Renal Effects of Saline-based 10% Pentastarch versus 6% Tetrastarch Infusion in Ovine Endotoxemic Shock


ABSTRACT
Background: Conflicting data exist on the renal effects of hydroxyethyl starch (HES) preparations. The current study evaluates the effects of saline-based 6% HES 130/0.4, 10% HES 200/0.5, and a balanced isotonic crystalloid on renal function and microscopic changes in ovine endotoxemic shock.

Methods: Thirty sheep were subjected to endotoxin infusion (Salmonella typhosa) at incremental doses until mean arterial pressure was less than 65 mmHg. Animals were randomized to receive fluid resuscitation with saline-based 6% HES 130/0.4, 10% HES 200/0.5, or a balanced isotonic crystalloid (n = 10 each). Animals surviving the 12-h intervention period were anesthetized and killed. Kidney samples were taken for microscopic analyses.

Results: Endotoxemia was associated with hemoconcentration, protein extravasation, and arterial hypotension. Fluid resuscitation established a hypotensive–hyperdynamic circulation with increased cardiac index and oxygen delivery and decreased afterload. Diuresis was lowest in animals treated with 10% HES 200/0.5. In addition, plasma creatinine and urea concentrations increased in sheep treated with 10% HES 200/0.5 (1.2 ± 0.1 and 19 ± 2 mg/dl) when compared with the other two groups (0.9 ± 0.1 and 15 ± 1 mg/dl, 6% HES 130/0.4; 0.9 ± 0.1 and 15 ± 1 mg/dl, crystalloids; each P < 0.05). Electron microscopic tubular injury score was highest in sheep treated with 10% HES 200/0.5 (P < 0.001 vs. 6% HES 130/0.4).

Conclusions: In ovine endotoxemic shock, saline-based 10% HES 200/0.5 was linked to impaired renal function and more pronounced tubular epithelial injury when compared with 6% HES 130/0.4 and balanced crystalloids.

What We Already Know about This Topic
- Starch-based colloid solution use during septic shock may be associated with renal dysfunction.
- It is unknown whether newer solutions of low substituted starch colloids (6% HES 130/0.4) also produce renal dysfunction.

What This Article Tells Us That Is New
- In ovine endotoxemic shock, 6% HES 130/0.4 produces less anatomic and functional renal injury than an older starch colloid (10% HES 200/0.5)

ADVERSE fluid resuscitation represents one of the key features of early hemodynamic optimization in patients with severe sepsis and septic shock.1 Although colloids reduce the total amount of fluids needed to achieve hemodynamic stabilization when compared with sole crystalloid infusion,2 it is still unclear whether crystalloids or colloids should be preferred in this indication. Previous clinical studies report negative renal effects associated with the infusion of “classic” hydroxyethyl starches (HES; 10% HES 200/0.5 = pentastarch or 6% HES 200/0.60 = hexastarch) in patients with sepsis. These adverse events include higher rates of acute renal failure and requirements for renal replacement therapy.3,4 Notably, these studies exclusively used HES preparations typically accumulating in the plasma after repetitive use and/or markedly exceeded the clinically recommended maximum doses.3,4 In addition, the exact pathomechanism of starch-induced renal injury is still unknown.5

The renal effects of modern, low-substituted, and medium–molecular weight preparations, that is, 6% HES 130/0.4 (tetrastarch), in the presence of severe sepsis have not yet been investigated. Likewise, few studies have directly compared the effects of HES 200/0.5, HES 130/0.4, and balanced crystalloids (acetate/malate based) on renal function,6,7 and none of these was conducted in the setting of sepsis or systemic inflammation.
Although 6% HES 130/0.4 has been suggested to be safe in patients who underwent nonseptic cardiac surgery, even in the presence of preexisting renal dysfunction, the recently published VISEP trial demonstrated that 10% HES 200/0.5 dose-dependently impaired renal function. In addition, a recent prospective observational study suggested that hyperoncotic colloids may increase the risk of renal failure in patients with shock.

We hypothesized that 10% HES 200/0.5 impairs renal function and tubular integrity when compared with 6% HES 130/0.4 or balanced crystalloids. The objective of the current study was, therefore, to compare the effects of saline-based 10% HES 200/0.5 (the study drug of the VISEP trial), saline-based 6% HES 130/0.4 (a modern third-generation tetrastarch), and a commonly used balanced, isotonic crystalloid on hemodynamics, colloid-osmotic pressure, surrogate variables of renal function, and light and transmission electron microscopic renal tubular injury, using an established and clinically relevant ovine model of endotoxic shock.

**Materials and Methods**

**Animals**

After approval by the Local Animal Care Committee (State Office for Nature, Environment, and Consumer Protection, Recklinghausen, Germany), 30 healthy adult ewes were chronically instrumented with strict adherence to the National Institutes of Health’s Guide and the American Physiologic Society’s Guide for the Care and Use of Laboratory Animals using an established protocol.

