Effects of Fluid Bolus Therapy on Renal Perfusion, Oxygenation, and Function in Early Experimental Septic Kidney Injury

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Objectives: To examine the effects of fluid bolus therapy on systemic hemodynamics, renal blood flow, intrarenal perfusion and oxygenation, Po_2, renal function, and fluid balance in experimental early septic acute kidney injury.

Design: Interventional study.

Setting: Research institute.

Subjects: Adult Merino ewes.

Interventions: Implantation of flow probes on the pulmonary and renal arteries and laser Doppler oxygen-sensing probes in the renal cortex, medulla, and within a bladder catheter in sheep. Infusion of *Escherichia coli* to induce septic acute kidney injury ($n = 8$). After 24, 25, and 26 hours of sepsis, fluid bolus therapy (500 mL of Hartmann's solution over 15 min) was administered.

Measurements and Main Results: In conscious sheep, infusion of *Escherichia coli* decreased creatinine clearance and increased plasma creatinine, renal blood flow (+46% ± 6%) and cortical perfusion (+25% ± 4%), but medullary perfusion (−48% ± 5%), medullary Po_2 (−56% ± 4%), and urinary Po_2 (−54% ± 3%) decreased ($p < 0.01$). The first fluid bolus therapy increased blood pressure (+6% ± 1%), central venous pressure (+245% ± 65%), cardiac output (+11% ± 2%), medullary Po_2 (+280% ± 90%), urinary Po_2 (+164% ± 80%), and creatinine clearance (+120% ± 65%) at 30 minutes. The following two boluses had no beneficial effects on creatinine clearance. The improvement in medullary oxygenation dissipated following the third fluid bolus therapy. Study animals retained 69% of the total volume and 80% of sodium infused. Throughout the study, urinary Po_2 correlated significantly with medullary Po_2.

Conclusions: In early experimental septic acute kidney injury, fluid bolus therapy transiently improved renal function and medullary Po_2, as also reflected by increased urinary Po_2. These initial effects of fluid bolus therapy dissipated within 4 hours, despite two additional fluid boluses, and resulted in significant volume retention. (*Crit Care Med* 2019; 47:e36–e43)

Key Words: acute kidney injury; fluid bolus therapy; hypoxia; sepsis

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Acute kidney injury (AKI) is common in septic patients, complicates their management, and is a significant independent predictor of mortality (1, 2). Hemodynamic management with fluid bolus therapy (FBT) is the recommended first-line therapy for sepsis and septic shock to improve organ perfusion and oxygenation (3). However, recent multicenter randomized clinical trials using early goal-directed therapy (EGDT) and its associated aggressive FBT-based resuscitation failed to show any improvements in renal outcomes (4–6). These findings contrast with the frequent clinical observation that serum creatinine falls, and urinary output increases after a fluid bolus. Thus, a better understanding of the mechanisms by which FBT might influence systemic hemodynamics, the renal macrocirculation, the renal microcirculation, renal function, and fluid, and sodium balance is important for the development of more evidence-based strategies to mitigate septic AKI (7, 8). Particularly, an understanding of how FBT impacts renal cortical and medullary perfusion and oxygenation appears fundamental.

Renal medullary hypoxia has been proposed as a critical mediator of AKI (9–11), which is supported by our findings that an early onset of medullary ischemia and hypoxia precedes the development of AKI in ovine sepsis (12–14). In ovine septic AKI, fluid resuscitation with a single bolus of crystalloids yields only transitory improvements in renal function, which dissipate within 45–60 minutes (15, 16). Resuscitation with balanced crystalloids is emerging as a safer alternative to unbalanced crystalloids in the setting of AKI (17). Accordingly, we hypothesized that repeated resuscitation with three sequential FBT of a balanced crystalloid (Hartmann's) solution would improve systemic hemodynamics and renal function, as well as intrarenal tissue perfusion and oxygen tension (P_{O_2}), and urinary oxygenation, in conscious sheep with septic AKI.

MATERIALS AND METHODS

Animals

The experiments were approved by the Animal Ethics Committee of the Florey Institute under guidelines laid down by the National Health and Medical Research Council of Australia. Twenty Merino ewes (35–40 Kg) underwent two surgical procedures under general anesthesia. First, a 20-mm transit-time probe was implanted around the pulmonary artery, and a carotid arterial loop was constructed (18). Second, a 4-mm transit-time probe was placed around the left renal artery, and the renal vein was directly cannulated. Fiber-optic probes (Oxford Optronix Ltd, Milton, United Kingdom) were inserted into the renal cortex and medulla (12, 13, 19) (for antibiotic and analgesia regimens, see **Online Data Supplement**, Supplemental Digital Content 1, http://links.lww.com/CCM/E168).

