ANESTHESIOLOGY

Resuscitation with Hydroxyethyl Starch Maintains Hemodynamic Coherence in Ovine Hemorrhagic Shock

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Both saline and hydroxyethyl starch can be used for fluid resuscitation of hemorrhagic shock
- Hemodynamic coherence is the concordant performance of macro- and microcirculation
- It is not known whether saline or hydroxyethyl starch resuscitation achieves better hemodynamic coherence

What This Article Tells Us That Is New

- In a sheep model of hemorrhagic shock, resuscitation with both saline and hydroxyethyl starch restored mean arterial pressure (macrocirculation)
- After fluid resuscitation with hydroxyethyl starch, microcirculatory perfused vessel density and microvascular flow index (microcirculation) both improved, whereas saline only marginally improved microvascular flow index and perfused vessel density decreased further
- Resuscitation with hydroxyethyl starch but not saline maintained hemodynamic coherence after hemorrhagic shock

ABSTRACT

Background: Fluid resuscitation in hemorrhagic shock aims to restore hemodynamics and repair altered microcirculation. Hemodynamic coherence is the concordant performance of macro- and microcirculation. The present study on fluid therapy in hemorrhagic shock hypothesized that the choice of fluid (0.9% sodium chloride [saline group] or balanced 6% hydroxyethyl starch 130/0.4 [hydroxyethyl starch group]) impacts on hemodynamic coherence.

Methods: After instrumentation, 10 sheep were bled up to 30 ml/kg body weight of blood stopping at a mean arterial pressure of 30 mmHg to establish hemorrhagic shock. To reestablish baseline mean arterial pressure, they received either saline or hydroxyethyl starch (each n = 5). Hemodynamic coherence was assessed by comparison of changes in mean arterial pressure and both perfused vessel density and microvascular flow index.

Results: Bleeding of 23 ml/kg blood [21; 30] (median [25th; 75th percentile]) in the saline group and 24 ml/kg [22; 25] ($P = 0.916$) in the hydroxyethyl starch group led to hemorrhagic shock. Fluid resuscitation reestablished baseline mean arterial pressure in all sheep of the hydroxyethyl starch group and in one sheep of the saline group. In the saline group 4,980 ml [3,312; 5,700] and in the hydroxyethyl starch group 610 ml [489; 615] of fluid were needed ($P = 0.009$). In hemorrhagic shock perfused vessel density (saline from 100% to 83% [49; 86]; hydroxyethyl starch from 100% to 74% [61; 80]) and microvascular flow index (saline from 3.1 [2.5; 3.3] to 2.0 [1.6; 2.3]; hydroxyethyl starch from 2.9 [2.9; 3.1] to 2.5 [2.3; 2.7]) decreased in both groups. After resuscitation both variables improved in the hydroxyethyl starch group (perfused vessel density: 125% [120; 147]; microvascular flow index: 3.4 [3.2; 3.5]), whereas in the saline group perfused vessel density further decreased (64% [62; 79]) and microvascular flow index increased less than in the hydroxyethyl starch group (2.7 [2.4; 2.8]; both $P < 0.001$ for saline *vs.* hydroxyethyl starch).

Conclusions: Resuscitation with hydroxyethyl starch maintained coherence in hemorrhagic shock. In contrast, saline only improved macro- but not microcirculation. Hemodynamic coherence might be influenced by the choice of resuscitation fluid.

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Traumatic injury is a major cause of death and morbidity,[1] leading to about 5 million deaths per year worldwide. This number is aggravated by the fact that mostly young people are affected. Hemorrhagic shock is responsible for approximately one third of deaths among patients with traumatic injury.[2] Microcirculatory disorders in the context of hemorrhagic shock have been shown to be associated with adverse outcome.[3]

In addition to causal therapy, fluid resuscitation is a common supportive treatment, at least in controlled hemorrhagic shock.[4] The rationale for fluid administration is to optimize

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cardiac output (*i.e.*, macrocirculation) and thereby to potentially restore microcirculation and cellular oxygen delivery. The positive association between changes in macro- and microcirculation during resuscitation has recently been described as hemodynamic coherence by Ince.[5] If, for various pathophysiologic reasons, the microcirculation does not improve despite normalization of the macrocirculation, hemodynamic coherence is lost. Loss of hemodynamic coherence is associated with increased rates of organ failure and death.[6]

There are only a few experimental studies investigating the choice of fluid for resuscitation in hemorrhagic shock in the context of hemodynamic coherence. In rodent models of hemorrhagic shock, evidence suggests that resuscitation with colloids as well as balanced crystalloid solutions may be more advantageous than 0.9% sodium chloride solution (saline).[7–9] However, saline is still the most commonly used resuscitation fluid.[10] The microvascular effects of modern colloid solutions compared with saline with respect to microcirculation and hemodynamic coherence have not been sufficiently investigated in the setting of acute hemorrhagic hypovolemia.[11] Nevertheless, colloids appear to have a higher efficacy in recruiting the microcirculation in other types of shock, *e.g.*, sepsis.[12]

The aim of the present study was to investigate the impact of resuscitation with saline and balanced 6% hydroxyethyl starch 130/0.4 (hydroxyethyl starch) on macro- and microcirculation in a clinically relevant large animal model of hemorrhagic shock. The primary aim was to investigate whether hydroxyethyl starch and saline produce concordant improvements on macro- and microcirculation and thus maintain hemodynamic coherence.