**Anesthesia and Instrumentation**

Induction of anesthesia was performed by intramuscular injection of S-ketamine (Ketanest® S, 10 mg/kg; Parke-Davis, Berlin, Freiburg, Germany) and midazolam (Dormicum®, 0.3 mg/kg; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany). After catheterization of a peripheral vein, ceftriaxone (1 g ceftriaxone; Rocephin®, Hoffmann-La Roche Germany). After catheterization of a peripheral vein, ceftriaxone (1 g; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany) and midazolam (Dormicum®, 0.3 mg/kg; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany) were administered intravenously as perioperative infection prophylaxis. Anesthesia was maintained using a continuous intravenous propofol infusion (Disopivran®, 4–8 mg · kg⁻¹ · h⁻¹; AstraZeneca, Schwetzingen, Germany). The ewes remained unconscious but were spontaneously breathing during the entire instrumentation period. All punctures were performed under sterile conditions after local anesthesia with 2% mepivacain (Scandicain® 2%; AstraZeneca GmbH, Wedel, Germany). An indwelling pulmonary artery catheter was inserted via the right jugular vein through an introducer sheath (7.5 French, Edwards Swan Ganz, Edwards Critical Care Division, Irvine, CA; 8.5 French, Catheter Introducer Set, pvb Medizintechnik GmbH, Kirchseeon, Germany). In addition, sheep were instrumented with a left femoral arterial catheter (18-gauge Leader Cath; Vygon, Aachen, Germany) and a Foley catheter (12 French, urinary catheter, Porgès S.A., Le Plessis Robinson-Cedex, France) to monitor arterial blood pressure and urinary output, respectively.

After the instrumentation, intravascular catheters were connected to a physiologic recorder (Hellige Servomed, Hellige, Freiburg, Germany) via pressure transducers (DTX pressure transducer, Ohmeda, Erlangen, Germany). The instrumentation was followed by a 24-h period of recovery. During this time, the sheep that were awake were housed in metabolic cages with free access to water and food until baseline measurements were performed.

**Hemodynamic and Oxygen Transport Variables**

Hemodynamic monitoring included mean arterial pressure (MAP), mean pulmonary arterial pressure, central venous pressure (CVP), and pulmonary arterial occlusion pressure. Heart rate was determined by calculating the mean frequency of arterial pressure curve peaks. The thermodilution technique (9520A cardiac output computer; Edward Lifescience, Irvine, CA) was applied to measure cardiac output by three-fold central venous injection of 10 ml of isotonic saline solution at a temperature of 2°C–5°C. Cardiac index (CI), systemic vascular resistance index, pulmonary vascular resistance index, stroke volume index, and left and right ventricular stroke work indices were determined using standard equations. Core body temperature was continuously measured by the thermistor positioned at the tip of the pulmonary artery catheter.

Arterial and mixed venous blood samples (0.5 ml each) were collected in heparinized tubes designed to determine blood gases (Sarstedt; Nümbrecht, Germany). Potentia hydrogenii (pH), and partial pressures of oxygen and carbon dioxide (PO₂ and PCO₂, respectively) were determined using an ABL 725 blood gas analyzer with SAT 100 calibration (Radiometer Copenhagen; Copenhagen, Denmark). In addition, hemoglobin concentration, hematocrit, arterial and mixed-venous oxygen saturation (SaO₂ and SvO₂, respectively), and arterial lactate concentrations were assessed. Oximetry-corrected base excess (BEₑₒ₉) was calculated from hemoglobin concentration, Pco₂, pH, and SaO₂. Systemic oxygen delivery index (DO₂I), oxygen consumption index, and oxygen extraction rate were determined by using standard formulae.

**Laboratory Analyses**

At specific time points (see Experimental Protocol), arterial blood (7.5 ml of lithium heparinate blood) was withdrawn and immediately centrifuged at 3,000 rpm for 10 min. The isolated plasma and urine samples (3 ml) were then immediately stored at −70°C for determination of creatinine, urea, electrolyte, and total protein concentrations (Hitachi 747 automatic analyzer, Roche Diagnostics GmbH, Mannheim, Germany) at a later time point (see Experimental Protocol). Plasma colloid osmotic pressure (COP) was measured using a colloid osmometer (Colloid Osmometer; Knauer, Berlin, Germany).
Experimental Protocol

Inclusion criteria for the current study were an initial heart rate less than 100 beats/min, core body temperature less than or equal to 39.8°C (normal range for sheep 38.5°C–39.8°C), mean pulmonary arterial pressure less than 20 mmHg, and arterial lactate less than or equal to 1 mmol/L. During the experimental protocol, all ewes were spontaneously breathing room air and were studied in a conscious state.