After 3 days, the carotid artery and jugular vein were cannulated following subcutaneous injection of local anesthetic (Lignocaine, 20 mg/mL; Troy Laboratories, Glendenning, NSW, Australia), and a fiber-optic probe was inserted into the bladder catheter to measure urinary P_{O_2} (13, 16). Analog signals for cardiovascular and renal variables were continuously recorded at 100 Hz on a computer using a CED micro 1,401 interface with Spike 2 software (Cambridge Electronic Design, Cambridge, United Kingdom). Arterial and renal venous blood was collected at predefined times for measurement of blood gases (ABL Systems-625; Radiometer, VIC, Australia), creatinine, and sodium. Simultaneous collections of urine were made for the measurement of creatinine and sodium.

Stroke volume (SV), total peripheral conductance (TPC), renal vascular conductance (RVC), renal perfusion pressure (RPP), creatinine clearance, fractional sodium excretion, renal oxygen delivery (RDO_2), renal oxygen consumption (RVO_2), renal oxygen extraction ratio, systemic oxygen delivery (DO_2), and fluid balance were calculated (Online Data Supplement, Supplemental Digital Content 1, http://links.lww.com/CCM/E168).

Experimental Protocols

Induction of Sepsis. After 24 hours of baseline measurements, sepsis was induced by IV infusion of live *Escherichia coli* (2.8×10^9 colony-forming units [CFUs] over 30-min followed by 1.26×10^9 CFU/hr for 30 hr). During 24 hours of sepsis, prior to treatment allocation, all sheep received Hartmann's solution (1 mL/Kg/hr) (Baxter, Brunswick, VIC, Australia; for composition, see Online Data Supplement, Supplemental Digital Content 1, http://links.lww.com/CCM/E168) as fluid replacement. All animals fulfilled the predefined criteria for hyperdynamic sepsis based on previously reported clinical criteria (20, 21) (i.e., mean arterial pressure [MAP] decreased ≥ 15 mm Hg; cardiac output [CO] increased ≥ 50%, heart rate [HR] increased ≥ 50%, core temperature increased to ≥ 41°C, and arterial lactate increased to ≥ 1.5 mmol/L and evidence of early AKI [urinary output ≤ 0.5 mL/Kg/hr for > 6 hr]).

Intervention. Sheep were randomly allocated to receive either FBT with Hartmann's solution (500 mL over 15 min) with repeated boluses at 24, 25, and 26 hours of sepsis or a maintenance infusion of Hartmann's solution at 1 mL/kg/hr from 24 to 30 hours of bacteremia, in the time-control group. No antibiotics or catecholamines were administered. At 30 hours of sepsis, animals were euthanized with pentobarbitone (100 mg/kg, IV) (Online Data Supplement, Supplemental Digital Content 1, http://links.lww.com/CCM/E168).

Statistical Analysis

All variables passed the tests for normality (D'Agostino and Pearson Omnibus) (13). Data are reported as mean ± SEM. Specific time-point comparisons were performed using a Student's paired *t* test. Variables during FBT or time-control treatment were analyzed using a Dunnett's test to make comparisons with the respective pretreatment period (24-hr sepsis) (GraphPad PRISM 6.0; GraphPad Software, La Jolla, CA). Lines of best fit were determined by ordinary products regression analysis (22). Two-sided *p* value of less than or equal to 0.05 was considered statistically significant.

RESULTS

Changes in Systemic Hemodynamics

Five sheep that reached predefined ethical endpoint criteria were euthanized between 12 and 24 hours following *E. coli* infusion (Online Data Supplement, Supplemental Digital Content 1, http://links.lww.com/CCM/E168) and were excluded from analysis.

After infusion of *E. coli* for 24 hours, a hyperdynamic state was established with a decreased MAP (80 ± 2 to 68 ± 2 mm Hg), peripheral vasodilatation (TPC: 49 ± 3 to 87 ± 6 mL/min/mm Hg) (both $p < 0.001$), increased CO (3.9 ± 0.2 to 6.1 ± 0.4 L/min), profound tachycardia (HR: 75 ± 3 to 144 ± 4 beats/min), reduced SV (53 ± 4 to 42 ± 3 mL), and increased DO_2 (488 ± 22 to 726 ± 39 mLO_2/min) (all $p < 0.05$) (**Figs. 1** and 3*E*). Sheep also developed tachypnea, hypocapnia ($PaCO_2$ < 27 mm Hg), fever (> 41.5°C), and ovine hyperlactatemia greater than 1.5 mmol/L (all $p < 0.05$) (Online Supplement, Supplemental Digital Content 1, http://links.lww.com/CCM/E168).

