Haemodynamic and renal effects of resuscitation of dogs from haemorrhagic shock with hypertonic saline or Lactated Ringer's solution combined with whole blood

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ABSTRACT: Despite numerous studies on haemorrhagic shock treatment, the choice of fluid and the rate of administration is a subject of controversy. Early haemodynamic effects, neuroendocrine response and renal function following acute sodium load with either hypertonic saline (HS) or Lactated Ringer's (LRS) were investigated in a model of controlled haemorrhagic shock. Six anaesthetized dogs were bled 30% of calculated blood volume, i.e., 27 ml/kg b.w. over 15 min and left untreated for 30 min. Afterward, the dogs were treated with either HS 4 ml/kg b.w. or LRS 81 ml/kg b.w., followed by whole blood. After a resting period of at least 30 days, the dogs underwent a similar second shock experiment, but with the second solution. Haemodynamics, renal effects and neuroendocrine response were studied during shock and at 15, 30, 45 and 60 min after administration of HS or LRS and 30 and 60 min after the addition of whole blood. LRS treatment resulted in significantly higher cardiac filling pressures and cardiac performance indexes than HS but, following whole blood administration, there were no differences between treatments, except that pulmonary capillary wedge pressure was higher in the LRS group. Urinary output and glomerular filtration rate index were restored to normal values by both treatments; however, they were significantly higher in the LRS group until whole blood was administered. LRS and HS restored plasma norepinephrine to baseline values with no difference between the treatments. Both solutions improved haemodynamics and renal function but their effects were short lived and whole blood was required for a favourable outcome, regardless of the initial solution used. In contrast to LRS, HS did not overload the cardiovascular system.

Keywords: haemorrhagic shock; fluid therapy; dog; haemodynamics; renal function; norepinephrine

Haemorrhagic shock is a clinical syndrome manifested by reduced perfusion of vital organs leading to inadequate delivery of oxygen and nutrients necessary for normal tissue and cellular function (Krausz, 2006). Haemorrhagic shock occurs when a patient loses 30% or more of its estimated blood volume. Venous return to the heart decreases and this in turn causes decreased cardiac output and activation of a variety of homeostatic mechanisms including hormonal modulation and cardiovascular neurologic reflexes. Compensatory mechanisms help improve blood volume, sustain the blood pressure, and maintain perfusion to the vital organs such as heart, brain and kidney. Perfusion can be maintained for a short period of time following mild to moderate volume loss, however, without intervention, compensatory mechanisms eventually fail and the complications of shock ensue. The goal of treatment is to improve tissue perfusion and oxygenation, and the mainstay of the treatment for haemorrhagic shock is the expansion of the intravascular volume by intravenous fluid therapy (Mandell and King, 1998).

Currently, isotonic crystalloids such as Lactated Ringer's are recommended to be used as the initial resuscitation fluid in the treatment of haemorrhagic shock. No more than one-fifth of unexcreted isotonic crystalloids remain within the plasma volume after

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equilibration, and for this reason, a large volume is necessary to effectively expand plasma volume and increase the preload (Svensen and Hahn, 1997). The poor intravascular retention of balanced salt solutions supports intravascular volume transiently but later can cause tissue oedema formation with impaired oxygen perfusion (Boldt, 2000).

Hypertonic saline (HS) solutions, with or without colloids, have been shown to expand plasma volume to three to four times the volume infused through endogenous fluid redistribution (Gala et al., 1991; Dubick and Wade 1994). The second possible outcome of HS action is a positive inotropic effect (Velasco et al., 1980; Rocha e Silva et al., 1987; Constable et al., 1991; Tyler et al., 1994). The increasing cardiac output is apparently mediated by a reflex arc involving the vagus nerve and a receptor in the lung (Velasco et al., 1980; Nerlich et al., 1983; Rocha e Silva et al., 1987). Mean arterial pressure is restored and peripheral and pulmonary vascular resistance are reduced (Rowe et al., 1972; Rocha e Silva et al., 1987; Muir and Sally, 1989; Bertone et al., 1990; Muir, 1990). The cardiovascular enhancement may in part be a non-specific neuroendocrine response including the effects of catecholamines as hyperosmotic infusion reduces plasma catecholamine concentrations in parallel with a significant increase in mean arterial pressure and cardiac output in haemorrhaged animals (Mermel and Boyle, 1986; Ronning et al., 1995).

The present study was conducted to determine the resuscitative efficacy and safety of 7.5% saline compared to Lactated Ringer's solution, both of them followed by administration of whole blood in haemorrhaged anaesthetized dogs. Specifically, the early cardiovascular haemodynamic effects, neuroendocrine response and renal function following acute sodium load were investigated. The protocol used resembled a clinical situation in which access to whole blood is usually delayed.

MATERIAL AND METHODS

Animals

Six adult, male Beagle dogs, weighing between 13.0 and 18.5 kg, were used in the study. The dogs were judged to be healthy based upon clinical examination, auscultation of the heart and thorax and normal blood work, i.e., complete blood count (CBC), white cell differential count (WCDC) and serum biochemistry profile including blood urea nitrogen, creatinine, phosphorus, total protein, albumin and electrolytes, potassium (K), sodium (Na), chloride (Cl) (data not shown).

The experiment was carried out under general anaesthesia. The dogs were randomly allocated to the treatment of haemorrhagic shock with 7.5% saline or Lactated Ringer's. After a resting period of at least 30 days, the dogs underwent a subsequent shock experiment using the same protocol except with the second solution. During the resting period, they were clinically checked daily and before entering the second episode of shock, the same blood profile as before the first shock episode was determined. No clinical abnormalities or deviations from a normal blood profile were found (data not presented). Before each experiment, food was withheld for 12 h, but unlimited access to water was allowed up to the time of premedication. All procedures complied with applicable Slovenian governmental regulations (Animal Protection Act UL RS, 43/2007) and were approved by the Ministry of Agriculture, Forestry and Food, Veterinary Administration of the Republic of Slovenia; license No 326-07-118/97.