After a baseline measurement in the healthy state (BL1), 4 ml·kg⁻¹·h⁻¹ of a balanced, isotonic crystalloid solution containing acetate/malate buffer (Sterofundin ISO, B. Braun Melsungen, Germany) were continuously infused to compensate for basal fluid requirements, because balanced electrolyte solutions (rather than isotonic saline) represent the standard of care for basal fluid substitution in Germany and other parts of Europe. In addition, all animals received a continuous infusion of 100 mg of Salmonella typhosa endotoxin (Sigma Chemicals, Deisenhofen, Germany, Catalogue number L6386) starting at a rate of 5 ng·kg⁻¹·min⁻¹. The endotoxin infusion rate was doubled every hour until MAP decreased less than 65 mmHg (shock time). At this time, the baseline measurement in endotoxemia (BL2) was performed, and endotoxin infusion was maintained at the dosage determined at shock time. Sheep were then randomly allocated to one of the three study groups, that is, 6% HES 130/0.4, 10% HES 200/0.5, and crystalloid (each n = 10). The 6% HES 130/0.4 and 10% HES 200/0.5 groups received volume resuscitation with repeated bolus infusions of 5 ml/kg saline-based 6% HES 130/0.4 (Voluven 6%; Fresenius Kabi, Bad Homburg, Germany) or saline-based 10% HES 200/0.5 (Hemohes 10%; B. Braun Melsungen), respectively. The crystalloid group received repeated bolus infusions of 10 ml/kg of Sterofundin ISO. Fluid resuscitation was performed according to the recommendations of the current sepsis guidelines, aiming to maintain CVP at 8–12 mmHg and pulmonary arterial occlusion pressure at 12–15 mmHg. After infusion of the maximum colloid dose of 20 ml/kg (i.e., recommended maximum dose for Hemohes 10%), only crystalloids were infused in all study groups. If MAP was less than predefined threshold values of 70 ± 5 mmHg despite fluid therapy, norepinephrine (Arterenol®; Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany) was continuously administered as a titrated infusion to achieve threshold values. Hemodynamic measurements and blood gas analyses were performed hourly. In addition, blood and urine samples were withdrawn for laboratory analyses at BL1 and BL2 and at 2, 4, 8, and 12 h after randomization.

Histologic Analyses

Animals surviving the 12-h intervention period were deeply anesthetized with propofol (4 mg/kg) and killed with a lethal dose of 100 ml of potassium chloride solution (7.45%). Subsequently, an autopsy was performed, and tissue samples from the left kidney were taken and fixated in 3.7% formaldehyde solution. At a later time point, kidney samples were stained with hematoxylin-eosin and analyzed by a pathologist, being unaware of the study protocol and grouping. Tissue injury was quantified using a modified score originally described by Cox et al., where 1 = normal tissue and 10 = necrosis.

**Transmission Electron Microscopy**

Kidney tissue samples were fixed for 12 h at 4°C in 3% cacodylate-buffered glutaraldehyde (pH 7.35) and then transferred into 5% sucrose for electron microscopy. Postfixation was performed with 1% osmium tetroxide and 50 mM potassium ferricyanide. Specimens were then washed with distilled water, dehydrated in graded alcohols, and embedded in araldite resin. The ultrathin sections (50 nm) were cut and placed on copper grids. The sections were stained with 5% uranyl acetate and 0.2% lead citrate. Transmission electron microscopic examination was performed using a Philips CM10 electron microscope (Philips, Eindhoven, The Netherlands) operating at 80 kV. Because there is no established quantitative scoring system for tubular injury in acute renal failure, kidney samples were analyzed as follows: in each of the 30 study animals, tubules of 10 fields of view were scored according to the following criteria by a pathologist unaware of the study protocol and group assignment: (1) vacuolar degeneration and swelling of organelle (0 = none; 1 = occasional; 2 = moderate; and 3 = ubiquitous), (2) dissociation of epithelium and basal membrane (0 = none; 1 = single cells; 2 = more than half of tubule; and 3 = whole tubule), (3) epithelial cell injury (0 = none; 1 = moderate; 2 = severe; and 3 = complete destruction), and (4) intratubular precipitation (0 = none; 1 = moderate protein precipitation; 2 = lumen obstructed by protein; and 3, intratubular cell organella). The sum of the four criteria was quantified as renal tubular injury score.

**Study Endpoints**

The study hypothesis and statistical analysis were two tailed. Primary study endpoints were differences in urinary output, plasma creatinine, and urea concentrations among study groups. Secondary endpoints included total volume requirements and electron microscopic tubular injury.

**Statistical Analysis**

Data are expressed as means ± SEM. Sigma Stat 3.10 software (Systat Software Inc., Chicago, IL) was used for statistical analysis. After confirming normal distribution of all variables (Kolmogorov-Smirnov test), overall differences between groups over the whole study period were analyzed using one-way ANOVA. Only if significant overall differences were detected, a two-way ANOVA for repeated measurements with group and time as factors was performed to analyze the different time points within and between groups using appropriate post hoc comparisons (Student-Newman-Keuls test). Time-independent variables and microscopic scores were analyzed by one-way ANOVA or one-way ANOVA on ranks, as appropriate, and post hoc comparisons (Student-Newman-Keuls test) were performed in case of significant overall differences. Correlation between electron mi-
croscopic scores and diuresis was tested using the Pearson Product Moment Correlation formula. For all statistical tests, an error probability of $P < 0.05$ was considered as statistically significant.

