64 (43–72)	59 (36–71)	56 (43–59)
	NS	84 (71–91)	78 (67–84)	62 (40–68)	54 (39–70)	44 (37–49)	66 (66–67)
NIRS StO_2,%	LR	78 (74–81)	74 (68–78)	69 (58–72)	61 (59–62)	57 (49–64)	49 (48–55)
	PlasmaLyte	77 (73–78)	70 (60–74)	66 (54–73)	60 (59–69)	62 (49–68)	51 (34–52)
	NS	79 (69–82)	70 (55–71)	59 (45–65)	45 (41–63)	43 (36–48)*†	46 (40–52)
fCO_2 gap, mmHg	LR	6 (4–10)	8 (5–9)	9 (8–11)	9 (8–11)	10 (8–13)	11 (11–16)
	PlasmaLyte	6 (4–9)	8 (6–10)	7 (7–14)	9 (9–18)	14 (11–16)	18 (15–35)
	NS	4 (3–8)	6 (6–9)	13 (9–16)	16 (14–20)*	20 (18–36)*	15 (15–16)
Muscle lactate, mmol/l	LR	1.7 (1.6–4.2)	3.9 (1.1–4.6)	3.7 (1.3–4.4)	3.8 (1.4–4.4)	4.0 (1.4–5.3)	4.0 (2.1–8.4)
	PlasmaLyte	3.0 (1.7–3.4)	3.0 (2.1–5.4)	3.8 (2.6–4.9)	5.7 (3.1–7.6)*	7.6 (5.3–7.9)*	8.7 (5.1–10.9)*
	NS	2.3 (1.1–3.9)	3.2 (2.2–4.2)	3.2 (3–4.3)	5.2 (4.4–6.0)*	9.2 (8.6–11.7)*	11.7 (9.1–14.2)*
Muscle pyruvate, µmol/l	LR	110 (49–166)	131 (73–247)	185 (125–211)	159 (106–217)	29 (12–248)	42 (20–144)
	PlasmaLyte	126 (43–149)	171 (48–225)	192 (51–310)	124 (110–358)	132 (95–234)	135 (63–166)
	NS	48 (31–112)	66 (39–195)	85 (18–192)	146 (11–214)	119 (8–215)	72 (7–136)
Muscle L/P ratio, %	LR	28 (9–39)	19 (12–30)	20 (14–25)	26 (10–30)	40 (22–335)	48 (27–191)
	PlasmaLyte	25 (17–50)	32 (15–44)	32 (11–64)	28 (19–72)	48 (33–62)	54 (37–348)
	NS	35 (30–48)	31 (21–50)	35 (30–166)	31 (24–565)	89 (43–1,129)	722 (104–1,339)

All values are given as median (25th to 75th percentiles).

*$P < 0.05$ *vs*. LR group. †$P < 0.05$ *vs*. PlasmaLyte group.

fCO_2 gap = femoral venoarterial PCO_2 difference; L/P = lactate/pyruvate; LR = lactated Ringer's; NIRS = near-infrared spectroscopy; NS = normal saline; StO_2 = tissue oxygen saturation; $SvfO_2$ = femoral vein hemoglobin oxygen saturation.