Materials and Methods

Anesthesia and Instrumentation

After approval by the local veterinary authority (North Rhine–Westphalia State Environment Agency, Duesseldorf, Germany, under the reference No. 84-02.04.2013.A003), 10 healthy female sheep of the species *Ovis orientalis aries* (body weight, 43 kg [interquartile range 41; 48], aged between 6 and 12 months) were anesthetized by intramuscular bolus injection of 10 mg · kg^{-1} S-ketamine and 0.3 mg · kg^{-1} midazolam. Experiments began between 6:00 AM and 7:00 AM. The animals were tested in sequential order. After endotracheal intubation, the animals were ventilated targeting an end-tidal carbon dioxide partial pressure of 35 mmHg. Balanced anesthesia was maintained by inhalational isoflurane with an expiratory fraction of 1.2 vol% as well as infusion of S-ketamine (1 mg · kg^{-1} · h^{-1}) and midazolam (0.3 mg · kg^{-1} · h^{-1}).

A central venous line was inserted into the right jugular vein to administer drugs and to conduct transpulmonary thermodilution as well as a 7.5 French catheter in the left jugular vein for blood withdrawal and fluid administration. A pulse contour cardiac output catheter (5 French PiCCO catheter, Pulsion Medical Systems, Germany) was placed in the left femoral artery to obtain hemodynamic variables and to take arterial blood samples. A Foley catheter was placed in the urinary bladder.

Afterward the sheep were turned into prone position. After a recovery period of 30 min, the experimental protocol was started.

Experimental Protocol

After baseline measurements ("baseline" time point; see below for details), 10 ml blood per kg body weight were withdrawn three times with a pause of 30 min after each step. When mean arterial pressure decreased under 30 mmHg during the current step, withdrawal was stopped. The resulting amount of blood loss (up to 30 ml per kg body weight) equals approximately 50 to 60% of total blood volume in sheep.[13] Thirty minutes after the last blood withdrawal, the "shock" time point was reached. The animals were then randomly assigned by envelope method to receive either 0.9% sodium chloride solution ("saline group"; 0.9% sodium chloride solution, Fresenius Kabi, Germany) or 6% hydroxyethyl starch 130/0.4 preparation in balanced carrier solution ("hydroxyethyl starch group"; Volulyte, Fresenius Kabi) as resuscitation fluid. The balanced carrier solution was Ringer's actetate. Blinding methods were not used regarding the type of fluid. The respective fluid was administered with an infusion rate of 60 ml · kg^{-1} · h^{-1} in the saline group and 30 ml · kg^{-1} · h^{-1} in the hydroxyethyl starch group until the individual baseline mean arterial pressure was reestablished. If, despite continuous fluid administration, mean arterial pressure did not further increase for 1 h or even dropped again, fluid administration was stopped for futility. When either the baseline mean arterial pressure was restored or the infusion had been stopped because of futility, the "resuscitation" time point measurements were conducted (see below for details). Thereafter anesthesia was deepened by intravenous injection of 4 mg · kg^{-1} propofol, and the animals were killed with an intravenous injection of 200 ml of potassium chloride solution (7.45%).

Measurements

For all sheep body weight was determined at baseline (before blood withdrawal) and after resuscitation. The difference in body weight between both time points was calculated. In addition, the time period from shock to resuscitation (ending of fluid resuscitation) was documented. Fluid balance was calculated as the difference of infused fluid volume and the sum of withdrawn blood and total urine volume.

At each time point (baseline, shock, and resuscitation), the macro- and microhemodynamic variables were measured, and arterial blood samples were analyzed. Hemodynamic measurements comprised mean arterial pressure, stroke volume index, and cardiac index. Mean arterial pressure was noted as presented by the PiCCO2 monitor (Pulsion Medical

Systems, Germany). Stroke volume index and cardiac index were obtained by threefold bolus transpulmonary thermodilution through the pulse contour cardiac output system (each 15 ml of 0.9% ice-cold saline) and calculated based on the body surface area of sheep as proposed by Quiring.[14] The blood samples were analyzed by an ABL 725 radiometer automatic blood gas analyzer (Radiometer, Denmark).

Strong ion difference was calculated by the formula:

$$[\text{Strong ion difference}] = [Na^+] + [K^+] + 2[Ca^{2+}] - [Cl^-] - [\text{lactate}]$$

Conjunctival microcirculation was measured in five different randomly chosen positions of the left eye at each of the three measurement time points. Measurements were conducted using an incident dark field imaging camera (CytoCam, Braedius Medical BV, The Netherlands).[15] The obtained videos consisted of 120 frames each (*i.e.*, 6 s) and were reviewed for quality according to the recommendations by Massey *et al.*[16] and discarded, if necessary. The remaining high quality videos were analyzed with dedicated software (AVA Software version 3.2, MicroVision Medical, The Netherlands).[17] Perfused vessel density, microvascular flow index, heterogeneity index, and percentage of perfused vessels were obtained as described by an independent expert panel[18] for each video. Vessel density is obtained by drawing (helped by a semiautomatic computed algorithm) each vessel in the video. Afterward the sum of their length is divided by the surface area of the microcirculatory measurement, which results in the total vessel density. Perfused vessel density is calculated by only including vessels with a flow at least classified as "sluggish" on the microvascular flow index scale in the previously described calculation of total vessel density. By dividing the video in four quadrants, associating each quadrant with the predominant type of flow in the respective area and averaging these four, the microvascular flow index is calculated. Flow is classified as absent (0), intermittent (1), sluggish (2), normal (3), or hyperdynamic (4). The percentage of perfused vessels is the percentage of vessels with a microvascular flow index classification of at least "sluggish" in relation to all vessels. Perfused vessel density values at shock and resuscitation time points were calculated as percentages of baseline values. All analyses of microcirculatory videos were conducted in a blinded manner regarding group and time point.