Anaesthetic protocol

The dogs were premedicated with ketamine (Ketanest 10%; Parke-Davis, Freiburg, Germany) 2 mg/kg b.w. and midazolam (Dormicum; F. Hoffmann-La Roche, Basel, Switzerland) 0.3 mg/kg b.w. mixed together and given intravenously. Anaesthesia was induced with propofol (Diprivan; Zeneca Pharmaceuticals, Wilmington, Delaware, USA) 3–4 mg/kg b.w. intravenously 1–2 min after premedication. After endotracheal intubation, anaesthesia was maintained with a continuous infusion of ketamine 2 mg/kg b.w./h, using a precision syringe pump (Perfusor® fm; B Braun, Melsungen AG, Germany), and isoflurane (Forane; Abbott Laboratories, Baar, Switzerland) in 100% oxygen at end-tidal isoflurane concentration (FE'ISO) 1.5%, which was reduced to 1.1% after the initiation of haemorrhagic shock and increased back to 1.5% after whole blood was given back to the dogs. Ketamine was given until 30 min before the end of experiment. The dogs were connected to an anaesthesia machine (Dräger Tiberius 800; Lübeck, Germany) using a circle circuit. Muscle relaxation was achieved with intravenous administration of 0.4 mg/kg b.w. atracurium (Tracrium; Glaxo Wellcome SpA, Parma,

Italy) repeated every 45–60 min at the dose of 0.2 mg/kg b.w. The dogs were mechanically ventilated (Ventilog; Dräger; Lübeck, Germany) with a tidal volume of 15–20 ml/kg b.w. and maximum airway pressure of 10 cm H_2O within the circuit. The end expiratory pressure was zero. Until the baseline measurements, the ventilatory settings were adjusted to obtain end-tidal CO_2 tensions ($PE'CO_2$) between 35 and 45 mmHg. After that they were left unchanged until the end of the experiment.

FE'ISO, $PE'CO_2$, arterial oxygen saturation measured with pulse oximetry (SpO_2), respiratory rate and airway pressure within the breathing circuit were continuously monitored during anaesthesia (RGM 5250; Ohmeda, Louisville CO, USA). Tidal volume and minute volume of respiration were measured by means of mechanical volumetry (Dräger Tiberius 800; Lübeck, Germany). A three-channel electrocardiograph (lead II) and esophageal thermometer were installed and heart rate (HR) and body core temperature were measured (HP Model 78354A; Hewlett Packard GmbH, Hamburg, Germany).

The dogs were given cefazolin (Cefamezin; Krka, Novo mesto, Slovenia) 20 mg/kg b.w. intravenously immediately after anaesthesia was induced. Cefazolin administration was repeated once 4 h later. Lactated Ringer's (Sestavljen natrijev laktat; B Braun Melsungen AG, Melsungen, Germany) was infused at 10 ml/kg b.w./h to all dogs during the experiment as maintenance fluid.

The dogs were positioned in a dorsal recumbency on a heated surgical table (33 °C) during the anaesthesia. The temperature of the air in the theatre was maintained at 20–24 °C. Anaesthesia lasted 4 h and 50 min on average (4 h and 30 min to 5 h and 15 min), depending upon the time required for the instrumentation of the dogs.

After the end of the experiment, all dogs were oxygenated until they were extubated and were left undisturbed on heating pads in a recovery room. LRS-treated dogs developed serous nasal discharge and dyspnea and were oxygenated until they were able to stand. Dyspnea was severe only in one dog, which was treated with furosemide (Edemid; Lek, Ljubljana, Slovenia) 2 mg/kg b.w. intravenously. Within two hours after the end of each experiment, all dogs, except the one in the LRS group, which required furosemide treatement and recovered slower, were able to walk, eat and drink water. They were then transferred to kennels and offered water and food (Hill's a/d; Hill's Pet Nutrition Inc, USA).

Instrumentation for experiments

A 20-gauge catheter was introduced into a cephalic vein for the administration of anaesthetics and maintenance fluid. Instrumentation was performed after the induction of anaesthesia. Both femoral arteries were percutaneously cannulated with 20-gauge catheters. The left femoral artery was used to measure systolic (SAP), diastolic (DAP) and mean arterial blood pressure (MAP), (HP Model 78354A; Hewlett Packard GmbH, Hamburg, Germany). The right femoral artery was used for arterial blood sampling and to perform bleeding. The right external jugular vein was percutaneously cannulated with a 17-gauge catheter that was used for venous blood sampling and administration of fluids and whole blood. A 7-F, 110 cm flow-directed Swan-Ganz catheter (131HF7; Baxter Healthcare Corporation, Irvine, CA, USA) was percutaneously introduced into the left external jugular vein and advanced into the pulmonary artery. The correct position was verified by observation of the appropriate pressure waveforms. A Swan-Ganz catheter was used to measure pulmonary artery pressures (systolic, diastolic and mean; PAP/S, PAP/D, PAP/M), pulmonary capillary wedge pressure (PCWP), central venous pressure (CVP) and cardiac index (CI); (HP Model 66S; Hewlett Packard GmbH, Hamburg, Germany). The Swan-Ganz catheter was also used for sampling mixed venous blood. A urinary catheter was introduced to collect urinary samples and to measure urinary output (UO). All the catheters were removed at the end of the experiment.

Experimental design

The experiment was divided into 12 phases (Table 1). After the instrumentation was completed, the dogs were allowed to stabilize for 30 min. Once a steady state was achieved, baseline measurements were taken (phase 1) and the dogs were bled 30% of calculated total blood volume, i.e., 27 ml/kg b.w. over 15 min (phase 2, blood withdrawal completed). The shed blood was collected in 60 ml syringes with CPDA-1, which was added to prevent coagulation. The blood was then injected into a collecting bag (Teruflex, Terumo corp., Tokyo, Japan) from which CPDA-1 was taken. The blood was kept at room temperature until it was transfused into the dogs. The dogs were left untreated for 30 min (phase 3, 15 min after the end of haemorrhage; phase 4,

Table 1. The timeline of events

Phases	Events	Time from the measurements (min)	
		baseline	previous
1	baseline measurements	0	0
Beginning of the blood withdrawal			
2	blood withdrawal completed	15	15
3	shock (15 min)	30	15
4	shock (30 min)	45	15
Fluid therapy initiated			
5	HS (4 ml/kg) or LRS (40 ml/kg) administration completed	50	5
6	LRS (total) administration completed	60	10
7	30 min after the commencement of fluid therapy	75	15
8	45 min after the commencement of fluid therapy	90	15
9	autotransfusion commenced	105	15
10	autotransfusion completed	120	15
11	30 min after the completion of the autotransfusion	150	30
12	60 min after the completion of the autotransfusion Anaesthesia terminated	180	30

30 min after the end of haemorrhage). Afterward, the dogs were randomly allocated to the HS or Lactated Ringer's (LRS) group. HS was prepared from 10% NaCl solution (Solutio natrii chloridi 10%, Zavod za transfuzijo krvi, Ljubljana, Slovenia) by dilution with distilled water (Aqua redestilata, Pliva, Zagreb, Croatia). The volume of infused resuscitation fluid was determined according to the sodium load, i.e. HS (4 ml/kg b.w.) or 40 m/kg b.w. of LRS that contained equal amounts of sodium were administered over 4 min. After the measurements (phase 5), the remainder of the LRS (41 ml/kg b.w.) was administered over 10 min in the LRS group. Phase 6 represents the end of LRS administration. Measurements were taken 30 min (phase 7), 45 min (phase 8) and 60 min (phase 9) subsequently. The shed blood was then transfused over 15 min in both treatment groups (phase 10, end of the transfusion). The dogs were left undisturbed for the following hour (phase 11, 30 min after the end of transfusion; phase 12, 60 min after the end of the transfusion). At the end of the follow-up hour, the dogs were allowed to recover from anaesthesia.

