

Plasma Volume Expansion with 5% Albumin Compared to Ringer's Acetate during Normal and Increased Microvascular Permeability in the Rat

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ABSTRACT

Background: It is believed that the effectiveness of colloids as plasma volume expanders is dependent on the endothelial permeability for macromolecules. The objective of this study was to test the hypothesis that the plasma volume expanding effect of 5% albumin relative to that of a crystalloid solution is reduced if microvascular permeability is increased.

Methods: A control group was resuscitated with either 5% albumin (8 ml/kg) or Ringer's acetate (36 ml/kg) immediately after a hemorrhage of 8 ml/kg ($n = 29$). In a second group, permeability was increased by inducing sepsis through cecal ligation and incision ($n = 28$). Three hours after cecal ligation and incision, the animals were resuscitated with either 5% albumin in a ratio of 1:1 relative to the volume of lost plasma, or Ringer's acetate in a ratio of 4.5:1.

Results: In the hemorrhage group, plasma volumes at 15 min after resuscitation with albumin or Ringer's acetate had increased by 9.8 ± 2.6 ml/kg (mean \pm SD) and 7.4 ± 2.9 ml/kg and were similar at 2 and 4 h. Plasma volume 3 h after cecal ligation and incision had decreased by approximately 7 ml/kg, and at 15 min after resuscitation with albumin or Ringer's acetate it had increased by 5.7 ± 2.9 and 2.4 ± 3.0 ml/kg, respectively ($P < 0.05$). At 2 and 4 h after resuscitation, plasma volumes did not differ between the groups.

Conclusion: This study does not support the hypothesis that the plasma-volume-expanding effect of albumin relative to that of crystalloids is decreased under conditions characterized by increased permeability. (*ANESTHESIOLOGY* 2014; 121:817-24)

MAINTENANCE of normal intravascular volume is universally considered to be a cornerstone in the treatment of hemodynamically compromised patients, but the optimal type of fluid used to reach this therapeutic goal has been debated for a long time.^{1,2} Proponents of colloids have argued that less volume is required for equal plasma volume expansion and that crystalloids may compromise organ function secondary to edema formation.

Based on studies in postoperative patients and trauma victims as well as in experimental models of hemorrhage, it has been suggested that 4 to 4.5 times the volume of crystalloid solution is required to obtain the same plasma volume expansion as with a given volume of albumin.³⁻⁵ The difference in the distribution volumes for the different fluids is often attributed to the fact that microvascular permeability to small solutes is high, whereas permeability to colloids is low. This means that under conditions of increased permeability such as sepsis, it is plausible that the distribution volume of a colloid approaches that of a crystalloid solution as also suggested previously.⁴ If so, this could explain the observation that in recent randomized controlled studies of intensive care patients, the ratio between the volumes of crystalloids and colloids given to achieve clinical resuscitation end points was found to be 1:1 to 1.3:1.⁶⁻⁸

What We Already Know about This Topic

- It is thought that the better volume-expanding effect of albumin relative to crystalloids is dependent on low endothelial permeability for albumin

What This Article Tells Us That Is New

- One group of animals were subjected to a 11% hemorrhage and then given either 5% albumin in a volume equal to the shed blood volume or Ringer's acetate at 4.5 times that volume
- Another group of animals were subjected to abdominal sepsis, and at 3 h, measured plasma volume loss was replaced with either 5% albumin or with Ringer's acetate in 4.5 times the measured loss
- Plasma volume expansion with albumin relative to Ringer's acetate did not differ between the two groups despite different etiologies for the decrease in plasma volume

Based on these considerations, the current study was designed to test the hypothesis that the difference between the volume of a colloid and the volume of a crystalloid required for equal plasma volume expansion decreases under conditions that are associated with increased permeability. For this purpose, rats subjected to either a controlled hemorrhage or abdominal sepsis were randomized to receive resuscitation with either 5% albumin at a ratio of 1:1 relative to the lost

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volume of blood or plasma, or Ringer's acetate at a ratio of 4.5:1 relative to the lost volume of blood or plasma. Plasma volume was measured for up to 4 h after resuscitation by measuring the initial distribution volume of radiolabeled albumin.