Statistical Analysis

Statistical analysis was performed with IBM SPSS statistics software version 20 (IBM, USA). All data are presented as medians and interquartile ranges. There were no missing data except one single stroke volume index value lost to technical reasons. Comparisons between groups were made using Mann–Whitney U test. Comparisons between time points within groups were conducted using Wilcoxon signed-rank test. Additional data and comparisons are available as Supplemental Digital Content 1 (http://links.lww.com/ALN/C65). No *a priori* sample size calculation was possible because of a lack of sufficient previous data to estimate the influence of different fluid solutions on microcirculation. Thus, the sample size was based on our experience with the experimental model of ovine hemorrhagic shock. The *P* values were adjusted according to Bonferroni. Correction was introduced *post hoc* during review process. Asymptotic two-sided *P* values smaller than 0.05 were assumed as statistically significant.

Results

Blood Withdrawal, Body Weight, Fluid Balance, and Urine Output

Blood withdrawal had to be stopped because of predefined safety measures (*i.e.*, mean arterial pressure less than 30 mmHg as detailed in the Materials and Methods) in 7 of 10 sheep (in 3 ewes of the saline group and 4 of the hydroxyethyl starch group). There was no difference in total blood withdrawal per body weight between the saline (23 ml/kg [21; 30]) and hydroxyethyl starch groups (24 ml/kg [22; 25]; $P = 0.916$).

A total amount of 4,980 ml [3,312; 5,700] of saline was administered in the saline group and 610 ml [489; 615] of hydroxyethyl starch in the hydroxyethyl starch group ($P = 0.009$). Time between shock and resuscitation was 178 min [173; 185] in the saline group and 76 min [74; 82] in the hydroxyethyl starch group ($P = 0.009$). Cumulative fluid balance was positive in the saline group (3,045 ml [1,742; 3,370]) but negative (–945 ml [–1,060; –398]) in the hydroxyethyl starch group ($P = 0.009$). The body weight difference between baseline and resuscitation in the saline group was positive (2.8 kg [2.5; 4.0]), whereas it was negative in the hydroxyethyl starch group (–1.0 kg [–1.5; –1.0]; $P = 0.009$). Total urine volume was lower in the hydroxyethyl starch group (440 ml [120; 550]) as compared with the saline group (690 ml [585; 700]; $P = 0.028$).

Hemodynamic Variables

Blood withdrawal induced a decrease in mean arterial pressure, stroke volume index, and cardiac index between baseline and shock in both groups to the same degree. After fluid administration, mean arterial pressure, stroke volume index, and cardiac index increased in both groups. There was no difference in hemodynamic variables between saline- and hydroxyethyl starch–resuscitated animals at any time point (fig. 1, A–C).

Baseline mean arterial pressure was reestablished by fluid resuscitation in all sheep of the hydroxyethyl starch group. In the saline group, only one sheep reached the Baseline mean arterial pressure, whereas in the other four sheep mean arterial pressure stopped increasing during fluid administration before baseline mean arterial pressure was reached (fluid

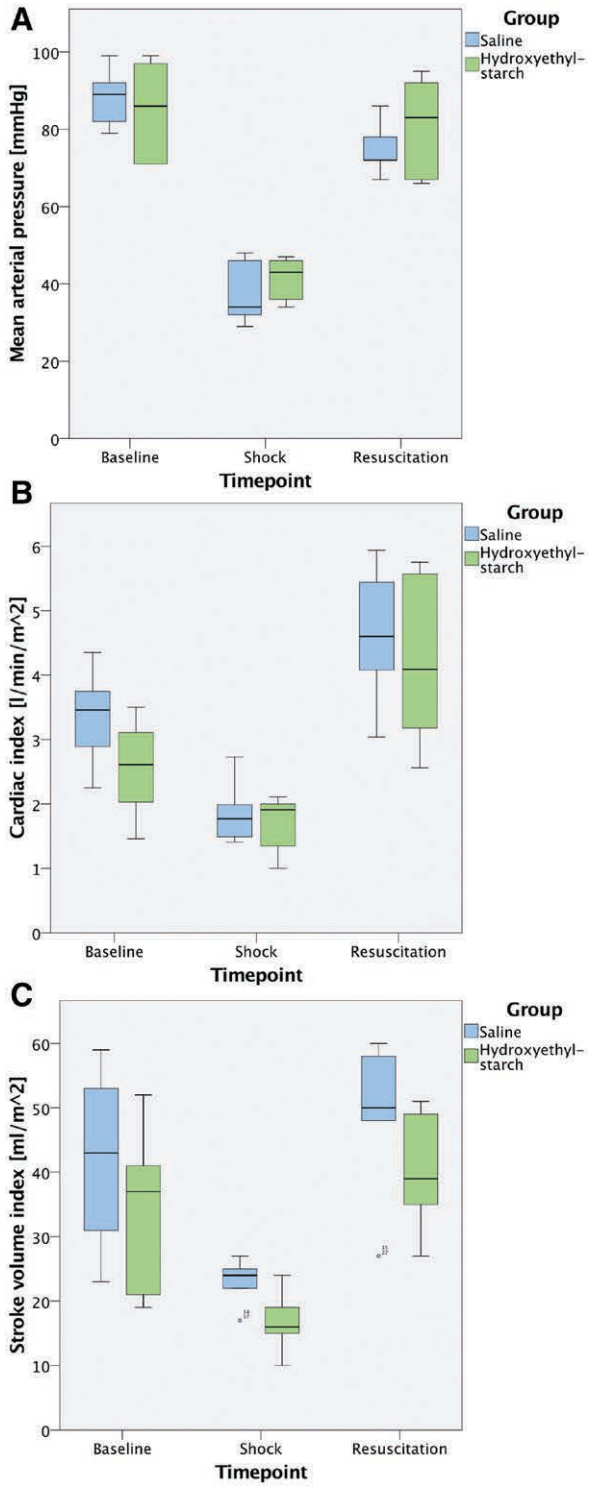

Fig. 1. Hemodynamic variables. The saline group received 0.9% sodium chloride solution. The hydroxyethyl starch group received 6% hydroxyethyl starch 130/0.4 in balanced carrier solution.