Sample collection and analysis

Blood samples for determination of CBC and WCDC, serum biochemistry profile and blood gas analysis were collected at all phases of the experiment. Urine samples for creatinine and electrolyte determination were collected at all phases of the experiment except at phase 5. Blood samples for plasma norepinephrine determination were collected at phases 1, 4, 5, 6 and 12.

Samples of venous blood for determination of CBC and WCDC were collected into Microtainer tubes containing the anticoagulant K_2EDTA (Becton Dickinson, NJ, USA) and analyzed immediately after collection, using an automated laser haematology analyzer Technicon H*1 (Siemens Bayer, Munich, Germany).

Samples of venous blood for determination of serum biochemistry profile were collected into serum separator tubes (Vacutainer, Becton Dickinson, NJ, USA). The samples stood for 15 min at room temperature to clot, followed by centrifugation at 1300g for 10 min at 4 °C. Serum was separated and stored at –20 °C until analysis. The concentration of electrolytes was determined with an electrolyte analyzer (ILyte, MA, USA). Other serum biochemistry profile parameters, such as blood urea nitrogen, creatinine, phosphorus, total protein and albumin were determined using an automated biochemistry analyzer Technicon RA-XT (Siemens Bayer, Munich, Germany).

Arterial (a) and mixed venous (mv) blood samples for blood gas analysis were collected anaerobically

in heparinized syringes and analyzed immediately (AVL Compact 3, Graz, Austria). pH and blood gas values were measured at 37 °C with correction for body temperature and haemoglobin concentration. Samples were analyzed for pH, partial pressure of CO_2 (PCO_2), partial pressure of oxygen (PO_2), actual bicarbonate concentration (HCO_3^-), base excess (BE_{ecf}) and oxygen saturation (SO_2). PCO_2, PO_2 and pH were measured directly; other variables included in the report were calculated.

Urine samples were collected into 15 ml centrifuge tubes and centrifuged at 2000g for 10 min at room temperature. Supernatants were collected and used immediately for determination of creatinine and electrolytes. For urine creatinine determinations, samples were diluted 1 : 20 or 1 : 40 prior to measurement with an automated biochemistry analyzer Technicon RA-XT (Siemens Bayer, Munich, Germany). Urine electrolytes were determined with an electrolyte analyzer (ILyte, MA, USA).

Urine creatinine concentration was determined in order to calculate endogenous creatinine clearance and glomerular filtration rate index (GFRI). Creatinine in serum and urine was measured by an automated biochemistry analyzer Technicon RA-XT (Siemens Bayer, Munich, Germany) using Jaffe's reaction kinetics.

GFRI (ml/min/kg b.w.) was calculated as glomerular filtration rate (GFR; ml/min)/body weight (kg), and GFR as $(V \times U_{crea})/S_{crea}$, where V is the volume of urine collected between the two phases, U_{crea} the urinary creatinine concentration (μmol/l), and S_{crea} the serum creatinine concentration (μmol/l). S_{crea} was calculated as the mean of serum creatinine concentrations from two samples taken in two consecutive phases.

Fractional urinary excretion of sodium (FE_{Na}) was calculated using the ratio of the clearance of the sodium to that of creatinine, where U indicates urinary values and S indicates serum values:

$$FE_{Na}\,(\%) = \frac{U_{Na} \times V/S_{Na}}{U_{crea} \times V/S_{crea}} \times 100 = \frac{U_{Na}/S_{Na}}{U_{crea}/S_{crea}}$$

Samples of venous blood for determination of norepinephrine concentration were collected into cold tubes (tubes were on ice) containing anticoagulant K_3EDTA (Greiner Bio-One GmbH, Kremsmünster, Austria) and imm ediately centrifuged at 1500g for 10 min at 4 °C. Plasma was harvested and stored at –20 °C until analysis. Norepinephrine concentrations were determined by RIA (Manz et al., 1990).

Hemodynamic monitoring and oxygen transport variables. Haemodynamic measurements, including HR, arterial blood pressure, CVP, PAP, PCWP and cardiac index (CI), were taken at all phases of the experiment. CI (cardiac output/body weight) was measured in triplicate by thermodilution with 3 ml of isotonic saline at 23 °C and the average value recorded. Stroke volume index (SVI) was calculated as CI/HR. Systemic vascular resistance (SVR) was calculated as (MAP – CVP)/CI and pulmonary vascular resistance (PVR) as (PAP/M – PCWP)/CI. Arterial and mixed venous oxygen contents (CaO_2 and $CmvO_2$, respectively) were calculated as $(SO_2 \times Hb \times 1.39) + 0.00314 \times PO_2$. Systemic oxygen delivery index (DO_2I) was calculated as CI × CaO_2 × 10 and systemic oxygen consumption index (VO_2I) as CI × (A-VdO_2) × 10, where A-VdO_2 is the arterio-venous difference in oxygen content ($CaO_2 - CmvO_2$).

Statistical analysis

The data were analyzed using commercial statistical software SPSS (SPSS 15.0 for Windows, Chicago, Illinois, USA). With the exception of renal parameters (UO, GFRI and FENa), values for all measured or calculated parameters were analyzed by use of parametric statistical methods. In each treatment group, HS and LRS, repeated measures ANOVA were used to determine the statistical significant changes of parameters during the experiment. Differences in parameters between two different treatments, LRS and HS, were calculated and subjected to repeated ANOVA measurements in order to compare them with baseline differences. All data analyzed by use of parametric methods are expressed as means ± SD (standard deviation).