**Results**

There were no significant differences among groups in any of the investigated variables at BL1 and BL2.

<table>
<thead>
<tr>
<th>HES 130/0.4</th>
<th>HES 200/0.5</th>
<th>Crystalloid</th>
<th>One-way ANOVA</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>41.5 ± 1.6</td>
<td>41.1 ± 1.3</td>
<td>40.6 ± 1.4</td>
<td>0.91</td>
</tr>
<tr>
<td>LPS dose, ng · kg$^{-1}$ · min$^{-1}$</td>
<td>160 [80; 160]</td>
<td>160 [80; 160]</td>
<td>160 [160; 160]</td>
<td>0.11</td>
</tr>
<tr>
<td>Time to shock onset, h</td>
<td>5.3 ± 0.3</td>
<td>5.9 ± 0.6</td>
<td>5.8 ± 0.3</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Data are presented as median (range 25–75% quartiles) for lipopolysaccharide (LPS) dose and means ± SEM for body weight and time to shock onset.

**Body Weight, Endotoxin Dose, and Shock Time**

Body weight, lipopolysaccharide dose, and time to the onset of shock were comparable between study groups (each $P > 0.05$; table 1).

**Hemodynamic and Oxygen Transport Variables**

The effects of endotoxin on cardiopulmonary and global oxygen transport variables are depicted in figure 1 and table 2, respectively. Endotoxin infusion was associated with in-

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Fig. 1. Hemodynamic effects of volume therapy in endotoxemic sheep. Data are presented as means ± SEM. BL = baseline; $DO_2I =$ systemic oxygen delivery index; HES = hydroxyethyl starch; HR = heart rate; MAP = mean arterial pressure; $SVI =$ stroke volume index. * $P < 0.05$ HES 130/0.4 versus HES 200. † $P < 0.05$ HES 200/0.5 versus crystalloid. ‡ $P < 0.05$ HES 130/0.4 versus crystalloid.

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Table 2. Hemodynamic Effects of Volume Therapy in Endotoxemic Sheep

<table>
<thead>
<tr>
<th>Variable</th>
<th>BL1</th>
<th>BL2</th>
<th>2 h</th>
<th>4 h</th>
<th>8 h</th>
<th>12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals alive, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HES 130/0.4</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>9 (90)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>HES 200/0.5</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>9 (90)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Crystalloid</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Cl, l·min⁻¹·m⁻²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HES 130/0.4</td>
<td>5.3 ± 0.2</td>
<td>5.4 ± 0.5</td>
<td>8.4 ± 0.8</td>
<td>7.1 ± 0.7</td>
<td>9.5 ± 0.4</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td>HES 200/0.5</td>
<td>5.6 ± 0.3</td>
<td>4.9 ± 0.4</td>
<td>7.7 ± 0.3</td>
<td>5.9 ± 0.5</td>
<td>7.2 ± 0.7</td>
<td>6.2 ± 0.4</td>
</tr>
<tr>
<td>Crystalloid</td>
<td>5.3 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>8.3 ± 0.6</td>
<td>5.9 ± 0.5</td>
<td>7.2 ± 0.5</td>
<td>8.3 ± 0.6</td>
</tr>
<tr>
<td>CVP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HES 130/0.4</td>
<td>6 ± 0</td>
<td>4 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 2</td>
<td>12 ± 1</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>HES 200/0.5</td>
<td>6 ± 0</td>
<td>4 ± 1</td>
<td>13 ± 1</td>
<td>14 ± 2</td>
<td>11 ± 2</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Crystalloid</td>
<td>5 ± 1</td>
<td>3 ± 1</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>PAOP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HES 130/0.4</td>
<td>10 ± 1</td>
<td>8 ± 0</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>HES 200/0.5</td>
<td>9 ± 0</td>
<td>9 ± 0</td>
<td>13 ± 1</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Crystalloid</td>
<td>9 ± 1</td>
<td>8 ± 1</td>
<td>12 ± 1</td>
<td>13 ± 1</td>
<td>12 ± 1</td>
<td>12 ± 0</td>
</tr>
<tr>
<td>SVRI, dyn·s⁻¹·cm⁻⁵·m⁻²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HES 130/0.4</td>
<td>1293 ± 52</td>
<td>933 ± 71</td>
<td>617 ± 72</td>
<td>737 ± 75</td>
<td>523 ± 23</td>
<td>642 ± 76</td>
</tr>
<tr>
<td>HES 200/0.5</td>
<td>1290 ± 116</td>
<td>1022 ± 80</td>
<td>642 ± 38</td>
<td>898 ± 80</td>
<td>769 ± 92</td>
<td>818 ± 62</td>
</tr>
<tr>
<td>Crystalloid</td>
<td>1319 ± 62</td>
<td>954 ± 40</td>
<td>639 ± 62</td>
<td>945 ± 83</td>
<td>743 ± 81</td>
<td>604 ± 44</td>
</tr>
<tr>
<td>MPAP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HES 130/0.4</td>
<td>17 ± 1</td>
<td>24 ± 2</td>
<td>28 ± 1</td>
<td>30 ± 2</td>
<td>31 ± 2</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>HES 200/0.5</td>
<td>18 ± 1</td>
<td>27 ± 2</td>
<td>31 ± 1†</td>
<td>31 ± 2</td>
<td>31 ± 2</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>Crystalloid</td>
<td>16 ± 1</td>
<td>21 ± 1</td>
<td>25 ± 1†</td>
<td>28 ± 1</td>
<td>26 ± 2</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>PVRI, dyn·s⁻¹·cm⁻⁵·m⁻²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HES 130/0.4</td>
<td>123 ± 16</td>
<td>249 ± 43</td>
<td>156 ± 31</td>
<td>206 ± 42</td>
<td>147 ± 11</td>
<td>212 ± 14</td>
</tr>
<tr>
<td>HES 200/0.5</td>
<td>130 ± 10</td>
<td>220 ± 41</td>
<td>192 ± 13</td>
<td>230 ± 20</td>
<td>225 ± 35</td>
<td>220 ± 37</td>
</tr>
<tr>
<td>Crystalloid</td>
<td>113 ± 10</td>
<td>203 ± 19</td>
<td>133 ± 12</td>
<td>225 ± 23</td>
<td>170 ± 31</td>
<td>142 ± 13</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM.
* P < 0.05 6% hydroxyethyl starch (HES) 130/0.4 versus 10% HES 200/0.5. † P < 0.05 10% HES 200/0.5 versus crystalloid.