Materials and Methods

Materials and Anesthesia

The study was approved by the Lund University ethics committee for animal research (M87-09), and the animals were treated in accordance with the guidelines of the National Institutes of Health for Care and Use of Laboratory Animals. Seventy male Sprague–Dawley rats weighing 354 ± 13 g were used in the study. The animals had free access to water and food until anesthesia was induced by placing them in a covered glass container with a continuous supply of isoflurane (Isoba® Vet; Intervet AB, Sollentuna, Sweden). After a tracheostomy, the animals were connected to a ventilator (Ugo Basile; Biological Research Apparatus, Comerio, Italy) and ventilated with tidal volumes of 6 ml/kg with a positive end-expiratory pressure of 3 to 4 cm H₂O. Anesthesia was maintained by inhalation of 1.6 to 1.8% isoflurane in humidified air through the tracheal cannula. Body temperature, measured rectally, was kept at 37.1° to 37.3°C *via* a feedback-controlled heating pad. End-tidal partial pressure of carbon dioxide was monitored continuously and kept between 34 and 42 mmHg (Capstar-1000; CWE, Ardmore, PA). The left femoral artery was cannulated for measurement of mean arterial pressure (MAP) and pulse pressure variation (PPV) and to obtain blood samples. The right jugular vein and the left femoral vein were cannulated and used for injections and to measure central venous pressure (CVP) intermittently. Following administration of an intravenous bolus of fentanyl (25 µg/kg; Braun Melsungen AG, Melsungen, Germany) and the start of a continuous fentanyl infusion ($0.5 \mu\text{g kg}^{-1} \text{min}^{-1}$), isoflurane concentration was reduced to 1.1 to 1.3%. After a 10-min equilibration period, MAP, CVP, and PPV were recorded, and baseline values for arterial blood gases, electrolytes, hematocrit, and lactate were measured (I-stat; Hewlett Packard, Böblingen, Germany). PPV was calculated by measuring PPV over a single respiratory cycle: $\text{PPV (\%)} = ((\text{PP}_{\text{max}} - \text{PP}_{\text{min}}) / ((\text{PP}_{\text{max}} + \text{PP}_{\text{min}}) / 2)) \times 100$ and is presented as the mean of 3 to 4 calculations. A PPV above 13% has previously been shown to be highly predictive of preload responsiveness in the rat.¹⁰

Urine was collected in a glass vial placed at the external meatus of the urethra, from the end of the preparation until the end of the experiment, when the bladder was emptied by external compression. Urine production is presented in ml kg⁻¹ h⁻¹ (total production divided by the length of the collection period). After the experiment, the animals were killed with an intravenous injection of potassium chloride.

Measurement of Plasma Volume

Plasma volume was estimated by determination of the initial volume of distribution for human serum albumin (CSL

Behring, King of Prussia, PA) labeled with ¹²⁵I as described in detail previously.^{11,12} This was accomplished by measuring the increase in radioactivity following injection of a known amount ¹²⁵I-human serum albumin (approximately 75 kBq/kg and 0.05 mg of albumin per kilogram) by subtracting the activity in a 250-µl blood sample taken just before the injection from the activity 5 min after the injection. The administered dose was calculated by subtracting the radioactivity in the emptied vial, in the syringe, and in the needle from the total radioactivity in the prepared dose. The volume of distribution for the tracer was then calculated by dividing the administered dose by the resulting change in concentration. The amount of unbound radioactivity in the injected ¹²⁵I-albumin was measured regularly after precipitation with 10% trichloroacetic acid and was found to be less than 1% in all experiments. All samples were counted in a gamma counter (Wizard 1480; LKB-Wallac, Turku, Finland).

Experimental Protocol

Animals were randomized with regard to experimental conditions and resuscitation fluid after preparation, and an investigator blinded to the type of experiments performed the analysis of the data. The investigator performing the experiments was not blinded to group assignment or treatment.

Hemorrhage Group

In the hemorrhage group, animals were bled a total of 8 ml/kg in 5 min. They were then resuscitated with either 5% albumin (8 ml/kg) (CSL Behring: 155 mmol/l sodium, 4 mmol/l caprylate, 4 mmol/l *N*-acetyltryptophan, and chloride at approximately 150 mmol/l) or Ringer's acetate (36 ml/kg) (Fresenius Kabi, Uppsala, Sweden: 131 mmol/l sodium, 4 mmol/l potassium, 2 mmol/l calcium, 1 mmol/l magnesium, 112 mmol/l chloride, 30 mmol/l acetate; osmolality 270 mosmol/kg) during a 30-min resuscitation period. Plasma volumes were measured at baseline, 15 min after resuscitation was completed, and again 2 h later. Arterial blood gases, lactate, hematocrit, and electrolytes were measured at baseline, after hemorrhage, and 2 h after resuscitation. Plasma volumes directly after hemorrhage were calculated as follows: $[\text{Baseline value} - (8 \text{ ml} \times (1 - \text{hematocrit}))]$. MAP, CVP, and PPV were measured at baseline, after hemorrhage, 15 min and 2 h after resuscitation (fig. 1). On a *post hoc* basis, a second set of experiments were performed in animals subjected to hemorrhage, using an identical protocol except that the last plasma volume measurement was performed 4 h instead of 2 h after resuscitation (fig. 1).