Changes in Renal Function and Renal Macrocirculation During Septic AKI

Early septic AKI developed, manifested by a ~60% reductionin urinary output (1.20 ± 0.08 to 0.50 ± 0.07 mL/Kg/min for > 6 hr), a

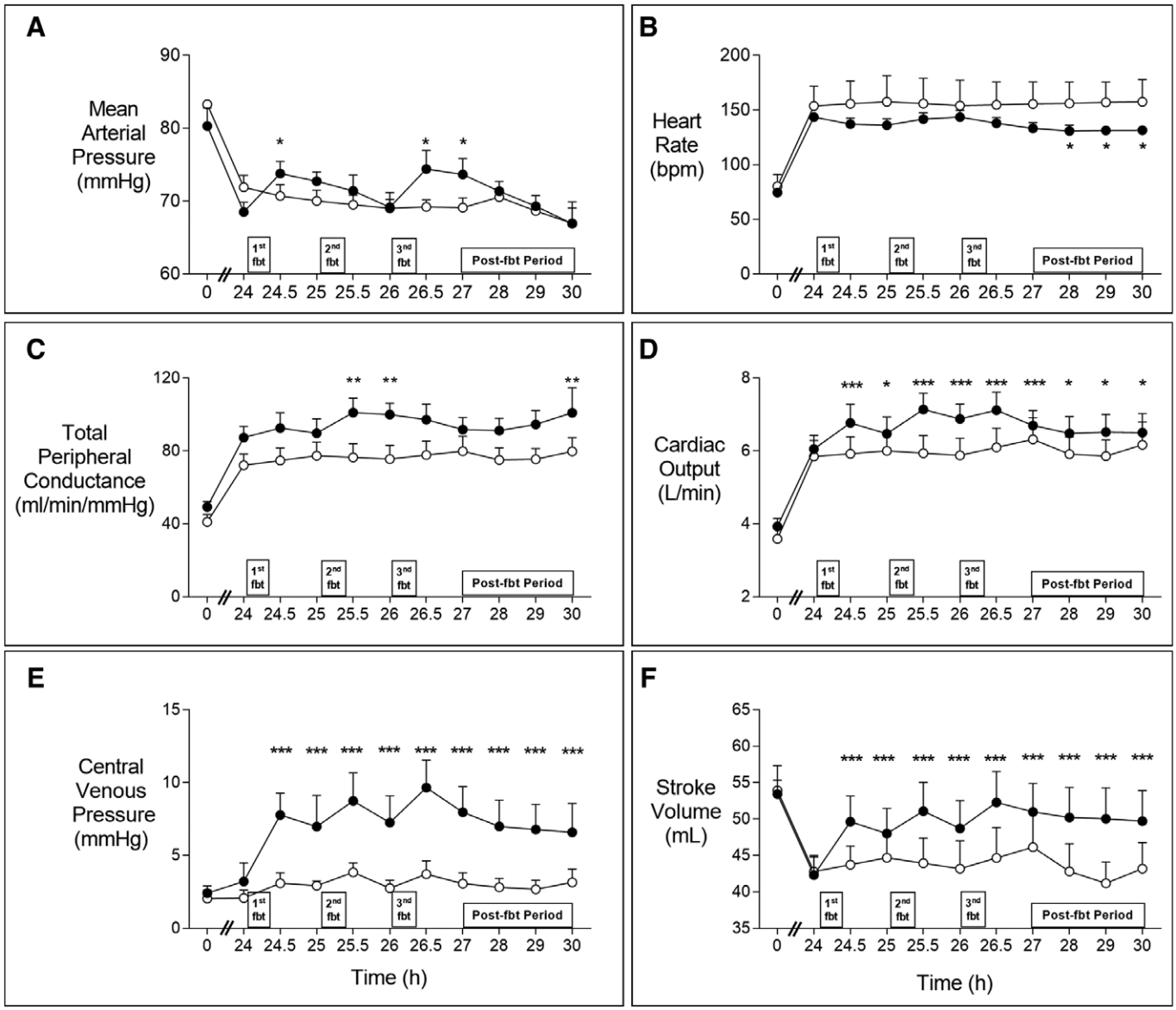

Figure 1. Mean arterial pressure (**A**), heart rate (**B**), total peripheral conductance (**C**), cardiac output (**D**), central venous pressure (**E**), and stroke volume (**F**) at baseline and during infusion of *Escherichia coli* from 0 to 30 hr and treatment with three successive fluid bolus therapies (FBTs, 500 mL of balanced crystalloid, administered over 15 min at 2,000 mL/hr) ($n = 8$, *filled circles*) or vehicle-saline (1 mL/Kg/hr) (*open circles*, $n = 7$) in conscious sheep. Time 0 is the mean of the 24 hr baseline period, time 24 is the mean of the 24th hr after commencement of *E. coli* infusion, and times 24–30 hours are means of 30-min periods. Values are mean ± SEM and $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ indicate significant differences between the 24th hr of sepsis and following three FBTs and a 3 hr post FBT period. *p* values represent the results of a Dunnett's test using absolute values.

50% reduction in creatinine clearance (from 81 ± 8 to 40 ± 6 mL/min), reduced fractional sodium excretion (from 1.2 ± 0.2 to 0.5 ± 0.1%), and a 60% increase in plasma creatinine (from 72 ± 4 to 116 ± 12 µmol/L) (all $p < 0.01$) (**Fig. 2**). These functional changes occurred despite increased global renal blood flow (RBF) (204 ± 7 to 300 ± 35 mL/min; $p = 0.02$), increased global RDO_2 (26 ± 1 to 36 ± 4 mL oxygen/min; $p = 0.03$), unchanged RVO_2 (3.6 ± 0.3 to 3.5 ± 0.7 mLO_2/min), and decreased renal oxygen extraction (13.1 ± 0.9 to 8.8% ± 1%; $p = 0.02$) (**Fig. 3**).