It is difficult to establish that the data have a particular distributional form by formal normality tests when the sample size is very small (six dogs in the present study; Petrie and Watson, 1999). Renal parameters, which showed great biological variability, were analyzed by use of nonparametric statistical methods. In each treatment group, HS and LRS, the Friedman test, a nonparametric equivalent to repeated measures ANOVA, was used to determine global interactions and the Wilcoxon Signed Rank test for detailed comparisons in order to determine significant changes of renal param-

eters during the experiment. Differences in renal parameters between different treatments were calculated and then subjected to the same nonparametric statistical analyses (Friedman test, Wilcoxon Signed rank test). Renal parameters are expressed as median and range. In all analyses, a value of $P < 0.05$ was considered significant.

RESULTS

Haemorrhage caused marked effects on haemodynamics (Table 2 and Figure 1–4). In both groups, CI, SVI, DO_2, MAP, CVP, PAP/M (except at 15 min of shock), and PCWP decreased, while HR, SVR and PVR increased during haemorrhage without significant differences between the groups when compared to the baseline difference. VO_2 decreased in the LRS group and increased in the HS group; however, this was not significant.

After the first part of resuscitation, that is when equal sodium load was administered (4 ml/kg of HS or 40 ml/kg of LRS; phase 5), MAP increased in both groups but there was no significant change in the difference between the groups. However, LRS restored MAP to above pre-haemorrhage values and HS to below them. On the other hand, CI and SVI increased to above pre-haemorrhage values with both treatments, but the change in the difference between the groups was not significant. CVP, PAP/M and PCWP increased more with LRS than with HS treatment and the change in the difference between the treatments was significant. SVR and PVR decreased and DO_2 and VO_2 increased after administration of both fluids with no significant change in the difference between the treatments.

After the second part of resuscitation, when the rest of LRS was administered (phase 6), the difference between the groups when compared to the baseline difference was significant for CI, SVI, MAP, CVP, PAP/M and PCWP, all the parameters except MAP reaching their peak values in the LRS group. CI and SVI values in HS-treated dogs decreased compared to the first part of resuscitation and were below pre-haemorrhage values. SVR and PVR decreased and DO_2 and VO_2 increased in LRS-treated dogs. On the other hand, SVR and PVR increased and DO_2 and VO_2 decreased in the HS group. However, the change in the difference between the groups in comparison with baseline difference was not significant.

After whole blood was administered (phase 10), the haemodynamic variables CI, SVI, DO_2, VO_2, MAP, CVP, PAP/M and PCWP, exceeded pre-haemorrhage values with both treatments, however, the change in the difference between the treatments in comparison with the basal difference was significant only for PCWP.

At the end of the experiment (phase 12), a significant change in the difference between the treatments was observed only for VO_2 and PCWP when compared to the baseline difference. VO_2 was higher in the HS group and PCWP in the LRS group.

Oxygen delivery/consumption ratio ($DO_2 : VO_2$) decreased below normal values of 4 : 1 (White, 1987) during haemorrhagic shock. Both treatments successfully increased the ratio during resuscitation, and it persisted above 4 : 1 until the end of the experiment. The $DO_2 : VO_2$ ratio was lower and the duration of the effect was shorter in HS-treated dogs. The difference between the treatments, when compared to the baseline difference, was significant only prior to the commencement of autotransfusion (phase 9). There was no significant change in the difference between the treatments at the end of the experiment.

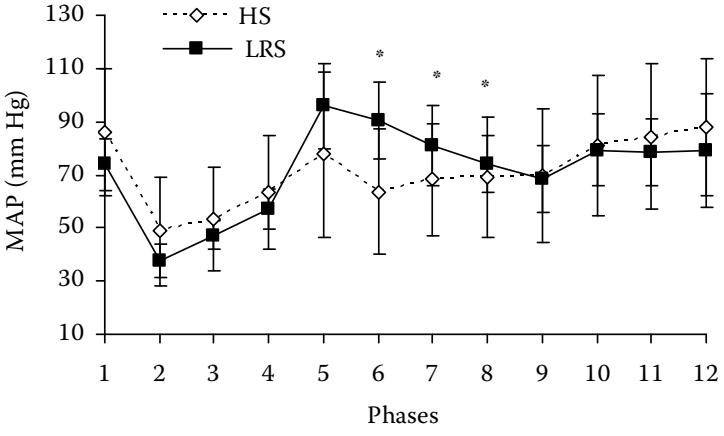

Figure 1. Mean arterial pressure (MAP; mm Hg) during the experiment. Values are mean ± SD for Lactated Ringer's (■ LRS; $n = 6$) and 7.5% saline (◇ HS; $n = 6$)

*significant change ($P < 0.05$) of LRS – HS difference in comparison with baseline difference (repeated measures ANOVA)

Table 2. Haemodynamic variables (mean ± SD) of dogs at baseline, during haemorrhagic shock and after resuscitation with Lactated Ringer's (LRS; $n = 6$) and whole blood (WB) or 7.5% saline (HS; $n = 6$) and whole blood (WB)