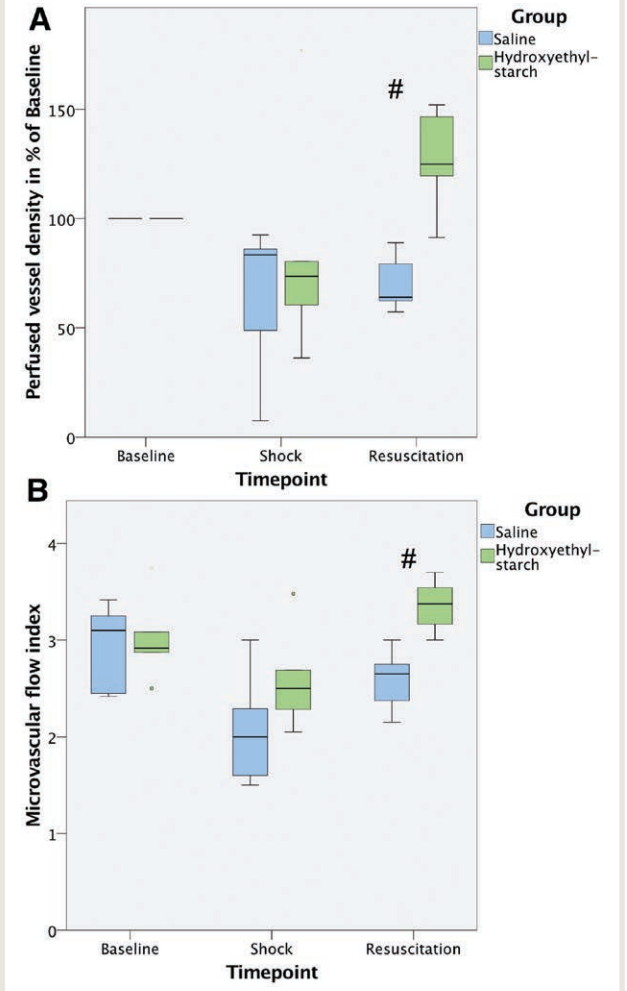

Fig. 2. Microcirculation. The saline group received 0.9% sodium chloride solution. The hydroxyethyl starch group received 6% hydroxyethyl starch 130/0.4 in balanced carrier solution. #Significant difference between groups with Bonferroni corrected $P < 0.05$.

stopped because of futility). The median mean arterial pressure at the resuscitation time point in percentage of baseline mean arterial pressure was 88% [79; 91] in the saline group and 95% [94; 96] in the hydroxyethyl starch group ($P = 0.048$).

Microcirculation

Perfused vessel density decreased from baseline to shock in both groups to the same degree. During resuscitation it further decreased in the saline group, whereas it increased in the hydroxyethyl starch group. At the resuscitation time point, perfused vessel density was significantly higher in the hydroxyethyl starch group than in the saline group (fig. 2A, $P = 0.027$). Microvascular flow index decreased

in both groups from baseline to shock and increased in both groups from shock to resuscitation (fig. 2B). At the resuscitation time point, the microvascular flow index was higher in the hydroxyethyl starch group compared with the saline group ($P = 0.036$). Heterogeneity index increased in both groups from baseline to resuscitation (saline: from 0.8 [0.6; 1.1] to 0.9 [0.7; 1.0]; hydroxyethyl starch from 0.7 [0.3; 0.8] to 0.7 [0.4; 0.8]). During resuscitation it decreased in the hydroxyethyl starch group and further increased in the saline group (saline: 1.0 [0.9; 1.1], hydroxyethyl starch: 0.3 [0.3; 0.3]). The difference at resuscitation was significant ($P = 0.027$). The percentage of perfused vessels decreased from baseline to shock and improved toward resuscitation in both groups (saline: from 92% [77; 95] to 73 [60; 85] and to 80 [75; 90]; hydroxyethyl starch from 88% [79; 98] to 73 [49; 73] and to 93 [92; 95]). Typical video captures of each time point are included in the Supplemental Digital Content (video 1: Baseline, http://links.lww.com/ALN/C66; video 2: Hemorrhagic shock, http://links.lww.com/ALN/C67; video 3: After resuscitation with saline, http://links.lww.com/ALN/C68; video 4: After resuscitation with hydroxyethyl starch, http://links.lww.com/ALN/C69).

Blood Gas Analysis

Hemoglobin concentration decreased in both groups from baseline to shock and further decreased from shock to resuscitation. There were no relevant differences between the two groups in hemoglobin concentration (for all blood gas analysis–derived variables see table 1). Lactate concentrations increased from baseline to shock in both groups. In the saline group, lactate concentration decreased between shock and resuscitation but further increased in the hydroxyethyl starch group. At resuscitation, lactate concentration in the hydroxyethyl starch group was higher than in the saline group but because of α-level correction was not significantly different. Chloride concentration increased from shock to resuscitation in the saline group but not in the hydroxyethyl starch group and was significantly different between both groups at resuscitation. Despite being statistically significant, base excess showed no physiologically relevant differences at baseline or at the other time points. Strong ion differences decreased in both groups from baseline to shock, decreased further from shock to resuscitation, but showed no differences between groups. No differences between groups were found regarding hydrogen ion concentration.