Sepsis Groups

Following surgical preparation and baseline measurements as described above, animals were subjected to a cecal ligation and incision (CLI) procedure. This procedure has been

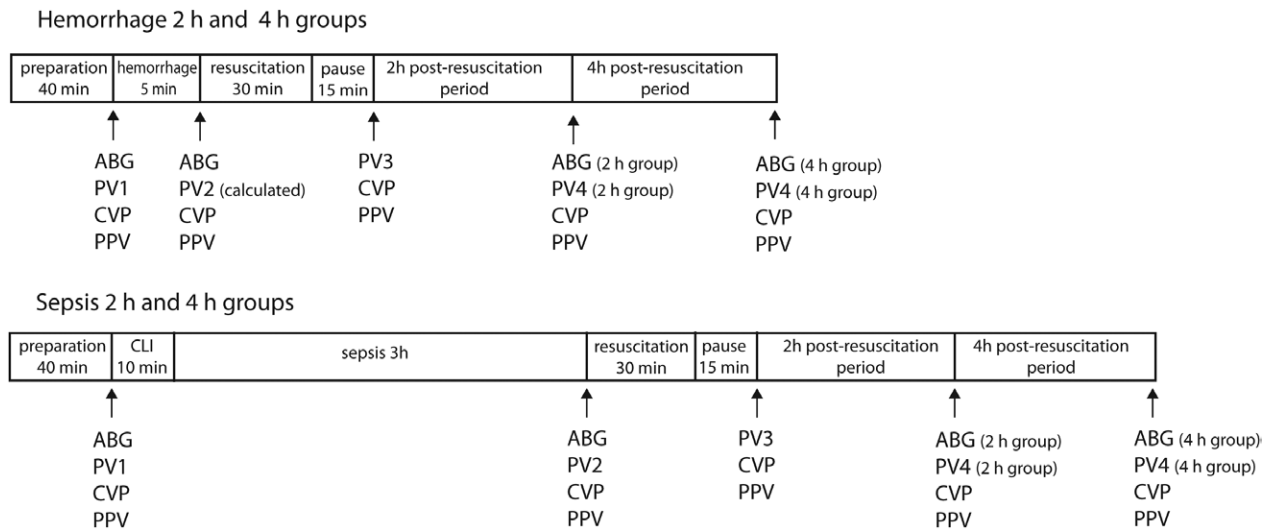


Fig. 1. Schematic diagram of the experimental protocol in the hemorrhage and sepsis groups. ABG = arterial blood gas; CLI = cecal ligation and incision; CVP = central venous pressure; PPV = pulse pressure variation; PV = plasma volume.

described in detail previously.^{11,13} Briefly, following a 3- to 4-cm midline abdominal incision, the cecum was mobilized while carefully avoiding hemorrhage. It then was ligated with a 3.5 silk ligature and a 1-cm incision was made with a scalpel blade. The abdomen was closed with metal clips. Three hours after the CLI procedure, MAP, CVP, and PPV were recorded and blood samples for analysis of plasma volume, arterial blood gases, electrolytes, hematocrit, and lactate were collected. Plasma volume loss was calculated, and the loss was replaced with the same volume of 5% albumin or with 4.5 times this volume of Ringer's acetate over the next 30 min. Plasma volume was measured again at 15 min and at 2 h after completion of the infusion (fig. 1). MAP, CVP, and PPV were measured immediately before plasma volume measurements, and arterial blood gases were measured again at the end of the experiments. On a *post hoc* basis, a second set of experiments were performed in septic animals, using an identical protocol except that the last plasma volume measurement was performed 4 h instead of 2 h after resuscitation (fig. 1). The change in plasma concentration of ¹²⁵I-human serum albumin from 15 min after resuscitation to either 2 h or 4 h after resuscitation was calculated to estimate differences in transcapillary leakage of albumin between the hemorrhage and sepsis groups.

Statistics

A sample size of a least eight in each of the 2-h groups was chosen based on previous studies using the same methodology demonstrating differences in plasma volume between different treatment groups.^{11,14} All data were analyzed by the Kolmogorov–Smirnov test and found to be normally distributed. Changes in plasma volumes and physiological data from baseline to 5 min after hemorrhage or 3 h after sepsis were analyzed with a paired Student *t* test. Effects of albumin and Ringer's acetate on plasma volume in the hemorrhage

and sepsis groups were evaluated by comparing the changes in plasma volume from baseline at the different time points with an unpaired Student *t* test. To test for differences in plasma volume between the hemorrhage and the sepsis animals immediately after resuscitation, an unpaired Student *t* test was used. Bonferroni correction was calculated by dividing the desired α -level with the number of comparisons within the sepsis and hemorrhage groups, respectively. All tests are two tailed. Data are presented as mean \pm SD. Prism 5.0c software was used for the analysis (GraphPad Software, La Jolla, CA)

Results

Hemorrhage Groups

Physiological and Laboratory Data. A total of 29 animals were included in the hemorrhage groups. In the 2-h group, eight and nine animals were resuscitated with albumin or Ringer's acetate, respectively. In the 4-h group, six animals were included in each group. No animals died during the experiment. MAP decreased and PPV increased after bleeding in both groups. After resuscitation, MAP and PPV returned to baseline values. There was a slight decrease in hematocrit already 5 min posthemorrhage. No difference between the resuscitation fluids on any of the physiological or laboratory data shown in tables 1 and 2 could be detected. Urine production was $2.3 \pm 1.0 \text{ ml kg}^{-1} \text{ h}^{-1}$ in the Ringer's acetate groups and $1.8 \pm 0.6 \text{ ml kg}^{-1} \text{ h}^{-1}$ in the albumin groups ($P < 0.05$).