Changes in Renal Microcirculation and Oxygenation

Septic AKI was associated with increased renal cortical PO_2 (40 ± 4 to 49 ± 2 mm Hg; $p = 0.03$) and a tendency toward increased cortical perfusion (1,050 ± 70 to 1,302 ± 113 Blood Perfusion Units [BPUs]; $p = 0.07$) (**Fig. 4**). In contrast, both medullary tissue perfusion (932 ± 74 to 482 ± 44 BPU) and medullary tissue PO_2 (43 ± 4 to 19 ± 4 mm Hg) decreased (Fig. 4), accompanied by a parallel fall in urinary PO_2 (44 ± 4 to 20 ± 3 mm Hg) (**Fig. 5*A***) (all $p < 0.01$).

Systemic Effects of FBT

During hypotensive sepsis, the first episode of FBT increased MAP (69 ± 2 to 74 ± 2 mm Hg), central venous pressure (CVP, 3.1 ± 0.8 to 7.8 ± 1.4 mm Hg), CO (6.1 ± 0.4 to 7.1 ± 0.4 L/min), and SV (42 ± 3 to 52 ± 4 mL) (**Fig. 1**). FBT was also associated with a 16% decrease in hemoglobin concentration (from

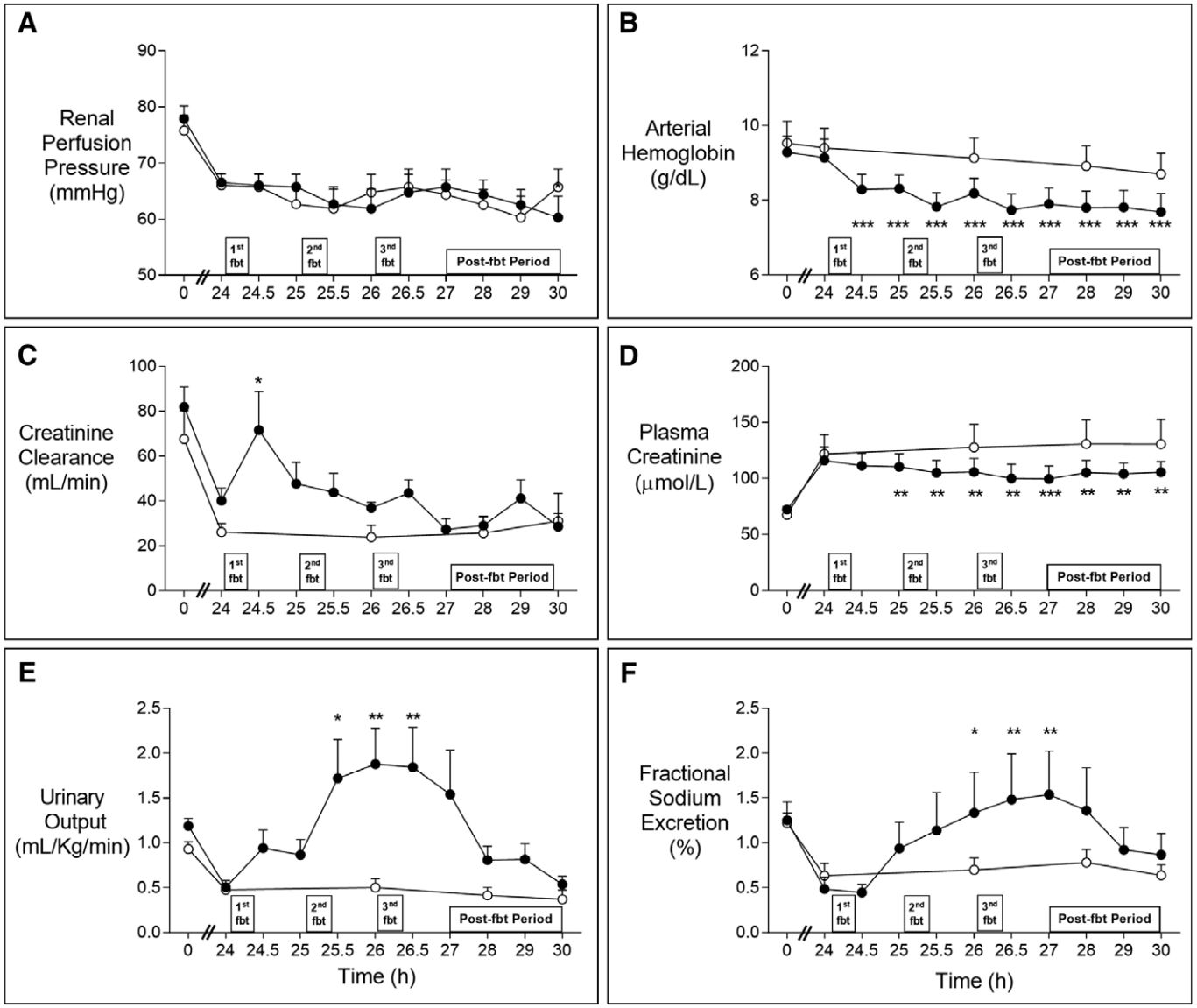

Figure 2. Renal perfusion pressure (**A**), arterial hemoglobin (**B**), creatinine clearance (**C**), plasma creatinine (**D**), urine output (**E**), and fractional excretion of sodium (**F**) at baseline and during infusion of *Escherichia coli* from 0 to 30 hr and treatment with three successive fluid bolus therapies (FBTs, 500 mL of balanced crystalloid, administered over 15 min at 2,000 mL/hr) ($n = 8$, *filled circles*) or vehicle-saline (1 mL/Kg/hr) (*open circles*, $n = 7$) in conscious sheep. Times, symbols, error bars, and *p* values are as for Figure 1.