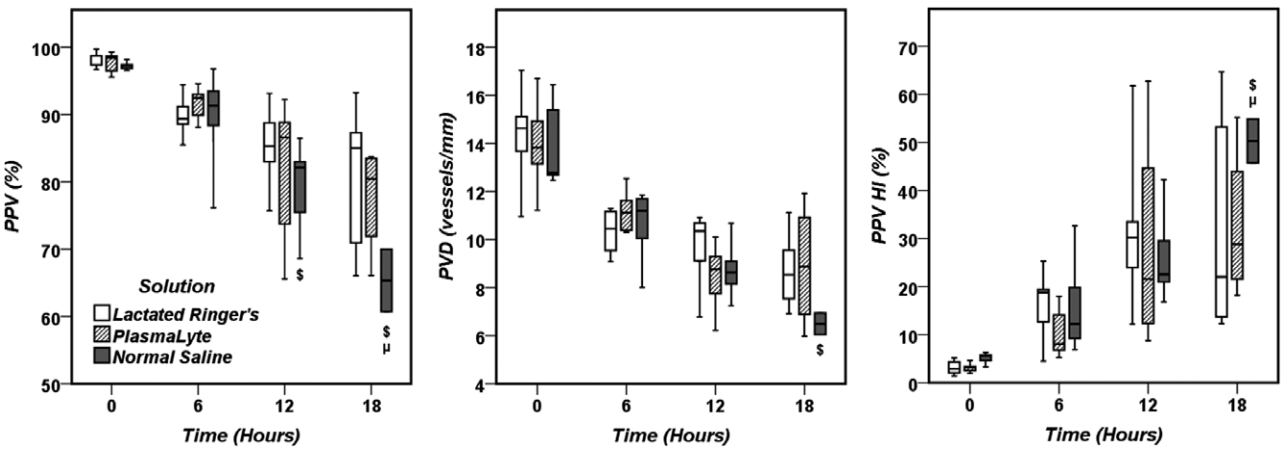

Fig. 2. Time course of sublingual microcirculatory variables. $*P < 0.05$ *versus* lactated Ringer's group, µ$P < 0.05$ *versus* PlasmaLyte group. PPV = proportion of perfused vessels; PPV HI = heterogeneity index of PPV; PVD = perfused vessel density.

the chloride-mediated renal vasoconstriction. In a hemorrhagic rat model, Aksu *et al.*[31] demonstrated that there were only minor differences in renal blood flow and corticomedullary kidney perfusion between PlasmaLyte or NS resuscitation compared to the large effect of the acute hemorrhagic shock insult. Nevertheless, infusion of NS solutions has been associated with a worsening of renal function in animals[8] and in patients.[10] We observed that creatinine levels increased earlier and diuresis and creatinine clearance decreased more rapidly with NS compared to PlasmaLyte or LR administration, but these alterations may also have been secondary to the more severe global hemodynamic alterations.

The role of hyperchloremic acidosis in the development of hemodynamic instability is controversial. Early physiologic studies on hydrochloric acid infusion in anesthetized dogs indicated that CO did not decrease until pH reached values less than 7.1.[32] However, our animals are also exposed to a severe septic process, and under these conditions, acidosis is not well tolerated. It is well known that the *in vitro* measured contractile tension of the smooth muscle of vessels decreases in proportion to the severity of acidosis,[33,34] a process partially mediated by an increase in nitric oxide.[35] Moreover, in a cecal ligation and puncture model of peritonitis in rats, Kellum *et al.*[36] reported a decrease in MAP when moderate hyperchloremic acidosis was induced. We found