Discussion

The main findings of the present study are that induction of hemorrhagic shock by controlled bleeding caused a parallel impairment of macro- and microcirculation. Resuscitation with either of the study fluids (0.9% sodium chloride solution [saline group] and 6% hydroxyethyl starch 130/0.4 in balanced solution [hydroxyethyl starch group]) improved macrohemodynamics. However, microvascular diffusion capacity, measured by perfused vessel density, was only improved in the hydroxyethyl starch group, whereas it further decreased in the saline group. Notably, only about one eighth of the fluid volume was required to correct mean arterial pressure in the hydroxyethyl starch group compared with the saline group. In addition, microvascular convective flow, *i.e.*, microvascular flow index, was higher in the hydroxyethyl starch than in the saline group. Resuscitation in the hydroxyethyl starch group required less time and markedly less volume of the respective resuscitation fluid. The concordant changes of macro- and microcirculation suggest maintained hemodynamic coherence in the hydroxyethyl starch group, whereas hemodynamic coherence was perturbed in the saline group.

Decreases of mean arterial pressure, stroke volume index, and cardiac index and an increase in lactate concentration caused by bleeding in the present model are expected findings in hemorrhagic shock, indicating that blood loss

Table 1. Blood Gas Analysis

Variable	Group	Baseline	Shock	Resuscitation
Hemoglobin concentration [g · dl^{-1}]	Saline	8.8 [8.7; 9.0]	7.9 [7.9; 8.2]	5.8 [5.6; 6.7]
	Hydroxyethyl starch	8.7 [8.6; 8.9]	8.1 [7.7; 8.4]	5.6 [5.5; 6.7]
Lactate concentration [mmol · l^{-1}]	Saline	0.6 [0.6; 1.1]	1.5 [1.5; 2.0]	1.5 [0.9; 1.7]
	Hydroxyethyl starch	1.1 [0.9; 1.2]	2.2 [1.7; 2.4]	2.3 [2.2; 2.7]
Chloride concentration [mmol · l^{-1}]	Saline	96 [96; 97]	96 [96; 98]	107 [104; 108]*
	Hydroxyethyl starch	95 [94; 96]	97 [93; 98]	96 [94; 98]*
Base excess [mmol · l^{-1}]	Saline	1.7 [1.6; 2.2]*	0.1 [0; 2.3]	−6.1 [−6.2; −2.0]
	Hydroxyethyl starch	−1.2 [−1.8; −0.6]*	−1.8 [−2.5; 0.4]	−2.9 [−3.3; 1.1]
Strong ion difference [mmol · l^{-1}]	Saline	48 [46; 48]	45 [45; 46]	41 [40; 42]
	Hydroxyethyl starch	47 [44; 47]	45 [43; 46]	42 [42; 44]
pH	Saline	7.36 [7.33; 7.39]	7.36 [7.35; 7.40]	7.29 [7.28; 7.31]
	Hydroxyethyl starch	7.31 [7.30; 7.33]	7.28 [7.26; 7.34]	7.34 [7.32; 7.45]

The saline group received 0.9% sodium chloride solution. The hydroxyethyl starch group received 6% hydroxyethyl starch 130/0.4 in balanced carrier solution.
*Significant difference between groups with Bonferroni corrected $P < 0.05$.

resulted in impaired macrocirculation ultimately leading to impaired cellular oxygen delivery and increased cellular stress response, thereby increasing lactate concentrations. Concordant to the decrease in stroke volume index and cardiac index from baseline to shock, microvascular flow (measured by microvascular flow index) also decreased, suggesting an intact coherence between macro- and microcirculation at this stage. These findings are consistent with previous experimental studies also demonstrating a reduced microvascular flow index in hemorrhagic shock.[19] The reduced total intravascular volume leading to decreases in stroke volume index, cardiac index, and microvascular flow index probably also caused the decrease in perfused vessel density from baseline to shock.

Mean arterial pressure is a common clinical target for fluid resuscitation in acute hemorrhagic shock mainly because of its broad availability. The underlying assumption is that by mean arterial pressure–guided fluid resuscitation, stroke volume index and cardiac index (which are not always measurable) are increased, which in turn leads to improvement of microcirculation. In the present study, fluid resuscitation targeting to reestablish baseline mean arterial pressure values likewise restored stroke volume index and cardiac index in both groups. The increase in stroke volume index and cardiac index during resuscitation was associated with an increased microvascular flow index in both groups. Notably, sheep in the hydroxyethyl starch group had a significantly more pronounced increase in microvascular flow index compared with the saline group. Given a similar cardiac index, this may be best attributed to improved blood rheology and decreased erythrocyte aggregation.[20,21] However, because only one microvascular organ site was measured, differential distribution of blood flow between organs may also explain the microvascular flow index differences between both groups. Moreover, the numeric (even if not significant) differences in mean arterial pressure post resuscitation between groups may also have impact on microvascular flow index.