9.1 ± 1.1 to 7.6 ± 0.4 g/dL) (Fig. 2*B*) and 45% decrease in arterial lactate (2.0 ± 0.6 to 1.1 ± 0.2 mmol/L; Online Data Supplement, Supplemental Digital Content 1, http://links.lww.com/CCM/E168). Systemic Do_2 remained unchanged (Fig. 3*E*) because of the progressive anemia (Fig. 2*B*). Despite an increase in MAP, there was no change in RPP because CVP increased (Fig. 2*A*). The initial increase in MAP rapidly dissipated, and it decreased to the same low levels as in time-control sheep, until a brief rise was induced by the third bolus after which MAP again returned to time-control values within 2 hours (Fig. 1*A*). In contrast, the modest increases in CO and SV, and marked increases in CVP, were sustained throughout the experimental period (Fig. 1).

Renal Effects of FBT

FBT did not affect global RBF, RVC, RDo_2, RVo_2, or renal oxygen extraction (Fig. 3). In contrast, FBT had selective, heterogeneous effects on different parts of the renal microcirculation. Although FBT had no effects on cortical tissue perfusion or oxygenation, it increased medullary tissue perfusion (482 ± 44 to 768 ± 116 BPU) and restored medullary (Po_2) to control levels (19 ± 4 to 42 ± 3 mm Hg) (Fig. 4). The changes in medullary oxygenation were mirrored by increases in bladder urinary Po_2, with a significant correlation between the two variables ($r^2 = 0.67$; $p < 0.01$) (**Fig. 5**). The improvements in renal medullary perfusion and oxygenation began with the first fluid bolus and were sustained (but not further increased) by subsequent boluses. However, they dissipated to pretreatment values by 2 hours after the last bolus (Fig. 4).

There were significant changes in renal function following FBT. Urinary output (0.50 ± 0.07 to 1.9 ± 0.41 mL/kg/min) and fractional excretion of sodium (0.5 ± 0.1 to 2.1 ± 0.9 %) increased to maximal levels after the third bolus; however,