Variable	Phase						
	1	4	5	6	9	10	12
	Baseline	Shock	HS 4 ml/kg or LRS 40 ml/kg completed	LRS total completed	WB commenced	WB completed	End of experiment
Heart rate (beats/min)							
LRS	121 ± 6	118 ± 11	114 ± 8	116 ± 8	124 ± 14	117 ± 9	122 ± 17
HS	128 ± 13	130 ± 15	133 ± 16•	121 ± 19•	127 ± 18	123 ± 13	126 ± 11
Cardiac index (ml/min/kg)				*	*		
LRS	221.9 ± 47.9•	95.8 ± 27.0•	263.9 ± 74.8▽	326.4 ± 51.6	273.1 ± 68.7	303.8 ± 67.5	309.4 ± 89.5
HS	210.4 ± 34.5•	108.0 ± 29.6•	226.7 ± 60.6•	162.7 ± 40.5	175.8 ± 28.8•	311.6 ± 61.6	327.8 ± 82.3
Stroke volume index (ml/beat/kg)				*	*		
LRS	1.83 ± 0.36•	0.82 ± 0.26•	2.31 ± 0.66	2.81 ± 0.50•	2.18 ± 0.37•	2.58 ± 0.49	2.49 ± 0.44
HS	1.66 ± 0.32•	0.84 ± 0.25•	1.72 ± 0.52	1.35 ± 0.31	1.41 ± 0.33•	2.56 ± 0.55	2.61 ± 0.69
Systemic vascular resistance (mmHg/ml/kg)							
LRS	0.309 ± 0.048•	0.579 ± 0.129•	0.334 ± 0.057•	0.236 ± 0.052	0.229 ± 0.040	0.225 ± 0.029	0.231 ± 0.045
HS	0.388 ± 0.147•	0.547 ± 0.205•	0.321 ± 0.155	0.353 ± 0.134	0.373 ± 0.197•	0.232 ± 0.091	0.254 ± 0.086
Pulmonary vascular resistance (mmHg/ml/kg)				*			
LRS	0.041 ± 0.012•	0.064 ± 0.027•	0.035 ± 0.017	0.026 ± 0.008	0.027 ± 0.007	0.026 ± 0.007	0.029 ± 0.013
HS	0.034 ± 0.008•	0.056 ± 0.015•	0.032 ± 0.005	0.037 ± 0.014	0.034 ± 0.005•	0.024 ± 0.005	0.025 ± 0.006
Oxygen delivery (ml O_2/kg/min)							
LRS	39.44 ± 10.64•	16.35 ± 5.29•	30.25 ± 11.33	31.17 ± 8.59	35.54 ± 13.09⊗	44.57 ± 12.84	47.40 ± 17.30
HS	39.28 ± 7.27•	18.72 ± 5.28•	33.16 ± 8.97•	24.00 ± 5.34	25.84 ± 4.08•	48.81 ± 8.37	53.11 ± 13.77
Oxygen consumption (ml O_2/kg/min))							*
LRS	4.82 ± 1.15•	4.29 ± 0.88•	4.72 ± 1.34•	5.67 ± 0.46	5.14 ± 1.25	5.69 ± 0.82	5.22 ± 1.22
HS	5.14 ± 1.17•	5.70 ± 1.03•	5.84 ± 1.57	5.36 ± 1.88	6.33 ± 1.59•	6.67 ± 1.18	7.30 ± 2.25
DO_2 : VO_2					*		
LRS	8.1 ± 1.1 : 1	3.8 ± 0.7 : 1	6.4 ± 1.6 : 1	5.6 ± 1.7 : 1	6.9 ± 2.1 : 1	7.8 ± 2.2 : 1	8.8 ± 2.4 : 1
HS	7.8 ± 1.3 : 1	3.3 ± 0.6 : 1	5.8 ± 1.0 : 1	5.1 ± 2.5 : 1	4.2 ± 0.7 : 1	7.4 ± 0.9 : 1	7.4 ± 1.0 : 1

*significant change ($P < 0.05$) in LRS-HS difference in comparison with baseline difference (repeated measures ANOVA)

•significant difference ($P < 0.05$) between two subsequent phases

⊗near significant difference ($P = 0.051$) between two subsequent phases

▽ near significant difference ($P = 0.054$) between two subsequent phases

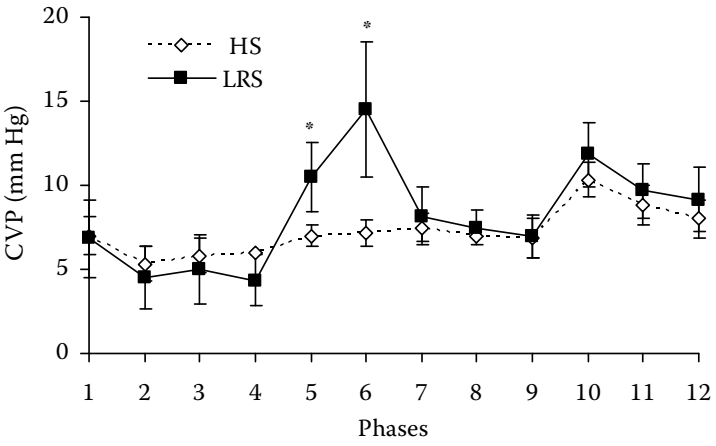

Figure 2. Central venous pressure (CVP; mmHg) during the experiment. Values are mean ± SD for Lactated Ringer's (■ LRS; n = 6) and 7.5% saline (◇ HS; n = 6)

*significant change (P < 0.05) of LRS – HS difference in comparison with baseline difference (repeated measures ANOVA)

Renal function is presented in Table 3. During haemorrhagic shock, urinary output decreased below the value of 1–2 ml/kg b.w./h required for normal kidney function (Mathews, 2006). Both treatments increased urinary output, LRS treatment significantly. Although LRS treatment resulted in higher urinary output than HS, the difference between the treatments was not significant in comparison with the basal difference. A statistically significant change in the difference between the treatments was observed only during resting period 1 (phases 6 to 9). There was no significant change in the difference between the treatments at the end of the experiment (resting period 2; phases 10 to 12).

Glomerular filtration rate index increased with both treatments; the difference between them was significantly changed during fluid administration (phases 4 to 6) and resting period 1 (phases 6 to 9). During whole blood administration (phases 9 to 10),

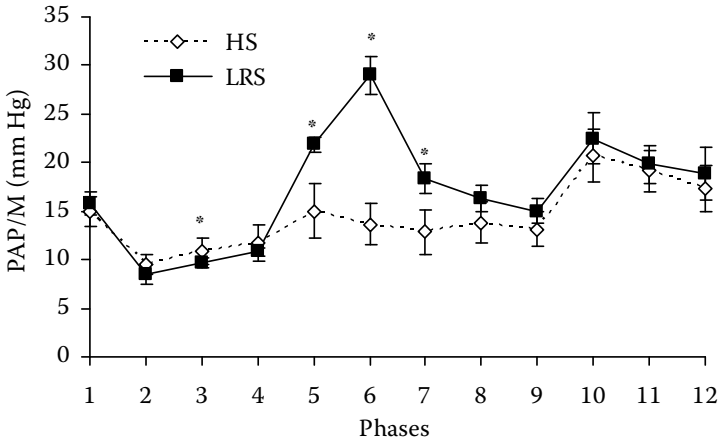

Figure 3. Mean pulmonary artery pressure (PAP/M; mm Hg) during the experiment. Values are mean ± SD for Lactated Ringer's (■ LRS; n = 6) and 7.5% saline (◇HS; n = 6)

*significant change (P < 0.05) of LRS – HS difference in comparison with baseline difference (repeated measures ANOVA)

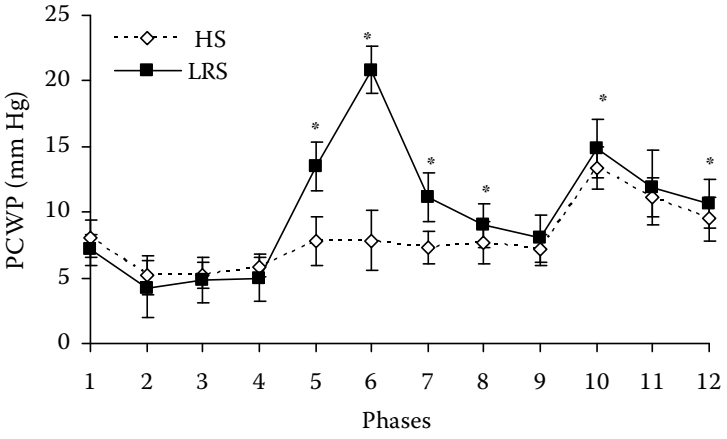

Figure 4. Pulmonary capillary wedge pressure (PCWP; mm Hg) during the experiment. Values are mean ± SD for Lactated Ringer's (■ LRS; n = 6) and 7.5% saline (◇HS; n = 6)