Table 3. Time Course of Renal, Coagulation, and Respiratory Variables

Variable	Group	Time, h 0	4	8	12	16	20
Urine output, ml kg^{-1} h^{-1}	LR	—	0.9 (0.6–2.2)	1.3 (0.5–2.3)	1.2 (0.3–2.9)	0.2 (0.0–1.3)	0.1 (0.0–0.4)
	PlasmaLyte	—	1.5 (1.0–2.2)	1.6 (1.1–4.8)	1.2 (0.6–3.1)	0.7 (0.0–1.7)	0.2 (0.1–0.5)
	NS	—	0.9 (0.7–1.2)*	1.3 (0.2–2.7)	0.1 (0.0–0.4)*†	0.0 (0.0–0.1)	0.2 (0.0–0.4)
Creatinine, mg/dl	LR	0.9 (0.7–1.2)	0.8 (0.6–1.0)	0.9 (0.6–1.0)	1.1 (0.7–1.2)	1.4 (0.9–2.0)	2.0 (1.4–2.7)
	PlasmaLyte	0.8 (0.7–0.9)	0.7 (0.6–0.8)	0.6 (0.5–0.7)	0.8 (0.5–0.9)	1.1 (0.7–1.7)	1.4 (1.2–1.7)
	NS	0.7 (0.6–1.0)	0.7 (0.6–0.8)	0.7 (0.7–0.9)	1.2 (0.8–1.6)	2.0 (1.1–2.5)	1.5 (1.4–1.6)
Renal blood flow, ml/min	LR	243 (213–350)	220 (167–340)	190 (175–236)	150 (80–182)	74 (50–178)	75 (40–119)
	PlasmaLyte	180 (130–310)	110 (100–190)†	80 (70–230)	40 (30–90)	25 (0–40)	15 (5–72.5)
	NS	280 (240–310)	230 (190–250)*	150 (130–260)	60 (10–130)	20 (0–40)†	20 (20–20)†
aPTT, s	LR	32 (28–36)	33 (32–41)	40 (35–47)	45 (40–53)	49 (40–59)	50 (40–61)
	PlasmaLyte	31 (30–42)	35 (32–47)	41 (37–52)	52 (40–56)	58 (44–61)	50 (42–72)
	NS	36 (24–42)	34 (27–42)	45 (37–47)	55 (46–55)	62 (50–135)	104 (58–150)†
INR	LR	1.4 (1.3–1.4)	1.5 (1.4–1.6)	1.7 (1.6–1.7)	2.0 (1.9–2.0)	2.2 (2.2–2.4)	2.4 (2.4–2.7)
	PlasmaLyte	1.4 (1.2–1.4)	1.5 (1.3–1.6)	1.7 (1.5–1.9)	1.8 (1.6–2.0)	2.3 (2.1–2.4)	2.8 (2.5–3.1)
	NS	1.4 (1.3–1.5)	1.5 (1.4–1.5)	1.7 (1.6–1.8)	2.2 (1.9–2.7)†	2.7 (2.3–4.4)*†	2.7 (2.5–2.9)†
Airway plateau pressure, cm H_2O	LR	24 (22–25)	25 (22–26)	25 (23–28)	27 (26–29)	28 (27–29)	29 (28–30)
	PlasmaLyte	26 (22–28)	24 (23–26)	24 (23–26)	27 (25–28)	28 (26–29)	31 (27–36)
	NS	24 (21–26)	25 (22–26)	25 (24–28)	29 (24–33)	32 (28–34)	33 (30–35)*

All values are given as median (25th to 75th percentiles).

*$P < 0.05$ *vs.* LR group. †$P < 0.05$ *vs.* PlasmaLyte group.

aPTT = activated partial thromboplastin time; INR = international normalized ratio; LR = lactated Ringer's; NS = normal saline.

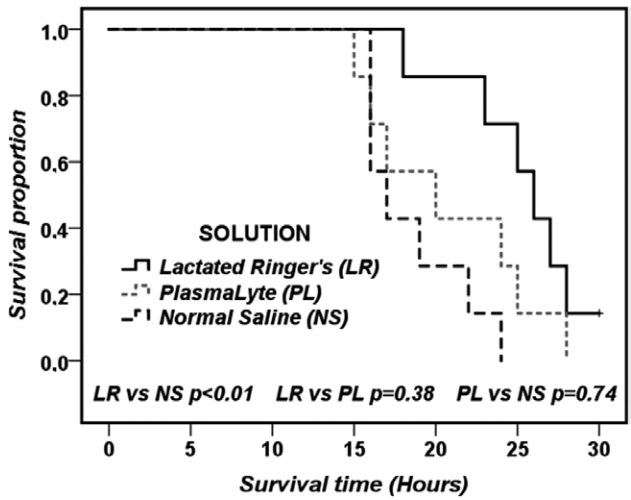

Fig. 3. Survival curves for the three cohorts.

lower CI, SVI, and left ventricular stroke work index in the NS group from 8 h when changes in pH were only moderate, a finding that suggests some degree of more severe septic cardiomyopathy. Unfortunately, we do not have data on cardiac biomarkers or inflammatory molecules that could help explain the physiopathologic mechanisms of our findings.