Plasma Volumes. Plasma volume was $41.4 \pm 2.5 \text{ ml/kg}$ at baseline and decreased to $37.0 \pm 2.4 \text{ ml/kg}$ after hemorrhage in the albumin groups, and corresponding values were $40.5 \pm 2.8 \text{ ml/kg}$ and $36.1 \pm 2.7 \text{ ml/kg}$ in the Ringer's acetate groups. In the albumin groups, plasma volume was $46.7 \pm 4.0 \text{ ml/kg}$ 15 min after resuscitation, $45.7 \pm 4.4 \text{ ml/kg}$ at the end of the experiment in the 2-h group, and

Table 1. Hemodynamics for Animals Resuscitated with either 5% Albumin or Ringer's Acetate

Hemodynamic Values	Hemorrhage		Sepsis	
	Albumin	Ringer's	Albumin	Ringer's
Baseline				
MAP (mmHg)	109±27	111±23	110±15	106±14
PPV (%)	11±3	10±5	8±2	8±3
CVP (mmHg)	1.5±0.7	2.0±0.8	1.6±0.5	1.5±0.3
5 min after hemorrhage/3h after CLI				
MAP (mmHg)	77±23*	80±24*	104±15	102±14
PPV (%)	17±7*	17±5*	15±4*	17±7*
CVP (mmHg)	1.1±0.8	1.7±0.7	1.5±0.6	1.4±0.4
15 min postresuscitation				
MAP (mmHg)	110±11	103±16	109±14	100±11
PPV (%)	9±6	9±4	12±3	13±5
CVP (mmHg)	1.8±0.9	2.2±0.9	1.5±0.4	1.5±0.4
2 h postresuscitation				
MAP (mmHg)	106±17	100±17	90±13	88±17
PPV (%)	9±2	13±5	17±6	21±9
CVP (mmHg)	1.8±0.7	2.3±0.9	1.4±0.5	1.3±0.6
4 h postresuscitation				
MAP (mmHg)	109±23	103±7	56±7	65±15
PPV (%)	9±3	7±2	30±10	18±8
CVP (mmHg)	2.1±0.8	2.4±0.5	1.0±0.6	1.0±1.0

Change in parameters from baseline to 5 min after hemorrhage or 3 h after CLI was analyzed with the paired Student *t* test.

* *P* < 0.05. Differences between albumin and Ringer's groups at the different time points in the hemorrhage or sepsis groups were evaluated using the unpaired Student *t* test with Bonferroni correction to adjust for multiple comparisons.

CLI = cecal ligation and incision; CVP = central venous pressure; MAP = mean arterial pressure; PPV = pulse pressure variation.

46.7±4.0 ml/kg at the end of the experiment in the 4-h group (fig. 2). In the Ringer's acetate group, plasma volume was 43.8±4.5 ml/kg 15 min after resuscitation, 45.5±6.2 ml/kg at the end of the experiment in the 2-h group, and 47.2±4.8 ml/kg at the end of the experiment in the 4-h group (fig. 2). At 15 min after resuscitation, the mean difference (±95% CI) between the mean of the albumin and Ringer's acetate groups was 3.0 (±3.3), and corresponding values at 2 and 4 h were 0.2 (±5.6) and 0.2 (±7.2), respectively (fig. 3).

Sepsis Groups

Physiological and Laboratory Data. A total of 28 animals completed the protocol and were included in the analysis. In the 2-h group, eight animals were included in each of the albumin or Ringer's acetate groups. In the 4-h group, six animals were included in each group. No difference in the effect of the resuscitation fluids on any of the physiological or laboratory data shown in tables 1 and 2 could be detected. Average MAP from the start of resuscitation until the end of the 2-h experiment was 107±10 mmHg in the albumin group and 93±12 in the Ringer's acetate group. The corresponding values for the 4-h experiments were 86±12 mmHg and 87±15 mmHg. Urine production was 0.8±0.1 ml kg⁻¹ h⁻¹ in the albumin group and 0.9±0.2 ml kg⁻¹ h⁻¹ in the Ringer's acetate group.