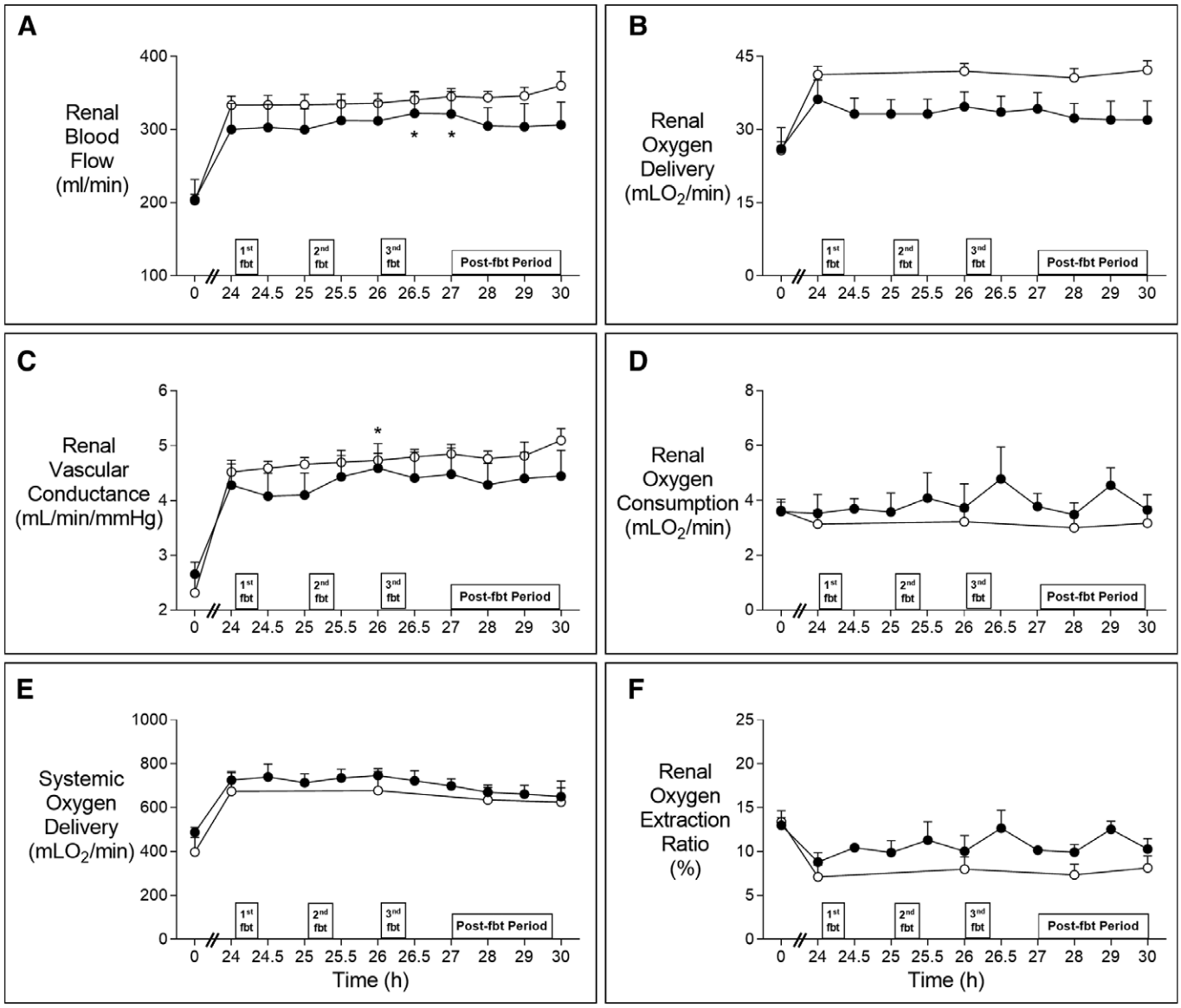

Figure 3. Renal blood flow (**A**) and vascular conductance (**C**), renal oxygen delivery (**B**), consumption (**D**) and extraction ratio (**F**), and systemic oxygen delivery (**E**) at baseline and during infusion of *Escherichia coli* from 0 to 30 hr and treatment with three successive fluid bolus therapies (FBTs, 500 mL of balanced crystalloid, administered over 15 min at 2,000 mL/hr) ($n = 8$, *filled circles*) or vehicle-saline (1 mL/Kg/hr) (*open circles*, $n = 7$) in conscious sheep. Times, symbols, error bars, and *p* values are as for Figure 1.

these changes dissipated after 2–3 hours (Fig. 2, *E* and *F*). Creatinine clearance (40 ± 6 to 72 ± 17 mL/min) increased immediately after the first bolus but then decreased with no further increases after either the second or third bolus (Fig. 2*C*), whereas plasma creatinine levels progressively decreased (116 ± 12 to 105 ± 10 µmol/L) (Fig. 2*D*). Finally, septic animals retained 69% ± 4% of the fluid and 80% ± 6% of the sodium administered (Online Data Supplement, Supplemental Digital Content 1, http://links.lww.com/CCM/E168).

DISCUSSION

In an experimental large animal model of sepsis, with a hemodynamic phenotype similar to early human septic AKI, we studied the effects of three successive boluses of balanced crystalloid solution on systemic and renal hemodynamics, renal function, and intrarenal tissue perfusion and oxygenation. These fluid boluses, of similar volume on a per kilogram basis to those used commonly in clinical practice, led to sustained increases in CO, CVP, and SV, but only a transient increase in MAP and no effects on RPP, global RBF, RDO_2, or cortical perfusion and oxygenation. However, FBT increased medullary perfusion and restored medullary PO_2 to normal levels, which was closely emulated by changes in bladder urinary PO_2. These increases in PO_2 were sustained during repeated FBT but dissipated shortly following the last bolus. Similarly, the transient increase in creatinine clearance occurred only after the first fluid bolus. Finally, although FBT increased urine output and fractional excretion of sodium, these effects also dissipated within 2 hours of the last bolus. Furthermore, fluid and sodium balance were markedly positive, and the progressive decline in plasma creatinine was