Sepsis is associated with a decrease in capillary perfusion and an increase in the heterogeneity of perfusion during sepsis.[37] Some data suggest that hyperchloremic acidosis may impair the microcirculation. In rabbit muscle microcirculation, hyperchloremic acidosis induced by hydrochloric acid infusion resulted in an 18% narrowing of the capillary diameter, probably related to endothelial cell swelling.[38] In a rat model of bacterial peritonitis, NS infusion was associated with decreased microcirculatory blood flow in the liver and a reduced number of capillaries with flow in the liver and intestine.[19] Our data with SDF videomicroscopy indicate that sepsis was associated with more severe alterations when NS was administered compared to PlasmaLyte or LR, and these changes were mainly characterized by diminished capillary density with increased perfusion heterogeneity. We also collected data on the peripheral regional blood flow (fco_2 gap),

metabolism (muscle microdialysis, femoral vein hemoglobin oxygen saturation), and oxygen saturation in the skeletal muscle microcirculation (NIRS StO_2), which were concordant with the SDF findings and, when considered together, indicated decreased peripheral perfusion in the NS group. All these parameters were rapidly altered after sepsis induction, but the differences between groups were only clear once marked hemodynamic differences were established.

We also demonstrated more coagulation abnormalities in the NS group than in the other two groups. This finding is concordant with previous reports in nonseptic conditions, in which NS was associated with more severe coagulopathy or increased blood losses than LR.[6,39] In a recent meta-analysis, we found increased blood loss and greater need for transfusion with NS than with LR in patients at high risk of bleeding.[2]

Survival time was markedly reduced with NS compared to LR but not compared to PlasmaLyte. In an early study in a pig model of severe hemorrhagic shock, Traverso *et al.*[40] reported a higher fatality rate with PlasmaLyte compared to NS or LR, perhaps related to the acetate or magnesium content of PlasmaLyte. Our data show that continuous PlasmaLyte infusions for prolonged periods led to some increase in magnesium levels, but still within normal limits. In a canine model of controlled right heart bypass, vascular tone was reduced by infusion of different acetate-containing solutions, even at low concentrations.[41] Acetate administration has also been associated with hemodynamic instability in chronically[16,42] and acutely[43] ill patients undergoing hemodialysis. We did not observe differences in the expansion power during a fluid challenge or lower systemic vascular resistance in the PlasmaLyte group but did transiently observe a decrease in oxygen transport in the PlasmaLyte group at 4 h, whereas in the other groups, it increased. Unexpectedly, we observed some decrease in renal blood flow in the PlasmaLyte group from the early phases, but the effects of acetate on renal blood flow are complex, with vasodilatation at low doses and vasoconstriction with higher doses.[44] In the PlasmaLyte group, we also observed an increased rate of hypokalemic episodes (mainly related to increased kaliuresis) and an early decrease in calcium levels (even when compared to NS). Rabinowitz *et al.*[45] showed in sheep that renal excretion of potassium was increased with acetate infusion, but the mechanism was unclear. Magnesium supplementation may also contribute to the development of hypocalcemia.[46]

Our study has some limitations that should be considered when trying to extrapolate the results to clinical practice. First, we did not use vasoactive agents to avoid this confounding factor; however, if they had been administered, it is likely that the NS group would have received them earlier than the other groups. Second, our results represent a condition in which hyperchloremic acidosis was severe and not compensated for by respiratory alkalosis; in clinical practice, physicians would likely have changed the fluid solution to limit the continued development of hyperchloremia or acidosis. Third, we included a limited number of animals per group, so the study potentially had insufficient power to detect some of the differences that may have existed between the fluids. For example, the survival curves for LR- and PlasmaLyte-treated animals separated from 16 h favoring the LR group, but this did not reach statistical significance. This may also explain, in part, the lack of a discernable pattern over time for some of the variables. Finally, our animal model, in which there is a continuum from normality through sepsis to septic shock after the abdominal injection of bacteria, tries to mimic the hemodynamic features of human bacterial peritonitis,[47,48] resulting in decreased systemic vascular resistance and a maintained CO. Nevertheless, caution must be exercised when extrapolating results from animal models of sepsis to clinical sepsis because of the clear differences between these two settings (differences in use of sedation, anesthesia, antibiotics, infused fluid volumes, control of the infectious source, presence of comorbidities, genetic differences between species, *etc.*).[49–51]

In conclusion, in our clinically relevant model of sepsis, NS infusion induced hyperchloremic acidosis, created more hemodynamic instability, altered the peripheral perfusion, impaired the microcirculation, and increased renal and coagulation dysfunction, leading to a shorter survival time compared to LR. The hemodynamic differences were evident from the early stages of resuscitation (when pH and chloride were only slightly altered), suggesting caution when considering NS infusion in sepsis resuscitation. We also identified some potential disadvantages with PlasmaLyte administration, but larger population studies are needed before we can draw definite conclusions about its safety profile.