In the 2-h group, four animals (20%) died, and in the 4-h group nine animals (36%) died prior to completion of

all measurements. The animals randomized to receive treatment with either Ringer's acetate (*n* = 7) or albumin (*n* = 6) that died prior to completion of the experimental protocol had a plasma volume loss of 9.7±2.6 ml/kg and 7.9±2.9 ml/kg, respectively, at 3 h after CLI. Plasma volume increased by 4.8 ml/kg to 36.3±1.7 at 15 min after resuscitation with Ringer's acetate and by 5.6 ml/kg to 38.0±1.8 ml/kg after resuscitation with albumin. Lactate in the Ringer's acetate group was 2.3±0.2 mmol/l at baseline and 4.0±1.0 mmol/l at 3 h after CLI, and corresponding values for the albumin resuscitated animals were 2.1±0.3 at baseline and 3.5±0.5 mmol/l. Data from the animals that died prior to completion of all measurements are not included in the analysis below.

Plasma Volumes. Plasma volume in the albumin groups was 40.4±2.1 ml/kg at baseline and decreased to 32.1±3.4 ml/kg 3 h after the CLI procedure. Corresponding values in the Ringer's acetate groups were 39.6±1.9 ml/kg and 32.7±2.8 ml/kg. In animals resuscitated with albumin, plasma volume was 37.8±3.6 ml/kg at 15 min, 29.6±3.2 ml/kg at 2 h, and 27.4±5.8 ml/kg at 4 h after resuscitation (fig. 2). In animals resuscitated with Ringer's acetate, plasma volume was 35.1±2.5 ml/kg at 15 min, 30.6±3.4 ml/kg at 2 h, and 28.3±4.2 ml/kg at 4 h after resuscitation (fig. 2). The relative increase in plasma volume at 15 min postresuscitation by albumin was higher than after resuscitation with Ringer's acetate (adjusted *P* < 0.05). No differences between albumin and Ringer's acetate could be detected after 2 or 4 h (fig. 3). At 15 min after resuscitation, the mean difference

Table 2. Arterial Blood Gas Values, Hematocrit, Lactate, and Potassium for Animals Resuscitated with either 5% Albumin or Ringer's Acetate

	Hemorrhage		Sepsis	
	Albumin	Ringer's	Albumin	Ringer's
Baseline				
pO ₂ (mmHg)	80.3±9.0	79.5±9.0	83.3±9.0	83.3±8.3
pCO ₂ (mmHg)	39.8±3.0	39.8±3.8	40.5±3.8	39.8±3.8
pH	7.46±0.04	7.47±0.3	7.47±0.04	7.48±0.05
Base excess (mEq/l)	4±2	5±1	6±2	5±2
Hct (%)	44±2	45±2	43±3	43±2
Lactate (mmol/l)	2.2±0.5	2.3±0.5	2.0±0.5	2.2±0.3
Potassium (mmol/l)	4.7±0.5	4.8±0.3	4.5±0.4	4.6±0.4
5 min after hemorrhage/3 h after CLI				
pO ₂ (mmHg)	78.8±7.5	78.8±12.0	81.8±10.5	81.0±6.0
pCO ₂ (mmHg)	39.0±3.0	38.3±3.8	4.8±12.8	39.8±3.0
pH	7.46±0.03	7.46±0.04	7.40±0.03	7.41±0.03
Base excess (mEq/l)	4±1	5±1	0±2*	1±2*
Hct (%)	41±2*	41±3*	48±3*	47±2*
Lactate (mmol/l)	2.3±0.6	2.6±0.9	3.5±0.9*	3.3±0.8*
Potassium (mmol/l)	5.2±0.3	5.2±0.3	4.8±0.3	4.8±0.3
15-min postresuscitation				
No blood gas analysis performed				
2-h postresuscitation				
pO ₂ (mmHg)	75.0±8.3	71.3±13.5	80.3±12.0	78.8±16.5
pCO ₂ (mmHg)	37.5±2.3	38.3±5.3	37.5±3.8	34.5±3.8
pH	7.45±0.02	7.42±0.04	7.38±0.05	7.37±0.05
Base excess (mEq/l)	2±2	0±2	-2±3	-4±2
Hct (%)	35±2	36±3	48±5	43±7
Lactate (mmol/l)	1.9±0.5	1.9±0.7	2.3±0.4	2.8±1.0
Potassium (mmol/l)	4.9±0.4	4.5±1.7	5.5±0.7	5.8±0.5
4-h postresuscitation				
pO ₂ (mmHg)	66.0±10.5	70.5±7.5	84.0±9.8	87.8±6.0
pCO ₂ (mmHg)	40.5±6.0	41.3±3.0	33.0±3.0	30.8±4.5
pH	7.42±0.03	7.42±0.03	7.28±0.11	7.36±0.06
Base excess (mEq/l)	1±1	2±1	-10±5	-7±3
Hct (%)	35±2	35±2	45±7	47±4
Lactate (mmol/l)	1.5±0.6	1.4±0.3	5.7±2.7	4.0±2.1
Potassium (mmol/l)	5.0±0.5	4.7±0.2	7.5±1.1	6.9±1.2

Change in parameters from baseline to 5 min after hemorrhage or 3 h after CLI was analyzed with a paired Student *t* test.