**BL = baseline; CI = cardiac index; CVP = central venous pressure; MPAP = mean pulmonary arterial pressure; PAOP = pulmonary arterial occlusion pressure; PVRI = pulmonary vascular resistance index; SVRI = systemic vascular resistance index.**

Increases in heart rate, mean pulmonary arterial pressure, and pulmonary vascular resistance index and reductions in CVP, left ventricular stroke work index, MAP, stroke volume index, and systemic vascular resistance index (each P < 0.001 BL2 vs. BL1). Volume resuscitation was associated with an increase in CVP, pulmonary arterial occlusion pressure, MAP, CI, and DO₂I in all three study groups (each P < 0.05 at 8 h vs. BL2). DO₂I was lowest in the HES 200/0.5 group when compared with the other two groups (P = 0.02 vs. HES 130/0.4; P = 0.04 vs. crystalloid). In addition, there was a strong trend toward a higher oxygen extraction rate in the HES 200/0.5 group when compared with the crystalloid group (P = 0.05). Sao₂ and Svo₂ were similar among groups (table 3).

**Volume Requirements**

Volume requirements of the three study groups are displayed in figure 2. The maximum dose of colloids was infused after 4 h in both HES-treated groups. Thus, from 4–12 h, only crystalloids were infused in all study groups. HES-treated animals required significantly less total fluids to achieve the predefined cardiac filling pressures when compared with the crystalloid group (P = 0.03, HES 200/0.5 vs. crystalloid, P = 0.04, HES 130/0.4 vs. crystalloid). In addition, crystalloid requirements were lower in the two saline-based HES groups when compared with the balanced crystalloid group (P = 0.002, HES 200/0.5 vs. crystalloid, P = 0.02, HES 130/0.4 vs. crystalloid).

**Norepinephrine Requirements**

There were no overall differences among groups in norepinephrine requirements over the whole study period (0.32 ± 0.07, 0.34 ± 0.08, and 0.37 ± 0.07 μg·kg⁻¹·min⁻¹ in the HES 130/0.4, HES 200/0.5, and crystalloid groups, respectively; P = 0.52, HES 130/0.4 vs. HES 200/0.5; P = 0.27, HES 130/0.4 vs. crystalloid; P = 0.28, HES 200/0.5 vs. crystalloid).

**COP and Plasma Protein Concentration**

Endotoxin infusion was linked to a significant decrease in total plasma protein concentration and COP in all three groups (each P < 0.001 BL2 vs. BL1; fig. 3). There was no overall difference between groups in total plasma protein concentrations. Within the initial 4 h of volume resuscitation...
Core Body Temperature, Electrolytes, and Acid–Base Balance

Core body temperature and arterial lactate increased (each P < 0.001 BL2 vs. BL1), whereas BEox decreased (P = 0.04, BL2 vs. BL1) in response to endotoxin infusion. The decrease in BEox was more pronounced in saline-based HES 200/0.5—than in saline-based HES 130/0.4—(P = 0.04, HES 130/0.4 vs. HES 200/0.5) and balanced crystalloid-treated sheep (P = 0.08, crystalloid vs. HES 200/0.5). Although plasma sodium concentrations did not significantly change, chloride concentrations increased with time in all groups (BL2 vs. 12 h: P < 0.001 in the HES 130/0.4 and crystalloid group; P = 0.03 in HES 200/0.5; table 3). However, there were no differences between groups at any time point.