*significant change (P < 0.05) of LRS – HS difference in comparison with baseline difference (repeated measures ANOVA)

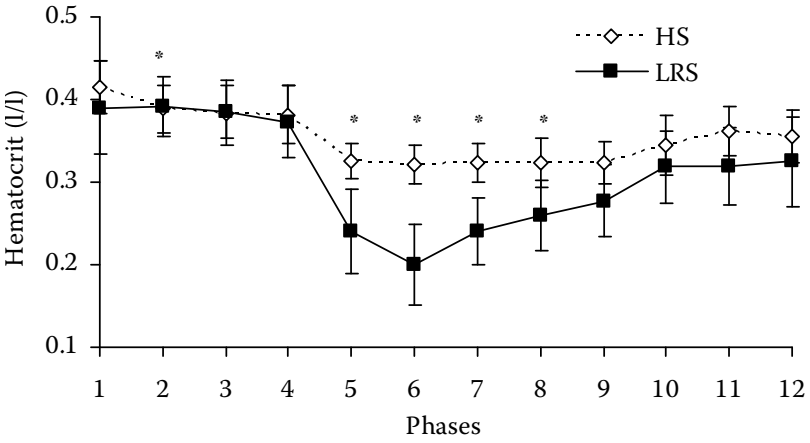

Figure 5. Hematocrit (l/l) during the experiment. Values are mean ± SD for Lactated Ringer's (■ LRS; n = 6) and 7.5% saline (◇ HS; n = 6)

*significant (P < 0.05) LRS – HS difference in comparison with baseline difference

GFRI decreased in LRS-treated dogs, while GFRI in HS-treated dogs increased. However, the change in the difference between the treatments was not significant. GFRI in HS-treated dogs was higher during the resting period 2 (phases 10 to 12), although the difference between the treatments was not significant when compared with baseline values.

Fractional excretion of sodium increased significantly with LRS treatment (phases 4 to 6). When compared to the baseline difference, the difference between the treatments changed significantly only during whole blood administration (phases 9 to 10), with fractional excretion of sodium higher in the LRS group.

Plasma norepinephrine did not change significantly with either treatment, nor was there a significant change in the difference between the treatments in comparison with the basal difference (Table 4).

Table 3. Renal variables (median, range) of dogs during haemorrhagic shock and after resuscitation with Lactated Ringer's (LRS; n = 6) and whole blood (WB) or 7.5% saline (HS; n = 6) and whole blood (WB)

Variable	Phase				
	1–4	4–6	6–9	9–10	10–12
	Shock	Fluid therapy with HS or LRS	Resting period 1	WB administration	Resting period 2
Urinary output (UO) (ml/kg/h)			*		
LRS	0.175 (0–0.66)•	5.255 (0.82–11.46) •	9.210 (4.46–11.68)•	5.715 (1.64–8.89)	6.485 (2.73–12.96)
HS	0.26 (0.15–0.47)	1.351 (0–5.33)	2.335 (0.09–4.82)•	4.81 (0.76–7.93)	7.205 (1.54–13.60)
Glomerular filtration rate index (GFRI) (ml/min/kg)		*	*		
LRS	0.884 (0–1.72)•	3.097 (0.78–10.28)	3.989 (2.05–5.47)⊗	2.443 (0.88–3.33)•	2.798 (2.25–3.40)
HS	0.557 (0.32–0.83)	1.53 (0–3.58)	1.846 (0.02–2.71)•	3.371 (1.22–3.95)	3.410 (1.78–10.39)
Fractional excretion of sodium (FE_{Na}) (%)				*	
LRS	0.24 (0–0.70)•	1.077 (0.85–4.13)	2.948 (2.47–6.62)	3.963 (2.07–4.34)	2.545 (1.44–6.40)
HS	0.904 (0.22–1.32)	0.863 (0–6.27)	3.225 (2.47–7.38)	3.44 (0.47–5.09)	3.568 (0.76–5.96)

*significant change (P < 0.05) in HS-LRS difference in comparison with difference during shock period (Friedman test, Wilcoxon Signed Rank test)

•significant difference (P < 0.05) between two subsequent measurement periods

⊗near significant difference (P = 0.058) between two subsequent measurement periods

Table 4. Norepinephrine and serum sodium concentrations (mean ± SD) of dogs at baseline, during the hemorrhagic shock and after resuscitation with Lactated Ringer's (LRS; n = 6) and whole blood (WB) or 7.5% saline (HS; n = 6) and whole blood (WB)

Variable	Phase				
	1	4	5	6	12
	Baseline	Shock	HS 4 ml/kg or LRS 40 ml/kg completed	LRS total completed	End of experiment
Norepinephrine (nmol/l)					
LRS	0.092 ± 0.019	0.108 ± 0.023	0.095 ± 0.018	0.095 ± 0.031	0.098 ± 0.045
HS	0.118 ± 0.045	0.157 ± 0.063	0.120 ± 0.027	0.108 ± 0.035	0.128 ± 0.042
Serum sodium (mmol/l)			*	*	*
LRS	146.2 ± 4.6	145.6 ± 1.7	144.1 ± 1.7	143.8 ± 2.0	144.0 ± 1.8
HS	143.3 ± 1.9	143.8 ± 1.4•	164.6 ± 1.8•	156.9 ± 4.2•	149.9 ± 1.7

*significant change ($P < 0.05$) in LRS-HS difference in comparison with baseline difference (repeated measures ANOVA)
• significant difference ($P < 0.05$) between two subsequent phases

Serum sodium concentration did not change greatly throughout the experiment in LRS-treated dogs, while it increased significantly after the administration of HS and progressively decreased until the end of experiment. The difference between the treatments changed significantly during administration of LRS or HS and remained significant until the end of the experiment (Table 4).

Hematocrit values (Figure 5) decreased more with LRS than with HS treatment. The difference between the treatments changed significantly during the administration of crystalloids; however, the hematocrit of LRS treated dogs was elevated only until the administration of whole blood, after which the difference between the treatments was no longer significant.

