EJA

Eur J Anaesthesiol 2017; **34:**89–97

ORIGINAL ARTICLE

Comparative analysis of resuscitation using human serum albumin and crystalloids or 130/0.4 hydroxyethyl starch and crystalloids on skeletal muscle metabolic profile during experimental haemorrhagic shock in swine

A randomised experimental study

Eric Noll, Michele Diana, Anne L. Charles, François Singh, Tong J. Gan, Julien Pottecher, François-Marie Moussallieh, Izzie J. Namer, Bernard Geny and Pierre Diemunsch

BACKGROUND Protection against acute skeletal muscle metabolic dysfunction and oxidative stress could be a therapeutic target in volume expansion for severely bleeding patients.

OBJECTIVES This experimental pilot study in swine aims at comparing 130/0.4 hydroxyethyl starch (HES) with 4% albumin along with crystalloid perfusion for first-line volume expansion in haemorrhagic shock with a particular emphasis on oxidative stress and muscular mitochondrial function.

DESIGN Randomised experimental study.

SETTING Digestive Cancer Research Institute Preclinical Laboratory, Strasbourg University Hospital, France, from February 2012 to June 2013.

ANIMALS Twenty large white pigs.

INTERVENTION Pressure-controlled haemorrhagic shock and volume resuscitation using either 4% human serum albumin or 130/0.4 HES along with crystalloid perfusion were performed in 20 large white pigs.

MAIN OUTCOME MEASURES Muscular biopsy of gastrocnemius muscle was performed for metabolomics screening, mitochondrial respiratory chain assessment and electron spin resonance reactive oxygen species production along with arterial and venous reactive oxygen species production at baseline, at the completion of shock, at 90 min and at 180 min after volume expansion.

RESULTS There was no difference between the two groups in measurements of skeletal muscle superoxide production. In a pooled analysis, there was a statistically significant decrease in gastrocnemius muscle creatine content from baseline to 90 min ($P < 0.05$) and 180 min ($P < 0.05$). Muscular lactate content and mitochondrial respiratory chain oxidative capacity remained constant at the respective time points.

CONCLUSION In this pilot experimental study in swine, during pressure-controlled haemorrhagic shock treated with either albumin or 130/0.4 HES in conjunction with crystalloid perfusion, skeletal muscle metabolic profile was unaltered.

ETHICAL APPROVAL NUMBER 38.2012.01.031.

Published online 28 October 2016

Introduction

Haemorrhagic shock is a major cause of morbidity and mortality in trauma and surgical practice.[1,2] Volume expansion is critical along with attempts at haemostasis, particularly before packed red blood cells are available.[3] The optimal plasma volume expander to be administered remains controversial.[4,5] In the critical care setting most

From the Anesthesia and Intensive Care, Hôpital de Hautepierre, University Hospitals of Strasbourg (EN, JP, PD), EA 3072, Laboratoire de Physiologie, Fédération de Médecine Translationnelle, Strasbourg University (EN, MD, ALC, FS, JP, BG, PD), IHU-Strasbourg, University Institute for Image-Guided Surgery, Strasbourg, France (EN, MD, JP, PD), Department of Anesthesiology, Stony Brook University, Stony Brook, New York, USA (TJG), Biophysics and Nuclear Medicine, Hôpital de Hautepierre, University Hospitals of Strasbourg (F-MM, IJN) and ICube, University of Strasbourg/CNRS UMR 7357, Strasbourg, France (F-MM, IJN)

Correspondence to Eric Noll, M.D., Anesthesia and Intensive Care, Hôpital de Hautepierre, Strasbourg University Hospital, 10 avenue Achille Baumann, Illkirch Graffenstaden 67400, France
Tel: +33 (0)3 88 12 70 76; e-mail: eric.noll@chru-strasbourg.fr

DOI:10.1097/EJA.0000000000000537

clinical trials fail to distinguish the effects of the resuscitative fluid itself from other interventions.[6]

Haemorrhagic shock and resuscitation are suspected as potential sources of oxidative stress,[7] which is closely related to mitochondrial dysfunction.[8] Skeletal muscle metabolism impairment has recently been shown to be involved in experimental haemorrhagic shock.[9,10] Hydroxyethyl starches (HES) have shown protective effects against oxidative stress in haemorrhagic shock models,[11,12] but the effects of human serum albumin have not been appropriately assessed. The primary aim of our study was to compare 130/0.4 HES with 4% albumin for first-line volume expansion associated with crystalloid perfusion in experimental haemorrhagic shock with a particular emphasis on oxidative stress. Our secondary objectives were to compare the effects of these first-line volume expansion strategies on muscular metabolomics and mitochondrial function. Our hypothesis was that there was a similar effect on oxidative stress levels and on skeletal muscle mitochondrial metabolism during haemorrhagic shock resuscitation with either 4% albumin or 130/0.4 HES associated with crystalloid perfusion.