From shock to resuscitation, perfused vessel density decreased in the saline group and increased in the hydroxyethyl starch group to levels higher than baseline values of perfused vessel density. This may be explained by the large amount of administered fluid in the saline group that probably led to interstitial edema, thereby relatively reducing space for perfused capillaries and increasing extramural pressure on these capillaries and postcapillary venules.[5] The different infusion rates in the study groups (saline was infused with the twofold velocity as compared with hydroxyethyl starch) may also have contributed to differences between groups. In this context, it has to be noted that slower infusion rates in the saline group would probably have resulted in underresuscitation, whereas the higher infusion rate we chose may have led to increased inflammatory response and extravasation. This is supported by the difference in heterogeneity index of microcirculation. Moreover, identical infusion rates of saline and hydroxyethyl starch would have further increased the time difference between groups from shock to resuscitation.

The volume needed to achieve mean arterial pressure goals in the present study was more than eight times higher in the saline than in the hydroxyethyl starch group. This might be expected because crystalloid fluid solutions leave the vascular system soon after administration, resulting in a volume effect of approximately 20% or less,[22–24] whereas the volume effect of isooncotic colloid solutions is nearly 100% in the absence of vascular leakage or hypervolemia.[25,26] The marked difference in administered fluid volumes is reflected by the difference in body weight gain, meaning that fluids were not eliminated *via* the kidneys but remained in the interstitial compartment. This is supported by the lack of differences in hemoglobin concentrations between groups after resuscitation, suggesting a similar macrovascular volume effect and hemodilution. In normovolemic hemodilution, hydroxyethyl starch compared with crystalloids has been shown to have a positive effect on microcirculation and function in the kidney despite the same degree of hemodilution.[27] The marked difference in administered fluid volumes may also partially explain the microcirculatory findings of the present study. Because high amounts of interstitial fluid may increase extramural pressure on capillaries, some of these capillaries may be excluded from circulation and therefore explain lower perfused vessel density in the saline group.

Notably, the hydroxyethyl starch group even showed a negative fluid balance. A higher amount of fluid or more precisely a positive fluid balance was previously shown to be a risk factor for increasing morbidity and mortality in critically ill patients.[28,29] Also, the presence of hyperchloremia as found in this study is known to produce several unfavorable effects such as renal afferent arteriolar vasoconstriction[30] or formation of active nitrogen species leading to systemic vasodilation and increased vascular leakage.[31] Hyperchloremic acidosis has likewise been associated with adverse outcome.[32]

The lower urinary output in the hydroxyethyl starch group might be caused by several factors. First, the time from shock to resuscitation was shorter than in the saline group; thus it might be possible that not as much urine was produced in this amount of time. In addition, hydroxyethyl starch solutions have a volume effect of nearly 100% as mentioned above; therefore its water remains in the vascular system and does not leave it, *e.g.*, *via* the kidneys as urine. On the other hand, the high amount of saline administered might be not only "lost" in the interstitium but also in part secreted *via* the kidneys as urine. Last, it is known that high amounts of hydroxyethyl starch solutions in critically ill patients may decrease kidney function, leading to decreased urine output. In the context of hemorrhagic shock, these findings are inconclusive[33] but need to be taken into consideration when regarding the results of the present study.

The difference in lactate concentration after resuscitation, despite not being statistically significant (rising lactate concentrations in the hydroxyethyl starch group compared with a decrease in the saline group), may be explained by the time delay of measurements. Because infusion rates were fixed and a lot more fluid had to be administered in the saline as compared with the hydroxyethyl starch group, the time until baseline mean arterial pressure was restored was much longer in the saline group (mean difference of 102 min). This happened despite anticipation of this phenomenon in the study protocol by infusing saline with a doubled infusion rate compared with hydroxyethyl starch; at first sight these results seem to contradict the ones of microcirculatory measurements, because high lactate levels are an indicator of impaired microcirculation. Notably, lactate clearance is time-dependent, because metabolic pathways are required. Moreover, lactate distributes to the complete extravascular space, which was markedly expanded in the saline group. Thus, dilution may be another explanation for higher lactate values in the hydroxyethyl starch group. A comparable effect has been shown for creatinine concentrations, which are also dependent on fluid balance.[34] Persistent microvascular dysfunction in other organs than the conjunctiva may at least theoretically be considered as a cause for higher lactate values in the hydroxyethyl starch group.

The ultimate goal of fluid resuscitation is to improve microvascular oxygen delivery, namely convection and diffusion.[35] Because convection is dependent on flow, whereas diffusion depends on the distance from erythrocyte to tissue cell, they can be characterized by microvascular flow index and perfused vessel density. Transcribed into the present study, resuscitation with hydroxyethyl starch was able to establish better convection and diffusion (by increasing microvascular flow index and perfused vessel density) than a large amount of saline. Thus, hemodynamic coherence (*i.e.*, the positive association of changes in macro- and microcirculation[5]) was only maintained in hydroxyethyl starch group and not in saline group when targeting macrohemodynamic variables, *i.e.*, mean arterial pressure in the present study. These results are supported by studies showing that colloidal fluids are advantageous more than 0.9% sodium chloride in restoring intestinal microcirculation.[7] Previous studies have shown that impairment of the microcirculation is associated with pronounced organ failure and adverse outcome,[3] emphasizing the importance of restoring the microcirculation. Based on these findings and the results of the present study, the choice of fluid type appears to be a critical factor in effectively restoring microcirculation and ultimately cellular oxygenation in hemorrhagic shock.