Renal Function

Surrogate variables of renal function are depicted in figure 4. Plasma creatinine concentrations significantly increased, and
creatinine clearance decreased after endotoxin infusion in all groups (each \( P < 0.001, \) BL2 vs. BL1). However, urinary output was lower during the initial 7 h of volume resuscitation in the saline-based HES 200/0.5 group when compared with the saline-based HES 130/0.4 (\( P = 0.04 \) at 7 h) and the balanced crystalloid group (\( P = 0.03 \) at 7 h). Plasma creatinine concentrations were higher in HES 200/0.5-treated sheep than in HES 130/0.4 over the entire intervention period (\( P = 0.02 \)). In addition, plasma creatinine concentrations were higher in the saline-based HES 200/0.5 than in the balanced crystalloid group from 4–8 h (\( P = 0.02 \) at 8 h). Plasma urea concentrations significantly increased in all study groups (each \( P < 0.001, \) BL2) and were significantly higher in the HES 200/0.5 group when compared with the HES 130/0.4 (\( P = 0.03 \)) and the crystalloid group (\( P = 0.001 \)) after 8 h. Creatinine clearance was 62% higher in the saline-based HES 130/0.4 group and 58% higher in the balanced crystalloid group than in the saline-based HES 200/0.5 group after 4 h of treatment. However, this trend was not statistically significant.

**Survival**
Six of 10 animals in the saline-based HES 130/0.4 and the balanced crystalloid group survived the 12-h intervention period when compared with 3 of 10 sheep in the saline-based HES 200/0.5 group (n.s., \( P = 0.34 \)).

**Histologic Examination**
Light microscopy of the kidney revealed acute tubular cell injury with intraluminal protein precipitation. These alterations were equally distributed among groups without significant differences (see fig. 5 for illustrative examples).

**Transmission Electron Microscopy**
Renal tubular injury score was inversely correlated with the diuresis rate determined during the last hour of the protocol \( r = -0.403; \) \( P = 0.03 \). Renal tubular injury score was higher in sheep treated with saline-based 10% HES 200/0.5 or sole balanced crystalloids when compared with saline-based 6% HES 130/0.4. In addition, intratubular precipitations and cellular injury were most pronounced after treatment with saline-based 10% HES 200/0.5 (table 4; fig. 6).

**Discussion**
The key finding of the current study is that in the setting of ovine endotoxemic shock, infusion of saline-based 10% HES 200/0.5, when given in pharmaceutically recommended doses, was associated with significantly impaired renal function and tubular integrity, whereas infusion of saline-based 6% HES 130/0.4 and a balanced crystalloids was not linked to such alterations.

Data were collected in an established large animal model of endotoxemic shock that closely reflects hemodynamic and metabolic derangements typically seen in the early stage of human septic shock. The observed alterations in response to endotoxin challenge are in accordance with previous data of our study group and others.
In the current study, all sheep suffered from vasodilatory shock (as indicated by reduced systemic vascular resistance index and MAP, and increased CI) and absolute hypovolemia (as reflected by increases in hematocrit before volume resuscitation). The reductions in COP and total plasma protein concentrations were indicative of endothelial barrier dysfunction with extravasation of plasma proteins.20,21 The increases in arterial lactate concentrations and decreases in BEox can best be explained by impaired tissue perfusion in response to arterial hypotension.22 These alterations were associated with increased surrogate markers of renal injury, suggesting the presence of organ dysfunction.23,24