* *P* < 0.05. Differences between albumin and Ringer's groups at the different time points in the hemorrhage or sepsis groups were evaluated using the unpaired Student *t* test with Bonferroni correction to adjust for multiple comparisons.

CLI = cecal ligation and incision; Hct = hematocrit; pCO₂ = partial pressure of carbon dioxide; pO₂ = partial pressure of oxygen.

(±95% CI) between the albumin and Ringer's acetate groups was 2.7 (±2.4), and corresponding values at 2 and 4 h were 1 (±3.5) and 0.9 (±6.5), respectively (fig. 3). Concentration of ¹²⁵I-human serum albumin decreased faster in the sepsis groups than in the hemorrhage groups (table 3).

Plasma volume changes 15 min following completion of resuscitation with albumin differed between the hemorrhage and sepsis groups and were 122±32% and 73±30% of the infused volume, respectively (unpaired Student *t* test, corrected *P* < 0.05). Also in the animals resuscitated with Ringer's acetate, plasma volume changes at 15 min postcompletion of resuscitation differed between the hemorrhage and sepsis groups and were 22±7% and 7±9% of the infused volume, respectively (unpaired Student *t* test, corrected *P* < 0.05).

Discussion

Our results show that resuscitation following hemorrhage with albumin or Ringer's acetate at 4.5 times the volume of albumin results in similar plasma volume expansion at 15 min, at 2 h, and at 4 h postresuscitation in rats. In sepsis animals, resuscitation with albumin or Ringer's acetate in the same ratio results in a higher plasma volume in the albumin group at 15 min, whereas plasma volumes at 2 and 4 h are similar. In sepsis, both albumin and Ringer's acetate are less efficient as plasma volume expanders at 15 min compared to the same time points after hemorrhage.

Plasma volume measurement using radiolabeled albumin is an established method, both experimentally and in clinical practice.^{11,12,15} As discussed previously, transcapillary escape

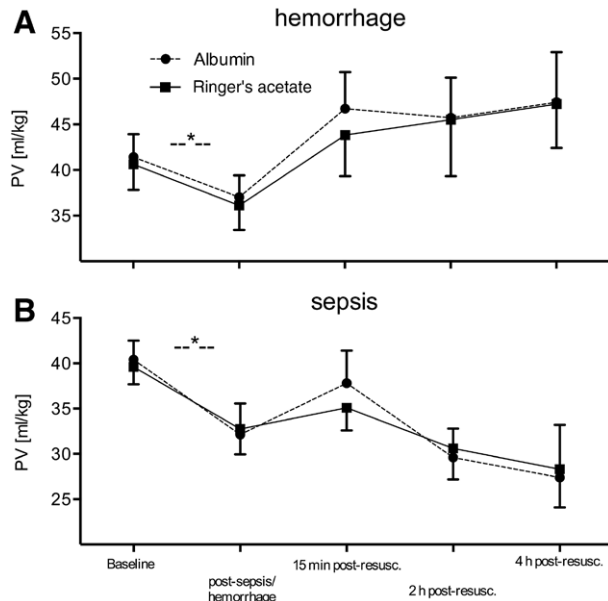


Fig. 2. (A) Absolute plasma volumes (PVs) at baseline, immediately following a controlled hemorrhage of 8 ml/kg, 15 min, 2 h, and 4 h after resuscitation with either 5% albumin or Ringer's acetate ($n = 29$ until 15 min after resuscitation, $n = 17$ for the 2-h group, and $n = 12$ for the 4-h group). (B) Plasma volumes at baseline, 3 h after cecal ligation and incision, at 15 min, 2 h, and 4 h after resuscitation with either 5% albumin or Ringer's acetate ($n = 28$ until 15 min after resuscitation, $n = 16$ for 2-h group, and $n = 12$ for 4-h group; $*P < 0.05$).

of albumin will cause an overestimation of true plasma volume during conditions of increased permeability.^{12,16} By measuring plasma concentrations of radiolabelled albumin at the earliest time point at which complete mixing of tracer probably has occurred, this source of error is minimized.¹² Based on our previous observation that transcapillary escape of albumin increases from approximately 15% per hour during basal conditions to approximately 20% per hour after the CLI procedure,¹¹ it can be calculated that the overestimation of plasma volume is small and in the range of 1.25 to 1.75% under basal or septic conditions, respectively. This in turn means that only 0.5% of the difference in plasma volume between the hemorrhage and sepsis groups can be attributed to a measurement error due to increased leakage of tracer. Free iodine in the injected tracer solution will rapidly distribute in the extracellular space, which may cause an overestimation of plasma volume. Given that free iodine was found to be less than 1% on all experiments, this error will also be small and similar in both hemorrhage and sepsis groups. Taken together, these sources of error are small and will not influence the conclusions made below.