Research Support

Support was provided solely from institutional and/or departmental sources.

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Vincent: Department of Intensive Care, Erasme University Hospital, Université Libre De Bruxelles, Route De Lennik 808, 1070 Brussels, Belgium. jlvincent@intensive.org.

References

1. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb SA, Beale RJ, Vincent JL, Moreno R; Surviving Sepsis Campaign Guidelines Committee Including the Pediatric Subgroup: Surviving sepsis campaign:

International guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med 2013; 41:580–637
2. Orbegozo Cortés D, Rayo Bonor A, Vincent JL: Isotonic crystalloid solutions: A structured review of the literature. Br J Anaesth 2014; 112:968–81
3. Raghunathan K, Shaw A, Nathanson B, Stürmer T, Brookhart A, Stefan MS, Setoguchi S, Beadles C, Lindenauer PK: Association between the choice of IV crystalloid and in-hospital mortality among critically ill adults with sepsis. Crit Care Med 2014; 42:1585–91
4. Reid F, Lobo DN, Williams RN, Rowlands BJ, Allison SP: (Ab) normal saline and physiological Hartmann's solution: A randomized double-blind crossover study. Clin Sci (Lond) 2003; 104:17–24
5. Scheingraber S, Rehm M, Sehmisch C, Finsterer U: Rapid saline infusion produces hyperchloremic acidosis in patients undergoing gynecologic surgery. ANESTHESIOLOGY 1999; 90:1265–70
6. Kiraly LN, Differding JA, Enomoto TM, Sawai RS, Muller PJ, Diggs B, Tieu BH, Englehart MS, Underwood S, Wiesberg TT, Schreiber MA: Resuscitation with normal saline (NS) *vs*. lactated ringers (LR) modulates hypercoagulability and leads to increased blood loss in an uncontrolled hemorrhagic shock swine model. J Trauma 2006; 61:57–64; discussion 64–5
7. Chowdhury AH, Cox EF, Francis ST, Lobo DN: A randomized, controlled, double-blind crossover study on the effects of 2-L infusions of 0.9% saline and plasma-lyte® 148 on renal blood flow velocity and renal cortical tissue perfusion in healthy volunteers. Ann Surg 2012; 256:18–24
8. Zhou F, Peng ZY, Bishop JV, Cove ME, Singbartl K, Kellum JA: Effects of fluid resuscitation with 0.9% saline *versus* a balanced electrolyte solution on acute kidney injury in a rat model of sepsis. Crit Care Med 2014; 42:e270–8
9. Kellum JA, Song M, Almasri E: Hyperchloremic acidosis increases circulating inflammatory molecules in experimental sepsis. Chest 2006; 130:962–7
10. Yunos NM, Bellomo R, Hegarty C, Story D, Ho L, Bailey M: Association between a chloride-liberal *vs* chloride-restrictive intravenous fluid administration strategy and kidney injury in critically ill adults. JAMA 2012; 308:1566–72
11. Williams EL, Hildebrand KL, McCormick SA, Bedel MJ: The effect of intravenous lactated Ringer's solution *versus* 0.9% sodium chloride solution on serum osmolality in human volunteers. Anesth Analg 1999; 88:999–1003
12. Levy B, Mansart A, Montemont C, Gibot S, Mallie JP, Regnault V, Lecompte T, Lacolley P: Myocardial lactate deprivation is associated with decreased cardiovascular performance, decreased myocardial energetics, and early death in endotoxic shock. Intensive Care Med 2007; 33:495–502
13. Bouzat P, Oddo M: Lactate and the injured brain: Friend or foe? Curr Opin Crit Care 2014; 20:133–40
14. Mochizuki M: 4-week oral toxicity study of sodium gluconate (FR2531). Final report no. BOZO/B-2966. Tokyo, Gotemba Laboratory, Bozo Research Center, 1995
15. Peekhaus N, Conway T: What's for dinner?: Entner-Doudoroff metabolism in *Escherichia coli*. J Bacteriol 1998; 180:3495–502
16. Thaha M, Yogiantoro M, Soewanto, Pranawa: Correlation between intradialytic hypotension in patients undergoing routine hemodialysis and use of acetate compared in bicarbonate dialysate. Acta Med Indones 2005; 37:145–8