Volume resuscitation was initiated as soon as MAP decreased less than the threshold values of 65 mmHg. Fluid therapy was guided to optimize CVP and pulmonary arterial occlusion pressure.15 Although the latter approach is recommended by the current sepsis guidelines,15 dynamic markers of volume responsiveness (i.e., variations in stroke volume or pulse pressure) may be more accurate variables to guide volume therapy. However, the latter dynamic markers are only reliable in mechanically ventilated subjects without spontaneous breathing activity,25 unlike in the current study. Passive leg lifting seems to be the most suitable method in awake, spontaneously breathing subjects,26 but it could not be applied in the current study because of the anatomy of sheep. Thus, filling pressures were chosen to guide resuscitation. Volume resuscitation established hemodynamic goal values in all groups within 1 h and contributed to a hyperdynamic circulation characterized by a low systemic vascular resistance index and an increase in CI,27 which resulted in higher DO2I in the HES 130/0.4 when compared with the HES 200/0.5 group. Reduced BEox values in the saline-based HES 200/0.5 group suggest that the reduction in DO2I may have negatively impacted on tissue oxygenation. This assumption is supported by a study of Chiara et al.,22 showing that base excess is a suitable marker of tissue hypoperfusion after hemorrhage in pigs. Marx et al.20 also conducted experiments in this area and reported similar hemodynamic differences after...
In the current study, administration of both saline-based 6% HES 130/0.4 and 10% HES 200/0.5 resulted in a significantly higher COP when compared with balanced crystalloid resuscitation. This may be explained by the different \textit{in vitro} COP of the compounds. Although 6% HES 130/0.4 is isooncotic (COP = 34–36 mmHg) and 10% HES 200/0.5 is hyperoncotic (COP = 60–80 mmHg), crystalloids are hypooncotic (COP = 0 mmHg). However, \textit{in vivo} COP values were relatively low in both colloid groups and decreased over time (fig. 3). This finding suggests the presence of pronounced vascular leakage with a subsequent transition of plasma proteins from the intravascular to the extravascular compartment.\textsuperscript{20} In this context, it is important to note that infusion of equivalent volumes of two colloids with markedly different \textit{in vitro} COP resulted in comparable \textit{in vivo} COP values. This finding may be explained (1) by a shift of extracellular protein-free water into the intravascular space after hyperoncotic pentastarch infusion and (2) by marked differences in pharmacokinetics between HES 200/0.5 and HES 130/0.4. Although the latter compound is rapidly degraded into many small fragments and thus exerts a high \textit{in vivo} COP, pentastarch is degraded slowly and, therefore, exerts an \textit{in vivo} COP that is lower than would be expected from the \textit{in vitro} molecular weight.\textsuperscript{5}

In the current study, administration of saline-based 10% HES 200/0.5 was associated with a significantly reduced urinary output when compared with the other two groups during the initial 7 h of volume resuscitation. This difference was accompanied by increases in plasma creatinine and urea concentrations (fig. 4). Thus, a reduced glomerular filtration rate contributed to the renal impairment noticed in the HES 200/0.5 group. It can be excluded that differences in sodium or chloride load between groups have contributed to the differences in renal function, because both HES groups received similar amounts of saline-based starch solutions, and plasma electrolyte concentrations were similar among groups (table

![Fig. 5. Histologic images of renal tubular injury in endotoxemic sheep. This figure represents illustrative examples of histologic findings in endotoxemic sheep. (A) 100-fold magnification; (B) 400-fold magnification.](image)

### Table 4. Electron Microscopic Evaluation of Renal Tubular Injury in Endotoxemic Sheep

<table>
<thead>
<tr>
<th>Group</th>
<th>Vacuolar Degeneration and Edema</th>
<th>Basal Dissociation</th>
<th>Cellular Injury</th>
<th>Tubular Precipitation</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>6% HES 130/0.4</td>
<td>0 (0–0)*†</td>
<td>1 (0–1)*</td>
<td>1 (1–2)*†</td>
<td>1 (1–2)*†</td>
<td>3.5 (3–4)*†</td>
</tr>
<tr>
<td>10% HES 200/0.5</td>
<td>2 (1–2)*†‡</td>
<td>1 (1–2)*</td>
<td>2 (2–2)*‡</td>
<td>2 (2–3)*‡</td>
<td>7 (6–8)*</td>
</tr>
<tr>
<td>Crystalloid</td>
<td>2 (1–2)*†‡</td>
<td>1 (1–1)</td>
<td>2 (1–2)*†‡</td>
<td>2 (2–2)*†‡</td>
<td>6 (5–8)*</td>
</tr>
<tr>
<td>ANOVA on ranks</td>
<td>P value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as median (range 25–75%). Vacuolar degeneration and edema, that is, vacuolar degeneration of tubular epithelial cells and/or swelling of cellular organelae: 0, none; 1, occasional; 2, moderate; and 3, ubiquitous; basal dissociation, that is, dissociation of tubular epithelial cells from the basal membrane: 0, none; 1, single cells; 2, more than 50% of tubular epithelium; and 3, complete tubule; cellular injury, that is, injury of tubular epithelial cells: 0, none; 1, moderate; 2, severe; and 3, complete destruction; intraluminal precipitation: 0, none; 1, moderate protein precipitation; 2, lumen obstructed by protein; and 3, intratubular cell organelle; Sum, sum of the latter four categories.

\* P < 0.05 for 6% hydroxyethyl starch (HES) 130/0.4 versus 10% HES 200/0.5. \† P < 0.05 for 6% HES 130/0.4 versus crystalloid. \‡ P < 0.05 for 10% HES 200/0.5 versus crystalloid.

ANOVA = analysis of variance.
3. Because MAP and COP were also comparable between the two colloid groups, it is unlikely that these variables impacted on the group differences in the current study. However, it cannot be excluded that differences in CI may have resulted in reduced glomerular filtration.