The CLI method has been shown to result in a bacteremia within hours with a high mortality rate.¹³ The observed decrease in plasma volume of approximately 7 ml/kg prior to resuscitation in combination with hemoconcentration suggests that the model induces plasma leakage secondary to increased microvascular permeability. The ongoing plasma

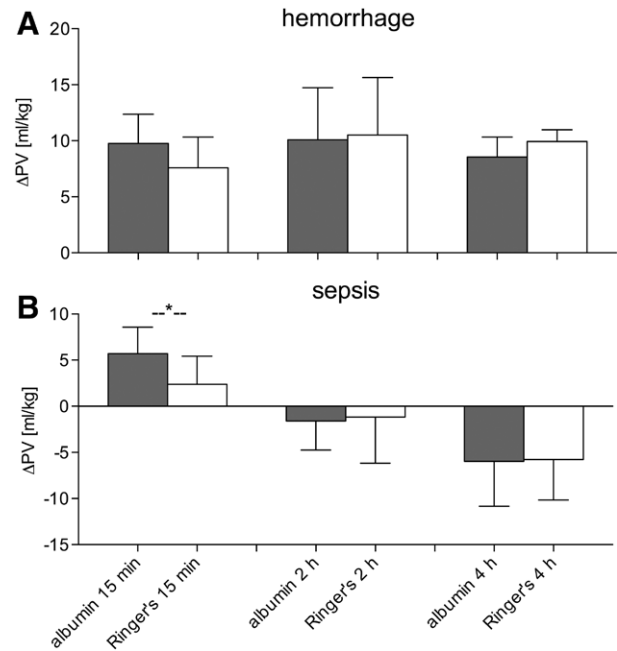


Fig. 3. (A) Change in plasma volume (PV) in animals subjected to a controlled hemorrhage of 8 ml/kg at 15 min, 2 h, and 4 h after resuscitation with either 5% albumin or Ringer's acetate ($n = 29$ until 15 min after resuscitation, $n = 17$ for the 2-h group, and $n = 12$ for the 4-h group). Unadjusted P values for comparison between Ringer's acetate and albumin are $P = 0.04$ at 15 min, $P = 0.86$ at 2 h, and $P = 0.54$ at 4 h. (B) Change in plasma volume in animals subjected to cecal ligation and incision at 15 min, 2 h, and 4 h after resuscitation with either 5% albumin or Ringer's acetate ($n = 28$ until 15 min after resuscitation, $n = 16$ for 2-h group, and $n = 12$ for 4-h group; $*P < 0.05$). Unadjusted P values for comparison between Ringer's acetate and albumin are $P = 0.006$ at 15 min, $P = 0.84$ at 2 h, and $P = 0.94$ at 4 h.

loss after resuscitation in the current study and the increase in the transcapillary escape rate of albumin after CLI in a previous study from our group further supports the hypothesis that microvascular permeability is increased.¹¹

It could be argued that hemorrhage may have induced a systemic inflammatory response syndrome, which could have increased microvascular permeability. However, a hemorrhage of 8 ml/kg is only 11% of the total blood volume in the rat and is unlikely to have increased permeability to a major degree. Our finding that plasma volumes remained

Table 3. Change in Concentration of ¹²⁵I-HSA from 15-min Postresuscitation to 2 or 4 h Postresuscitation, Respectively, in the Hemorrhage and Sepsis Groups

	Hemorrhage	Sepsis
2-h group	14.6 ± 2.6% ($n = 17$)	19.2 ± 4.5% ($n = 16$)*
4-h group	25.2 ± 4.3% ($n = 12$)	32.3 ± 5.1% ($n = 12$)*

Differences between the hemorrhage groups and sepsis groups at the different time points were analyzed using the unpaired Student t test.

* $P < 0.05$.

HSA = human serum albumin.

stable after resuscitation and that concentrations of radiolabeled albumin decreased at a higher rate in the sepsis groups supports this assumption and we conclude that the hemorrhage and sepsis groups differed with regard to permeability as intended when designing the protocol.