The present study has some limitations that need to be mentioned. First of all, because no outcome-related parameters were measured, it is not possible to conclude on long-term implications of the present findings. In addition, it is unclear whether the observed differences between groups are attributable to 6% hydroxyethyl starch 130/0.4 solution being a colloidal solution and/or a balanced solution. However, the aim of this study was to compare the most commonly used fluid worldwide (*i.e.*, 0.9% saline) with a modern solution with several potential advantages. In addition, the difference in time between groups needed to finish fluid resuscitation and the different administration rates might have influenced the microcirculation. The relevance of the single components (colloid and carrier solution) needs to be answered by further studies. In addition, the sample size is rather small. Nevertheless, the results are clearly significant and unlikely to change if the sample were increased. Moreover, only videos of conjunctival microcirculation were investigated, whereas other sites (sublingual, intestinal, renal) would have also been of interest.[3,17] The reasons for monitoring only the conjunctival microcirculation were its good and reproducible accessibility and visualization in a very time-critical and fast resuscitation protocol. Recently, experimental studies demonstrated a convincing correlation between sublingual and conjunctival microcirculation.[36,37]

Conclusions

Hemorrhagic shock was associated with coherent deteriorations in macro- and microcirculation in the present sheep model. Resuscitation with balanced 6% hydroxyethyl starch led to an improvement in both macrocirculatory variables, as well as microvascular flow and diffusion capacity, thereby preserving hemodynamic coherence. In contrast, resuscitation with 0.9% saline nearly normalized macrohemodynamics but further deteriorated diffusion capacity. The present results suggest that in acute hemorrhagic shock, the type of resuscitation fluid is relevant for hemodynamic coherence. Future studies are warranted to investigate the optimal type of fluid and carrier solution for resuscitation in hemorrhagic shock with respect to the microcirculation.

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Competing Interests

Dr. Arnemann received research grants from Fresenius Kabi (Bad Homburg, Germany). Dr. Rehberg received travel reimbursements from Astellas Pharma (Munich, Germany) and Orion Pharma (Hamburg, Germany) and works as a medical advisor for Fresenius Kabi. In the last 2 years, Dr. Ince has received honoraria and independent research grants from Fresenius Kabi and Prolong (South Plainfield, New Jersey). In addition, Dr. Ince has developed sidestream dark field imaging and is listed as inventor on related patents commercialized by MicroVision Medical under a license from the Academic Medical Center. He receives no royalties or benefits from this license. He has

been a consultant for MicroVision Medical in the past but has not been involved with this company for more than 5 years and holds no shares or stock. Braedius Medical, a company owned by a relative of Dr. Ince, has developed and designed a hand held microscope called CytoCam-IDF imaging; however, Dr. Ince has no financial relation with Braedius Medical of any sort, *i.e.*, never owned shares or received consultancy or speaker fees from Braedius Medical. Drs. Kampmeier and Ertmer received travel reimbursement and research grants from Fresenius Kabi. The other authors declare no competing interests.

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References

1. Krug EG, Sharma GK, Lozano R: The global burden of injuries. Am J Public Health 2000; 90:523–6
2. Kauvar DS, Lefering R, Wade CE: Impact of hemorrhage on trauma outcome: An overview of epidemiology, clinical presentations, and therapeutic considerations. J Trauma 2006; 60:S3–11
3. Tachon G, Harrois A, Tanaka S, Kato H, Huet O, Pottecher J, Vicaut E, Duranteau J: Microcirculatory alterations in traumatic hemorrhagic shock. Crit Care Med 2014; 42:1433–41
4. Spahn DR, Bouillon B, Cerny V, Coats TJ, Duranteau J, Fernández-Mondéjar E, Filipescu D, Hunt BJ, Komadina R, Nardi G, Neugebauer E, Ozier Y, Riddez L, Schultz A, Vincent JL, Rossaint R: Management of bleeding and coagulopathy following major trauma: An updated European guideline. Crit Care 2013; 17:R76
5. Ince C: Hemodynamic coherence and the rationale for monitoring the microcirculation. Crit Care 2015; 19:S8
6. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL: Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. Crit Care Med 2004; 32:1825–31
7. Wu CY, Chan KC, Cheng YJ, Yeh YC, Chien CT; NTUH Center of Microcirculation Medical Research: Effects of different types of fluid resuscitation for hemorrhagic shock on splanchnic organ microcirculation and renal reactive oxygen species formation. Crit Care 2015; 19:434
8. Wu CY, Yeh YC, Chien CT, Chao A, Sun WZ, Cheng YJ; NTUH Center of Microcirculation Medical Research (NCMMR): Laser speckle contrast imaging for assessing microcirculatory changes in multiple splanchnic organs and the gracilis muscle during hemorrhagic shock and fluid resuscitation. Microvasc Res 2015; 101:55–61
9. 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
10. Shaw AD, Bagshaw SM, Goldstein SL, Scherer LA, Duan M, Schermer CR, Kellum JA: Major complications, mortality, and resource utilization after open abdominal surgery: 0.9% saline compared to Plasma-Lyte. Ann Surg 2012; 255:821–9
11. Myburgh JA, Mythen MG: Resuscitation fluids. N Engl J Med 2013; 369:1243–51
12. Dubin A, Pozo MO, Casabella CA, Murias G, Pálizas F Jr, Moseinco MC, Kanoore Edul VS, Pálizas F, Estenssoro E, Ince C: Comparison of 6% hydroxyethyl starch 130/0.4 and saline solution for resuscitation of the microcirculation during the early goal-directed therapy of septic patients. J Crit Care 2010; 25:659.e1–8
13. Hansard SL: Residual organ blood volume of cattle, sheep and swine. Proc Soc Exp Biol Med 1956; 91:31–4
14. Quiring DP: Surface area determination, Medical Physics Yearbook. Edited by Glasser O. Chicago, 1955, pp 1490–4
15. Aykut G, Veenstra G, Scorcella C, Ince C, Boerma C: Cytocam-IDF (incident dark field illumination) imaging for bedside monitoring of the microcirculation. Intensive Care Med Exp 2015; 3:40
16. Massey MJ, Larochelle E, Najarro G, Karmacharla A, Arnold R, Trzeciak S, Angus DC, Shapiro NI: The microcirculation image quality score: Development and preliminary evaluation of a proposed approach to grading quality of image acquisition for bedside videomicroscopy. J Crit Care 2013; 28:913–7
17. Dobbe JG, Streekstra GJ, Atasever B, van Zijderveld R, Ince C: Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. Med Biol Eng Comput 2008; 46:659–70
18. De Backer D, Hollenberg S, Boerma C, Goedhart P, Büchele G, Ospina-Tascon G, Dobbe I, Ince C: How to evaluate the microcirculation: Report of a round table conference. 2007, p R101
19. Dubin A, Pozo MO, Ferrara G, Murias G, Martins E, Canullán C, Canales HS, Kanoore Edul VS, Estenssoro E, Ince C: Systemic and microcirculatory responses to progressive hemorrhage. Intensive Care Med 2009; 35:556–64
20. Neff TA, Fischler L, Mark M, Stocker R, Reinhart WH: The influence of two different hydroxyethyl starch solutions (6% HES 130/0.4 and 200/0.5) on blood viscosity. Anesth Analg 2005; 100:1773–80