The phenomenon that differences in renal function diminished during the late phase of the intervention period (fig. 4) represents another interesting finding of the current study and may be explained by the fact that the maximum dose of colloids was already infused after 4 h. Thereafter (from hours 5 to 12, when the maximum HES dose had been reached), only colloids were infused in all study groups. Thus, it seems that impaired renal function associated with saline-based 10% HES 200/0.5 has partially been offset by prolonged administration of balanced crystalloids.

Tubular injury, as assessed by standard histologic techniques, was comparable between groups. However, cellular and subcellular tubular epithelial injury was more pronounced in sheep treated with saline-based 10% HES 200/0.5 when compared with 6% HES 130/0.4. In particular, infusion of 10% HES 200/0.5 was associated with vacuolar degeneration of tubular cells. These pathologic alterations were not seen in sheep treated with 6% HES 130/0.4. Interestingly, intracellular edema and cellular injury accounted for marked tubular injury in sheep treated only with balanced crystalloids. Extracellular overhydration caused by maximum amounts of crystalloids may have resulted in tubular cell edema and subsequent cellular injury. However, these hypotheses need to be tested in future (experimental) studies.

Kidney injury in response to HES 200/0.5 infusion has been noticed recently in a randomized controlled clinical trial comparing fluid therapy with 10% HES 200/0.5 and a modified lactated Ringer’s solution (45 mM lactate) in 537 patients with severe sepsis. Notably, patients allocated to the HES 200/0.5 group had a higher incidence of acute renal failure and were more likely to require renal replacement therapy. Future studies are now needed to evaluate the hypothesis that modern, third-generation tetrasaccharide solutions do not negatively impact on renal function in critically ill patients. In this context, Boldt et al. demonstrated the safety of 6% HES 130/0.4 in several small-scale studies, even in elderly patients with preexisting renal dysfunction undergoing coronary artery bypass grafting. To date, however, only one clinical comparison of 6% HES 130/0.4 and 20% human albumin has been performed. In the latter study, patients treated with HES 130/0.4 had a significant reduction in Acute Physiology and Chronic Health Evaluation II Score and an improvement in gas exchange when compared with patients treated with 20% human albumin. However, these results may have been influenced by the fact that 20% human albumin is hyperoncotic and has been reported to contribute to organ dysfunction.

The mechanisms by which saline-based 10% HES 200/0.5 may have impaired renal function in the current study cannot be entirely described by the data but seem to include increased tubular epithelial injury. The large spectrum of HES molecules with diverse molecular weights in 10% HES 200/0.5 may have been associated with excessive glomerular filtration and reabsorption of starch molecules by tubular epithelial cells, which might partly explain vacuolar degeneration. In this context, it has been...
reported that renal impairment after colloid infusion is dependent on the concentration (with 10% dextran 40 kD posing the highest risk among all synthetic colloids). In the latter experimental study, it has also been shown that infusion of 10% HES 200/0.5 increases urine viscosity by a factor of 2.5. Using a porcine model of hemodilution, Eisenbach et al. noticed that tissue storage of hexastarch is more pronounced when compared with pentastarch. However, considerable amounts of all three colloids (6% solutions of HES 200/0.62, HES 200/0.5, and HES 100/0.5) were detected in kidney tissue.

The current study has some limitations. (1) Because we did not analyze HES plasma concentrations, the exact amount of oncotically active HES molecules remains unknown. However, it has to be considered that COP more accurately reflects the amount of oncotically active molecules than HES plasma concentrations per se. (2) Cardiac filling pressures were used to guide volume therapy. Although the latter approach is recommended by the current sepsis guidelines, filling pressures are associated with considerable weaknesses as detailed earlier. (3) Because of the high mortality among the study subjects, the sample size decreased over time. This may have contributed to the absence of significant differences in some variables at the end of the experiment. (4) Finally, the electron microscopic tubular injury score used in the current study has not yet been validated in healthy subjects or subjects with standardized acute kidney injury, respectively. Therefore, the current electron microscopic data should be interpreted with caution.

Conclusions

In summary, the current study directly compared the effects of saline-based 6% HES 130/0.4, saline-based 10% HES 200/0.5, and a balanced crystalloid on renal function and tubular injury in fulminant ovine endotoxemia. The data provide evidence that 10% HES 200/0.5 is associated with negative effects on renal function and tubular epithelial integrity in contrast to 6% HES 130/0.4. Because of significant pharmacologic and physicochemical differences among second- and third-generation HES preparations, the negative effects of 10% HES 200/0.5 noticed in clinical studies should not be extrapolated to modern tetrastarch preparations. Randomized clinical trials elucidating the effects of third-generation tetrastarch solutions versus crystalloids in patients with severe sepsis and septic shock (e.g., CRYSMAS and CHEST) are currently ongoing and will hopefully shed more light on this important issue.

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