17. Young P, Bailey M, Beasley R, Henderson S, Mackle D, McArthur C, McGuinness S, Mehrtens J, Myburgh J, Psirides A, Reddy S, Bellomo R; SPLIT Investigators; ANZICS CTG: Effect of a buffered crystalloid solution *vs* saline on acute kidney injury among patients in the intensive care unit: The SPLIT Randomized Clinical Trial. JAMA 2015; 314:1701–10
18. Kellum JA: Fluid resuscitation and hyperchloremic acidosis in experimental sepsis: Improved short-term survival and acid-base balance with Hextend compared with saline. Crit Care Med 2002; 30:300–5
19. Schick MA, Isbary JT, Stueber T, Brugger J, Stumpner J, Schlegel N, Roewer N, Eichelbroenner O, Wunder C: Effects of crystalloids and colloids on liver and intestine microcirculation and function in cecal ligation and puncture induced septic rodents. BMC Gastroenterol 2012; 12:179
20. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals: Guide for the Care and Use of Laboratory Animals, 8th edition. Washington, The National Academies Press, 2015
21. Wang Z, Su F, Bruhn A, Yang X, Vincent JL: Acute hypercapnia improves indices of tissue oxygenation more than dobutamine in septic shock. Am J Respir Crit Care Med 2008; 177:178–83
22. Galatos AD: Anesthesia and analgesia in sheep and goats. Vet Clin North Am Food Anim Pract 2011; 27:47–59
23. De Backer D, Hollenberg S, Boerma C, Goedhart P, Buchele G, Ospina-Tascon G, Dobbe I, Ince C: How to evaluate the microcirculation: report of a round table conference. Crit Care 2007; 11:R101
24. Berman A: Effects of body surface area estimates on predicted energy requirements and heat stress. J Dairy Sci 2003; 86:3605–10
25. Calzavacca P, Ishikawa K, Bailey M, May CN, Bellomo R: Systemic and renal hemodynamic effects of intra-arterial radiocontrast. Intensive Care Med Exp 2014; 2:32
26. Schetz M, Gunst J, Van den Berghe G: The impact of using estimated GFR *versus* creatinine clearance on the evaluation of recovery from acute kidney injury in the ICU. Intensive Care Med 2014; 40:1709–17
27. Su F, Huang H, Akieda K, Occhipinti G, Donadello K, Piagnerelli M, De Backer D, Vincent JL: Effects of a selective iNOS inhibitor *versus* norepinephrine in the treatment of septic shock. Shock 2010; 34:243–9
28. He X, Su F, Velissaris D, Salgado DR, de Souza Barros D, Lorent S, Taccone FS, Vincent JL, De Backer D: Administration of tetrahydrobiopterin improves the microcirculation and outcome in an ovine model of septic shock. Crit Care Med 2012; 40:2833–40
29. Schaller MD, Waeber B, Nussberger J, Brunner HR: Angiotensin II, vasopressin, and sympathetic activity in conscious rats with endotoxemia. Am J Physiol 1985; 249(6 pt 2):H1086–92
30. Wilcox CS: Regulation of renal blood flow by plasma chloride. J Clin Invest 1983; 71:726–35
31. Aksu U, Bezemer R, Yavuz B, Kandil A, Demirci C, Ince C: Balanced *vs* unbalanced crystalloid resuscitation in a near-fatal model of hemorrhagic shock and the effects on renal oxygenation, oxidative stress, and inflammation. Resuscitation 2012; 83:767–73
32. Clowes GH Jr, Sabga GA, Konitaxis A, Tomin R, Hughes M, Simeone FA: Effects of acidosis on cardiovascular function in surgical patients. Ann Surg 1961; 154:524–55
33. Rooke TW, Sparks HV Jr: Effect of metabolic *versus* respiratory acid-base changes on isolated coronary artery and saphenous vein. Experientia 1981; 37:982–3
34. Celotto AC, Capellini VK, Baldo CF, Dalio MB, Rodrigues AJ, Evora PR: Effects of acid-base imbalance on vascular reactivity. Braz J Med Biol Res 2008; 41:439–45
35. Hattori K, Tsuchida S, Tsukahara H, Mayumi M, Tanaka T, Zhang L, Taniguchi T, Muramatsu I: Augmentation of NO-mediated vasodilation in metabolic acidosis. Life Sci 2002; 71:1439–47
36. Kellum JA, Song M, Venkataraman R: Effects of hyperchloremic acidosis on arterial pressure and circulating inflammatory molecules in experimental sepsis. Chest 2004; 125:243–8
37. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL: Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med 2002; 166:98–104