21. Villela NR, Tsai AG, Cabrales P, Intaglietta M: Improved resuscitation from hemorrhagic shock with Ringer's lactate with increased viscosity in the hamster window chamber model. J Trauma 2011; 71:418–24
22. Carey JS, Scharschmidt BF, Culliford AT, Greenlee JE, Scott CR: Hemodynamic effectiveness of colloid and electrolyte solutions for replacement of simulated operative blood loss. Surg Gynecol Obstet 1970; 131:679–86
23. Lamke LO, Liljedahl SO: Plasma volume changes after infusion of various plasma expanders. Resuscitation 1976; 5:93–102
24. Jacob M, Chappell D, Hofmann-Kiefer K, Helfen T, Schuelke A, Jacob B, Burges A, Conzen P, Rehm M: The intravascular volume effect of Ringer's lactate is below 20%: A prospective study in humans. Crit Care 2012; 16:R86
25. Baron JF, De Kegel D, Prost AC, Mundler O, Arthaud M, Basset G, Maistre G, Masson F, Carayon A, Landault C: Low molecular weight hydroxyethyl starch 6% compared to albumin 4% during intentional hemodilution. Intensive Care Med 1991; 17:141–8
26. Ertmer C, Rehberg S, Van Aken H, Westphal M: Relevance of non-albumin colloids in intensive care medicine. Best Pract Res Clin Anaesthesiol 2009; 23:193–212
27. Konrad FM, Mik EG, Bodmer SI, Ates NB, Willems HF, Klingel K, de Geus HR, Stolker RJ, Johannes T: Acute normovolemic hemodilution in the pig is associated with renal tissue edema, impaired renal microvascular oxygenation, and functional loss. Anesthesiology 2013; 119:256–69
28. Malbrain ML, Marik PE, Witters I, Cordemans C, Kirkpatrick AW, Roberts DJ, Van Regenmortel N: Fluid overload, de-resuscitation, and outcomes in critically ill or injured patients: A systematic review with suggestions for clinical practice. Anaesthesiol Intensive Ther 2014; 46:361–80
29. Gou DY, Zhu YF, Jin Y, Chen LY: Spontaneous diuresis and negative fluid balance predicting recovery and survival in patients with trauma-hemorragic shock. Chin J Traumatol 2003; 6:382–4
30. Thurau K, Boylan JW: Acute renal success: The unexpected logic of oliguria in acute renal failure. Am J Med 1976; 61:308–15
31. Pedoto A, Nandi J, Oler A, Camporesi EM, Hakim TS, Levine RA: Role of nitric oxide in acidosis-induced intestinal injury in anesthetized rats. J Lab Clin Med 2001; 138:270–6
32. Gunnerson KJ, Saul M, He S, Kellum JA: Lactate *versus* non-lactate metabolic acidosis: A retrospective outcome evaluation of critically ill patients. Crit Care 2006; 10:R22
33. Ertmer C, Annane D, Van Der Linden P: Is the literature inconclusive about the harm from HES?: Yes. Intensive Care Med 2017; 43:1520–2
34. Macedo E, Bouchard J, Soroko SH, Chertow GM, Himmelfarb J, Ikizler TA, Paganini EP, Mehta RL; Program to Improve Care in Acute Renal Disease Study: Fluid accumulation, recognition and staging of acute kidney injury in critically-ill patients. Crit Care 2010; 14:R82
35. Ince C: The rationale for microcirculatory guided fluid therapy. Curr Opin Crit Care 2014; 20:301–8
36. Yin L, Yang Z, Yu H, Qian J, Zhao S, Wang J, Wu X, Cahoon J, Tang W: Changes in sublingual microcirculation is closely related with that of bulbar conjunctival microcirculation in a rat model of cardiac arrest. Shock 2016; 45:428–33
37. Hessler M, Arnemann P-H, Zamit F, Seidel L, Kampmeier T-G, Kvathöfer U, Alnaweiseh M, Tchaichian S, Rehberg S, Ertmer C: Monitoring of conjunctival microcirculation reflects sublingual microcirculation in ovine septic and hemorrhagic shock. Shock 2019; 51:479–86