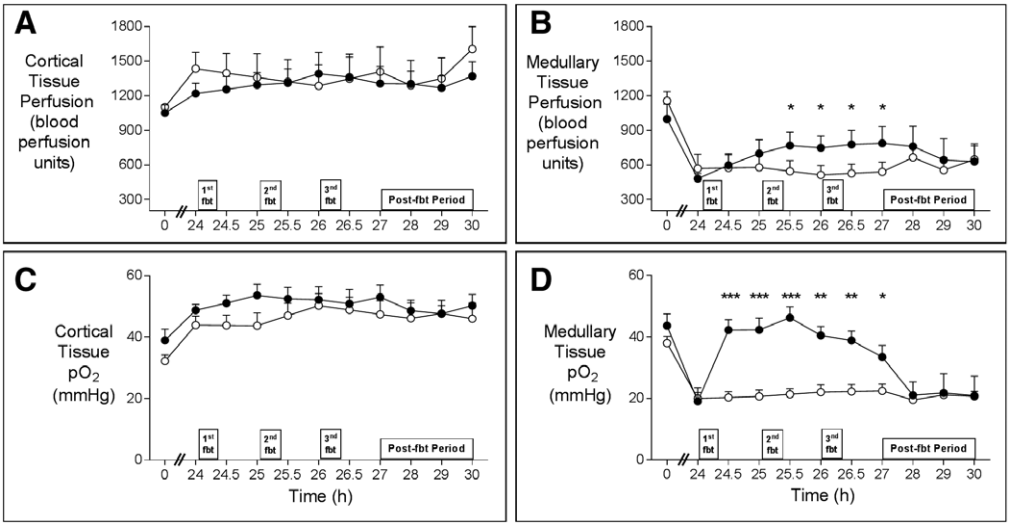

Figure 4. Cortical (**A**) and medullary (**B**) tissue perfusion and cortical (**C**) and medullary (**D**) tissue Po_2 at baseline and during infusion of *Escherichia coli* from 0 to 30 hr and treatment with three successive fluid bolus therapies (FBTs, 500 mL of balanced crystalloid, administered over 15 min at 2,000 mL/hr) ($n = 8$, *filled circles*) or vehicle-saline (1 mL/Kg/hr) (open circles, $n = 7$) in conscious sheep. Times, symbols, error bars, and p values are as for Figure 1.

dissociated from creatinine clearance and, instead, mirrored the progressive decrease in hemoglobin concentration over time.

Relationship to Previous Studies

At 24 hours of sepsis, prior to FBT, stage 1 AKI had developed according to the Kidney disease Improving Global Outcomes guidelines (23), consistent with our studies in ovine sepsis (12–14) and studies in septic patients where AKI develops within the first 24 hours in 64% of the cases (1). Histologic assessment of kidneys from human (24, 25) and ovine (26, 27) septic AKI demonstrate a general absence of acute tubular necrosis or extensive apoptosis. However, early onset of renal medullary hypoxia in sepsis could initiate a vicious cycle of oxidative stress and inflammation leading to mitochondrial dysfunction, tubular cell injury, and reduced kidney function (28–30). Ovine septic AKI is characterized by redistribution of intrarenal perfusion, with medullary ischemia and hypoxia but preserved cortical perfusion and oxygenation (12–14). The differences between the sepsis-induced changes in intra-RBF in sheep compared with those reported in other species is likely due to the effect of anesthesia in other studies, since we have shown that general anesthesia causes a large, 50%, reduction in RBF (31).

We recently reported that in ovine sepsis reductions in medullary oxygenation precede the increases in serum creatinine and urinary neutrophil gelatinase-associated lipocalin, by at least 8 hours (14), and the renal dysfunction by 12 hours (12). In the present study, FBT effectively restored medullary oxygenation in septic sheep, but this dissipated within 2 hours of the last bolus, and it failed to induce any sustained improvements in renal function. These findings suggest that although medullary hypoxia early in sepsis may contribute to the development of AKI, in established sepsis mechanisms independent of hypoxia may sustain the AKI (32). The lack of a sustained improvement in renal function following FBT is in accord with a study evaluating the effects of protocolized EGDT on renal functional indices in septic patients, which also found no effect of FBT on the development or progression of AKI (33). The fluid-induced reductions in renal sympathetic nerve activity (34–36) and components of the renin-angiotensin system (37) may contribute to the natriuresis and increased perfusion and oxygenation in the renal medulla, independent of changes in RPP and creatinine clearance.

We found a positive correlation in the pattern of responses between urinary Po_2 and renal medullary tissue Po_2 in response to FBT, confirming previous findings that urinary Po_2 may be a useful surrogate measure of medullary oxygenation. Similar observations have been reported in human septic AKI, where urinary hypoxia was ameliorated by fluid therapy (38). Similar correlations between medullary and urinary Po_2 have also been observed in sheep (13, 14), rabbits (39), and humans (38) in response to vasoactive drugs. Finally, FBT may delay the recognition and progression of AKI in patients due to the dilution of creatinine in plasma (40). Accordingly, our findings of a progressive reduction in plasma creatinine in response to FBT despite a decrease in creatinine clearance suggest a dilutional effect of a positive fluid balance (41).

Study Strengths and Limitations

Our study has several strengths. Our animal model closely replicates the clinical phenotype of early human septic AKI (3, 42). The study is a comprehensive, temporal assessment of systemic and global renal hemodynamics and function, as well as intrarenal perfusion and oxygenation and the relationship between medullary and urinary Po_2 during repeated FBT. FBT was administered at a clinically appropriate time-point, when at least two clinical triggers for fluid resuscitation were present (i.e., HR > 90 beats/min; MAP < 75 mm Hg; CVP < 10 mm Hg; urinary output < 0.5 mL/Kg/hr), as reported in the Saline versus Albumin Fluid Evaluation (SAFE) Study (20) and Crystalloid versus Hydroxyethyl Starch Trial (CHEST) (21). The volume of fluid administered (1,500 mL in 40 Kg sheep) was similar on a per kilogram basis (3,000 mL in 80 Kg humans) to that commonly administered in clinical practice (4–6). We studied unanesthetized sheep to remove the confounding effects of anesthesia, which significantly reduces RBF (31). However, as with any experimental studies, caution must be exercised when extrapolating our findings to the clinical setting. In contrast to human sepsis, our sheep did not have preexisting comorbidities. Five of the sheep with more severe hemodynamic and metabolic deterioration reached ethical endpoint criteria and were euthanized