38. Mazzoni MC, Cragoe EJ Jr, Arfors KE: Systemic blood acidosis in low-flow ischemia induces capillary luminal narrowing. Int J Microcirc Clin Exp 1994; 14:144–50
39. Brummel-Ziedins K, Whelihan MF, Ziedins EG, Mann KG: The resuscitative fluid you choose may potentiate bleeding. J Trauma 2006; 61:1350–8
40. Traverso LW, Lee WP, Langford MJ: Fluid resuscitation after an otherwise fatal hemorrhage: I. Crystalloid solutions. J Trauma 1986; 26:168–75
41. Olinger GN, Werner PH, Bonchek LI, Boerboom LE: Vasodilator effects of the sodium acetate in pooled protein fraction. Ann Surg 1979; 190:305–11
42. Aizawa Y, Ohmori T, Imai K, Nara Y, Matsuoka M, Hirasawa Y: Depressant action of acetate upon the human cardiovascular system. Clin Nephrol 1977; 8:477–80
43. Vincent JL, Vanherweghem JL, Degaute JP, Berré J, Dufaye P, Kahn RJ: Acetate-induced myocardial depression during hemodialysis for acute renal failure. Kidney Int 1982; 22:653–7
44. Steffen RP, O'Neill JT, Haddy FJ: Effect of theophylline on renal vasoactivity of acetate and adenosine. J Cardiovasc Pharmacol 1988; 11:682–6
45. Rabinowitz L, Sarason RL, Tanasovich C, Mendel VE, Brockman RP: Effects of glucagon, insulin, propionate, acetate, and HCO3 on K excretion in sheep. Am J Physiol 1984; 246(2 pt 2):R197–204
46. van den Bergh WM, van de Water JM, Hoff RG, Algra A, Rinkel GJ: Calcium homeostasis during magnesium treatment in aneurysmal subarachnoid hemorrhage. Neurocrit Care 2008; 8:413–7
47. Vincent JL, Weil MH, Puri V, Carlson RW: Circulatory shock associated with purulent peritonitis. Am J Surg 1981; 142:262–70
48. Jardin F, Eveleigh MC, Gurdjian F, Delille F, Margairaz A: Venous admixture in human septic shock: Comparative effects of blood volume expansion, dopamine infusion and isoproterenol infusion on mismatching of ventilation and pulmonary blood flow in peritonitis. Circulation 1979; 60:155–9
49. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, Finnerty CC, López CM, Honari S, Moore EE, Minei JP, Cuschieri J, Bankey PE, Johnson JL, Sperry J, Nathens AB, Billiar TR, West MA, Jeschke MG, Klein MB, Gamelli RL, Gibran NS, Brownstein BH, Miller-Graziano C, Calvano SE, Mason PH, Cobb JP, Rahme LG, Lowry SF, Maier RV, Moldawer LL, Herndon DN, Davis RW, Xiao W, Tompkins RG; Inflammation and Host Response to Injury, Large Scale Collaborative Research Program: Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci USA 2013; 110:3507–12
50. Takao K, Miyakawa T: Genomic responses in mouse models greatly mimic human inflammatory diseases. Proc Natl Acad Sci USA 2015; 112:1167–72
51. Doi K: How to replicate the complexity of human sepsis: Development of a new animal model of sepsis. Crit Care Med 2012; 40:2722–3