The ratio between volumes of Ringer's acetate and albumin of 4.5 to 1 resulted in a similar plasma volume expansion in the hemorrhage groups. The adequacy of this crystalloid to colloid ratio is supported by a previous study showing that if Ringer's acetate is administered in a lower-volume ratio to that of 5% albumin, plasma volume expansion will be significantly lower than in the group resuscitated with albumin.¹⁴ Similar ratios between 0.9% NaCl and 5% albumin have been reported in hemorrhage models, indicating that, with regard to plasma volume expansion, 0.9% NaCl and Ringer's acetate have similar efficacy.⁵ Also, clinical studies support that crystalloid solutions have poor plasma-expanding properties and that only approximately 20% of 0.9% NaCl and Ringer's solutions remain intravascularly immediately after resuscitation.^{3,4,9,17}

Our hypothesis that 5% albumin would be a relatively less potent plasma volume expander than a crystalloid in sepsis with increased plasma leakage compared to conditions with a normal microvascular permeability was not supported by our result that plasma volume was equal in the Ringer's acetate and albumin groups 2 h after resuscitation, and initially even higher in the albumin-treated animals. Based on the concern that 2 h was too short a time for an increased permeability to affect the plasma-volume-expanding properties of albumin, we added a second group of sepsis animals in which plasma volume was evaluated 4 h after resuscitation. Also the 4-h data failed to demonstrate a difference in plasma volume between the Ringer's acetate group and the albumin group. It could be argued that a difference in the plasma-volume-expanding effect might have been detected by measuring plasma volume at even later time points after resuscitation. However, given that more than one-third of the animals died before the end of the 4-h period, longer experiments were not considered feasible.

It could be argued that the lack of difference in plasma volume expansion between albumin and Ringer's acetate could be attributed to low statistical power. On average, sepsis animals were resuscitated with 8 ml/kg of albumin. If our hypothesis that the 1:1.4 ratio of albumin to Ringer's acetate observed in the "Saline versus Albumin Fluid Evaluation" study could be explained by a decreased plasma volume expansion by albumin this corresponds to approximately 6-ml/kg difference in plasma volume between animals resuscitated with albumin and animals resuscitated with Ringer's acetate. As can be seen from the 95% CI of the difference between the groups this is highly unlikely. However, we cannot exclude that a smaller change in plasma volume expansion by albumin could have been detected at 2 and 4 h after resuscitation if we had included a higher number of animals.

Our result of lower plasma volume expansion by both albumin and Ringer's acetate in the sepsis group than in the

hemorrhage group at 2 and 4 h was expected and probably reflects the ongoing loss of plasma in the septic animals. However, our finding of an almost 50% lower plasma-volume-expanding effect of both albumin and Ringer's acetate during sepsis compared to that observed after a hemorrhage already 15 min after resuscitation is unlikely to be fully explained by ongoing plasma leakage at the rate observed prerescuscitation. Sepsis animals lost approximately 7 ml/kg of plasma on average prior to resuscitation, and even if we assume that this loss occurred during the last hour preceding resuscitation, when animals were most strongly affected by the sepsis, this could at most account for 35% of the observed difference in the plasma volume expansion of approximately 5 ml/kg between the sepsis and the hemorrhage group, and other mechanisms most likely contributed to this result. Following hemorrhage, homeostatic mechanisms such as activation of the baroreceptor reflex will strive to normalize intravascular volumes by mobilizing fluid from the extravascular compartment. This hypothesis is supported by our result that the iso-oncotic 5% albumin solution increased plasma volume by more than the infused volume and that hematocrit had decreased already prior to resuscitation. The mobilized fluid is added to that given during the resuscitation and contributes to the measured plasma volume expansion. In contrast, sepsis disrupts homeostatic mechanisms such as autoregulation of capillary pressure. This means that the increased blood pressure seen during resuscitation will be transferred to the exchange vessels and may transiently increase plasma leakage, thereby contributing to the reduced volume-expanding properties of both colloids and crystalloids in sepsis.^{18,19}

Our rat model differs from human sepsis in several aspects. We chose to fluid-resuscitate the animals at one single occasion according to measured loss of plasma in order to make the groups comparable and to isolate effects of fluid resuscitation on plasma volume. This contrasts to clinical practice in which plasma volume is rarely measured and fluid resuscitation is a continuous process with end points such as MAP, lactate, and central venous oxygen saturation and is often combined with the use of inotropes and vasopressors. The latter may influence permeability and transcapillary hydrostatic pressures, which in turn may differentially influence the plasma-volume-expanding properties of colloids and crystalloids.²⁰ Normal transcapillary escape of albumin in humans is approximately 5% per hour in healthy subjects and is reported to increase to approximately 15% per hour in sepsis, and corresponding values in the rat are 15% per hour and 20% per hour, respectively.^{11,21} These differences in transcapillary escape of albumin suggest that also species differences may be of importance for the clinical relevance of this study.

Conclusion

The current study does not support the hypothesis that pathophysiological conditions associated with increased

microvascular permeability change the plasma-volume-expanding properties of 5% albumin relative to that of crystalloids and indicates that also in severe sepsis the ratio of albumin to crystalloid for equal plasma volume expansion is approximately 1 to 4.5.

The findings indicate that mechanisms other than changes in permeability may influence the plasma volume expansion of albumin relative to that of crystalloids and further research in this area is warranted.

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Competing Interests

The authors declare no competing interests.

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