DISCUSSION

The short-term effects of fixed fluid bolus administration of Lactated Ringer's or 7.5 % NaCl solution combined with the administration of whole blood were studied in a model of controlled haemorrhagic shock in anaesthetized dogs, with the emphasis on haemodynamics and renal variables. The volume of crystalloid solutions used in the present study simulates the initial approach to fluid resuscitation in patients with haemorrhagic shock. HS, alone or in combination with dextran, promptly restores central haemodynamics and tissue perfusion for a short period following resuscitation (Velasco et al., 1980; Lopes et al., 1981; Rocha e Silva et al., 1987). Haemodynamics and intestinal blood flow are not maintained unless HS is supplemented with the return of the shed blood. Early administration of blood with HS is essential for full restoration of central haemodynamics and tissue perfusion (Zakaria et al., 2006); thus, addition of whole blood to the initial treatment with crystalloids was carried out in the present study.

The volumes of HS and LRS used for the initial resuscitation in the present study were adjusted according to the sodium load, i.e., the volume of fluid that contains an equal amount of sodium was administered over the same period (4 min). HS was administered with a flow rate of 1 ml/kg b.w./min because administration of HS with a higher flow rate may cause hypotension, as demonstrated by Kien et al. (1991a), who claimed that hypotension is mediated by the decrease in total peripheral resistance. Administration of isotonic crystalloid with a flow rate of 6 ml/kg b.w./min to haemorrhaged dogs, in a volume three times that of shed blood used by Dula et al. (1985), produced a rise in CI, while the CVP and PCWP remained within the optimal range. With flow rates at 15 ml/kg b.w./min the cardiovascular system appeared to be overloaded, with CVP of 23.2 mm Hg and PCWP of 31 mm Hg measured during the administration of fluids. On the other hand, flow rates of 4 ml/kg b.w./min appeared to result in a suboptimal rise in filling pressures and cardiac performance indexes (Dula et al., 1985). In the present study, the

initial administration of LRS with a flow rate of 10 ml/kg b.w./min overloaded the cardiovascular system as CVP, PAP/M and PCWP increased above normal values. CI exceeded the pre-haemorrhage value; however, a small volume of HS was equally effective in restoring CI. Chest radiographs taken at peak CVP, PAP/M and PCWP values revealed pulmonary congestion only in LRS-treated dogs. One dog required treatment with furosemide at the end of the experiment, while in the rest of them pulmonary congestion spontaneously resolved and dogs recovered well with supplemental oxygen. However, pulmonary congestion could be detrimental in dogs with pre-existing cardiopulmonary pathology, which probably would not be able to compensate marked elevations in filling pressures caused by rapid administration of large volumes of isotonic crystalloids. On the other hand, the cardiovascular system was not overloaded in dogs treated with a small volume of HS as CVP, PAP/M and PCWP remained within the optimal range during the treatment, despite increased CI.

Initial HS resuscitation resulted in a modest increase in MAP above the threshold level known to cause renal injury (Di Bartola, 2006). LRS also increased MAP, but the difference between the treatments, significant due to higher MAP in LRS-treated dogs, was short-lived because LRS redistributed into the extravascular space within one hour as reported previously (Shoemaker, 1976; Moss et al., 1981; Modig, 1986). Another increase in MAP, with no difference between the treatments, was observed after the administration of whole blood in both groups of dogs. These results are in agreement with the study of Zakaria et al. (2006) in which the return of shed blood 60 min after the initial HS treatment promptly restored MAP and intestinal flow in anaesthetized haemorrhaged rats.

The amount of oxygen delivered optimally is four times the amount of oxygen consumed, and the oxygen supply/demand balance ($DO_2 : VO_2$) is therefore 4 : 1. Because tissues extract 25% of the delivered oxygen, the SvO_2 of 75% reflects the 4 : 1 balance and an oxygen extraction of 25% (Oblouk Darovic and Yacone-Morton, 1987). In the present study, $DO_2 : VO_2$ decreased below 4 : 1 during haemorrhagic shock. Both treatments successfully restored $DO_2 : VO_2$ above 4 : 1; however the effects of HS were short-lived and the difference between the treatments changed significantly prior to the administration of whole blood, due to decreasing DO_2 in HS group. Nevertheless, the $DO_2 : VO_2$ balance did not decrease below 4 : 1 at any time during resuscitation with HS.

Haemodilution has been shown to reduce the resistance to blood flow in vascular beds due to the decrease in blood viscosity that accompanies reductions in hematocrit (Jan et al., 1980; Cowley, 1992; Pries et al., 1992, 1994). In the present study, hematocrit decreased significantly ($P < 0.05$) after administration of both crystalloids with lower values in LRS-treated dogs. Because isotonic crystalloids cross capillary membranes freely, approximately 75–80% of the fluid moves to the interstitial space within the first hour after intravenous administration (Griffel and Kaufman, 1992; Day and Bateman, 2006), which is consistent with progressive increase of hematocrit in the LRS group within the first hour after the administration in the present study. The poor intravascular retention of isotonic crystalloids supports intravascular volume transiently; therefore, three to four times the amount of crystalloids, when compared to the blood loss, should be administered to support circulation (Griffel and Kaufman, 1992; Day and Bateman, 2006). On the other hand, HS acts through endogenous fluid redistribution (Gala et al., 1991; Dubick and Wade, 1994) and expands plasma volume to three to four times the volume infused. Hematocrit and total plasma protein levels were found to drop after infusion of HS in dogs (Velasco et al., 1980; Rocha e Silva et al., 1987; Schertel et al., 1990) swine (Maningas et al., 1986) and conscious or anaesthetized sheep (Nakayama et al., 1984; Smith et al., 1985; Walsh and Kramer, 1991). In the study of Nakayama et al. (1984), replacement of 10% of the shed blood with 7% NaCl solution (3–4 ml/kg b.w.) caused the hematocrit level to fall from 0.27 l/l to 0.23 l/l 30 min after resuscitation. Ninety minutes later, hematocrit began to increase, but remained below the post-haemorrhage values. These changes were attributed not only to dilution of the circulating blood by the saline solution, but also to the osmotic effects of the solution. The results of the present study are consistent with those already published, as hematocrit in the present study decreased from 0.38 l/l (phase 4) to 0.33 l/l (phase 5) following HS administration and did not change significantly until the administration of whole blood.