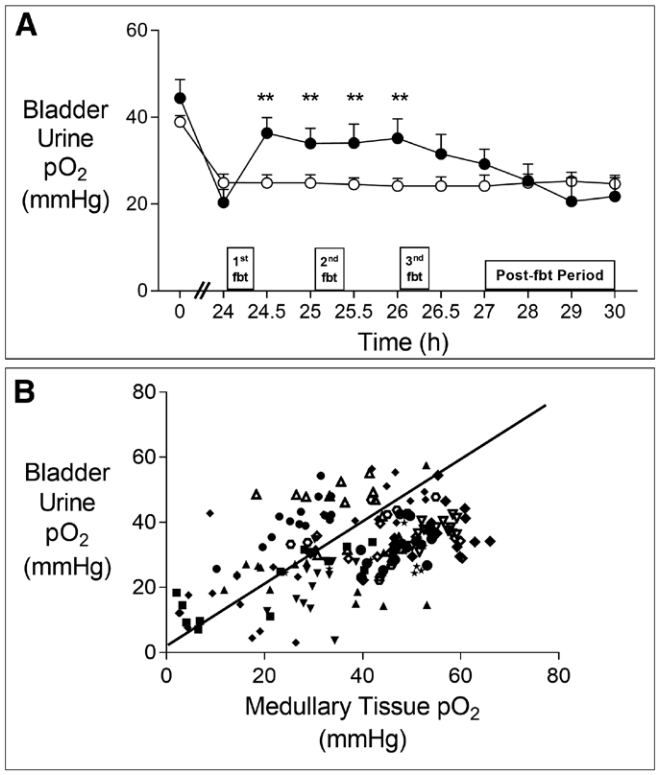

Figure 5. A, Bladder urinary oxygen tension at baseline and during infusion of *Escherichia coli* from 0 to 30 hr and treatment with three successive fluid bolus therapies (FBTs, 500 mL of balanced crystalloid, administered over 15 min at 2,000 mL/h) ($n = 8$, *filled circles*) or vehicle-saline (1 mL/Kg/hr) (*open circles*, $n = 7$) in conscious sheep. Times, symbols, error bars, and p values are as for Figure 1. **B**, Scatterplot of the relationship between medullary tissue Po_2 and urinary Po_2 in septic sheep treated with three successive 500 mL boluses of balanced crystalloid (2,000 mL/hr; $n = 8$) or vehicle-saline (1 mL/Kg/hr; $n = 7$). Data for 15 different sheep are shown by different symbols (FBT group: *closed symbols*; vehicle-saline: *open symbols*). The line of best fit, determined by ordinary least-product regression analysis, had a slope of 0.75 with a 95% CI (0.64–0.87) and an X intercept of 3.09 mm Hg (−1.57 to 7.75 mm Hg) ($r^2 = 0.67$; $p < 0.001$).

prior to study completion, so the remaining animals available for assessment did not fulfill the Sepsis-3 consensus criteria. However, sustaining sepsis over 30 hours to increase blood lactate to greater than 2 mmol/L to fulfill such criteria, in conscious animals without sedation or anesthesia, is challenging and would result in an ethically unacceptable loss of study animals. Our study was confined to the assessment of systemic and renal hemodynamics, intrarenal perfusion and oxygenation, and kidney function in response to FBT over a 6-hour period. Thus, we cannot comment on any longer term renal effects of repeated FBT and its associated improvements in medullary oxygenation. However, all systemic, intrarenal and renal functional effects of FBT had dissipated by the end of the observation period, making it unlikely that differences would be seen if the observation period had been extended for another 24 hours.

CONCLUSIONS

Our findings indicate that in established ovine septic AKI, FBT caused effective hemodynamic resuscitation as indicated by sustained increases in CO, CVP, and SV, but the improvement in MAP was only transient. Interestingly, although FBT did not change RPP, global RBF, RDo_2, cortical perfusion or cortical Po_2, it restored medullary Po_2 to healthy levels, although this increase waned shortly after the final fluid bolus. Creatinine clearance increased only after the first fluid bolus, despite medullary Po_2 being maintained at normal levels throughout the three fluid boluses. We also demonstrated that during FBT, there was a close correlation between renal medullary tissue and bladder urinary Po_2. Following FBT, there was a positive fluid balance resulting in a dilutional reduction in plasma creatinine, despite a decrease in creatinine clearance. These findings imply that in early experimental septic AKI, repeated FBT does not cause an extended improvement in renal function; however, additional studies are required to define the impact of additional fluid boluses or of a continuous infusion.

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