Severe haemorrhage, with a drop in arterial blood pressure and deficit in vascular volume, primarily sensed by the baroreceptors, provides a strong stimulus to mobilize catecholamines (Ronning et

al., 1995). Decreased vasopressin and norepinephrine levels after administration of hypertonic solutions to haemorrhaged animals might account partially for the diminished peripheral resistance (Kramer et al., 1986). In the study of Gala et al. (1991), norepinephrine, which increased during haemorrhagic shock in woken dogs, decreased rapidly and returned to the range of resting values at 40 min in dogs treated with 0.9% NaCl (full resuscitation) or 3.0% NaCl (full resuscitation) and at 150 min in the dogs treated with a small volume of 0.9% NaCl (equal to the volume of 3% NaCl used). Norepinephrine did not decrease in non-resuscitated dogs. In haemorrhaged conscious swine, 7.5% HS/6% dextran 70 rapidly improved cardiovascular function and acutely decreased epinephrine and norepinephrine levels (Wade et al., 1991). Our data are in agreement with those published, since treatment with both tested crystalloid solutions decreased norepinephrine, which increased non-significantly during shock. However, when compared to the effects of isotonic and hypertonic crystalloid solutions, an appropriate volume of both fluids should be used because of their different mechanisms of action. The volume of LRS used in the present study was three times greater than the volume of blood loss, and the volume of HS was 4 ml/kg. In contrast, Ronning et al. (1995) used an equal small volume of hypertonic and isotonic crystalloid solutions to treat haemorrhagic shock in anaesthetized pigs. The increased plasma adrenaline and noradrenaline concentrations returned to the reference ranges 20 min after the hypertonic infusion, and were significantly lower from the catecholamine concentrations observed in the isotonic treatment group. Ten minutes after the end of hypertonic infusion, the PVR and PAP/M were increased compared with those in the isotonic group, which the authors explained as a possible neurogenic reflex or a neurohormonal release that facilitates cardiac inotropia (Lopes et al., 1981; Ronning et al., 1995). Norepinephrine levels slightly increased at the end of the experiment in the present study in both groups of dogs, and this might presumably be a consequence of the administration of collected blood, most probably containing a high level of norepinephrine, being released into the blood stream during haemorrhagic shock or of the destruction of platelets during collection and manipulation with collected blood, since platelets actively concentrate catecholamines during their life span (Katoh et al., 1992).

Urinary output and glomerular filtration rate are reduced during haemorrhagic shock. Urinary output was restored to normal (Walsh and Kramer, 1991), or to at least three times the baseline level (Nakayama et al., 1984), in haemorrhaged sheep given 2–4 ml/kg b.w. of 7–7.5% NaCl. This effect was explained by improved renal blood flow. Following resuscitation with 4–5 ml/kg b.w. of 7.5% NaCl, the renal blood flow increased in haemorrhaged dogs, but remained below baseline (Rocha e Silva et al., 1986; Kien et al., 1991b). Treatment with 7.5% NaCl/6% Dextran produced transient increases in urine flow and electrolyte/osmotic excretion in conscious haemorrhaged swine (Sondeen et al., 1990). In the present study both treatments increased urinary output and glomerular filtration rate. Urinary output increased much more with LRS ($P < 0.05$) than HS; however, in both cases it was far above the minimum value of 1–2 ml/kg b.w./h required for normal kidney function (Mathews, 2006). Interestingly, there was no difference between the treatments at the end of experiment, suggesting that early administration of whole blood is necessary, regardless of the initial crystalloid used.

The values for endogenous creatinine clearance used to estimate glomerular filtration rate index in dog and cat are approximately 2–5 ml/min/kg b.w. (Finco, 1971; Bovee and Joyce, 1979; Finco et al., 1993). In most clinical pathology laboratories, creatinine is measured by the alkaline picrate reaction, which is not entirely specific for creatinine and measures another group of substances collectively known as noncreatinine chromatogens. The same method was used in our laboratory, thus the results are comparable with the reference limits for endogenous creatinine clearance. GFRI in the present study increased after the treatment with HS or LRS, the change in the difference between the treatments being significant on the account of higher GFRI in LRS-treated dogs. Administration of whole blood improved GFRI in HS-treated dogs to the upper limit of normal values, confirming again the necessity of whole blood administration together with the initial administration of HS.

Pre-renal factors that decrease the blood flow to the kidneys, including all causes of circulatory shock, may lead to acute renal failure. Pre-renal conditions do not cause permanent renal damage and hence are potentially reversible, unless hypoperfusion is severe enough to cause tubular ischemia and acute tubular necrosis. Hypoperfusion of an

otherwise functioning kidney leads to enhanced reabsorption of sodium and water, resulting in oliguria with high urine osmolality and low urine sodium concentration (Elliot, 1996). Fractional excretion of sodium is one of the urinary diagnostic indices that may be helpful in distinguishing pre-renal azotemia and acute tubular necrosis (< 1% pre-renal, > 1% tubular injury) (Gleadhill and Michell, 1996). The observed range for urinary excretion of sodium in healthy non-anaesthetized Greyhound dogs is 0–0.77%. Expressed as percentages, the suggested reference limit for fractional excretion of sodium is less or equal to 0.72 (Bennett et al., 2006). The range in non-Greyhound dogs published by Di Bartola et al. (1980) is 0.019–0.793%. In the present study the fractional excretion of sodium was, expectedly, below 1% during the hemorrhagic shock in both groups of dogs due to hypoperfusion of kidneys. After the treatment with LRS, it was between 0.85 and 4.13% (median value of 1.077%). On the other hand, in HS-treated dogs, fractional excretion of sodium showed great variability (median value of 0.863%, range between 0 and 6.27%). The values of fractional excretion of sodium obtained after fluid therapy with either of the solutions used may not be useful to distinguish pre-renal azotemia from acute tubular necrosis, because therapy with fluids or diuretics alters sodium secretion (Gleadhill and Michell, 1996).

In conclusion, both solutions used in this study improved hemodynamic and renal function in a short term period after the haemorrhagic shock. Rapid administration of a large volume of Lactated Ringer's resulted in pulmonary congestion, while the cardiovascular system was not overloaded during treatment with a small volume of hypertonic saline. Large volumes of isotonic crystalloids should be given over a longer period of time, which may favour the use of small volumes of hypertonic saline that can be administered over a much shorter period of time to achieve comparable results. However, the effects of hypertonic saline are short lived, which is why it should be combined with the administration of whole blood as soon as possible, that is, within one hour after the beginning of the fluid therapy. The addition of whole blood to the initial fluid therapy showed that there was no difference between the treatments at the end of the experiment in terms of haemodynamic and renal effects, suggesting that the administration of whole blood is required for the favourable outcome regardless of the initial crystalloid used.

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