Material and methods

Animals

After institutional approval by the Institutional Ethical Committee on Animal Experimentation (IRCAD, Strasbourg, France, approval number: 38.2012.01.031 on the 6 February 2012), 20 large white pigs were studied under standardised general anaesthesia. Animals were sampled in a 1 : 1 female to male ratio farmed cohort. All animals used in the experimental laboratory were managed according to the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines, French laws for animal use and care as well as directives of the European Community Council (2010/63/EU). To stick to the Replacement, Reduction and Refinement rule and reduce the number animals under study, this study was *a priori* nested in extended experimental work focussing on haemorrhagic shock-induced coagulopathy. None of the analytical data presented in this manuscript will be used in the coagulation-based manuscript except for standard monitoring values. Pigs were fasted for 24 h before surgery with free access to water. Premedication by intramuscular injection of ketamine (20 mg kg^{-1}) and azaperone (2 mg kg^{-1}) was administered 1 h before surgery. After arrival at the surgical laboratory, animals were weighed, a 22-gauge i.v. catheter was inserted into an auricular vein and the line was maintained with saline (0.9% sodium chloride) to ensure a perfusion rate of 12 ml kg^{-1} h^{-1} during the entire experimental procedure. General anaesthesia was induced with i.v. propofol (3 mg kg^{-1}) and tracheal intubation (Portex Blue Line, Smiths Medical, Dublin, USA; 6 mm) was facilitated with rocuronium (1 mg kg^{-1}).

Anaesthesia was maintained with 2% end-tidal isoflurane concentration with 60% nitrous oxide in oxygen.[13] Analgesia was provided with i.v. tramadol (2 mg kg^{-1}) every 5 h. Mechanical ventilation was controlled with an Aisys Carestation (GE Healthcare, Aulnay Sous Bois, France) to reach an end-tidal CO_2 concentration between 35 and 45 mmHg.[14]

Experimental setting

A central venous line (Arrow international, Diegem, Belgium) was inserted into the left external jugular vein for central venous blood sampling. An arterial catheter (Leader cath, Vygon) was placed into the right femoral artery for invasive arterial pressure monitoring and arterial blood sampling. Pressure-controlled haemorrhagic shock was induced by progressive blood withdrawal via the jugular vein to reach a mean arterial blood pressure (MAP) target of 40 ± 5 mmHg. When required, additional arterial blood subtractions were performed to maintain the target MAP at 40 mmHg for 2 h. After 2 h of hypotension, colloidal fluid therapy was initiated with a randomised use of either 4% human serum albumin (Vialebex, LFB Biomedicaments, Courtaboeauf, France; $n = 10$) or 130/0.4 HES (Voluven Fresenius-Kabi, Finland; $n = 10$). Colloidal volume expansion was added to the basal crystalloid perfusion to compare the two mixed crystalloid-colloidal volume expansion strategies as might be used in the clinical setting. Fluid therapy was stopped after reaching 90% of baseline MAP and the experiment proceeded for additional 3 h. At the end of the procedure, pigs were sacrificed according to our protocol with an intravenous injection of a lethal dose of potassium chloride under deep general anaesthesia.

Monitoring

Monitoring included ECG, oxygen saturation by pulse oximetry (SpO2) and core temperature monitoring with an oesophageal probe (Odam Physiogard SM785 TM, Odam Physiogard, Wissembourg, France). Normothermia was maintained with an under and upper body warming blanket. Heart rate, MAP, core temperature, SpO_2, were recorded every 15 min for all animals.

Blood sampling

Arterial and central venous blood samples were harvested before the induction of haemorrhagic shock (baseline, T0), after 2 h of bleeding and completion of haemorrhagic shock (T1), 90 min after fluid therapy (T2) and 180 min after fluid therapy (T3) for planned analysis [arterial blood gas, arterial and venous electron spin resonance (EPR) analysis].

Arterial blood gas analysis

Arterial blood gas and arterial lactate[15] content were analysed using the Siemens Rapidlab 865 Blood Gas Analyser, Siemens Healthcare, Saint-Denis, France.

Blood gas and lactate measurement were performed on only 13 animals for technical reasons.

Mitochondrial respiratory chain functional assessment

Gastrocnemius muscle samples were harvested at T0, T1, T2 and T3. Immediately after harvesting, the mitochondrial respiratory chain was assessed in the gastrocnemius samples using the saponin-skinned fibres technique. This technique, as previously described,[16] allows an in-depth study of mitochondria in their tissue environment. After recording the tissue baseline oxygen consumption (V0), the complex I-linked (CI-linked) substrate state respiratory rate was monitored with the adjunction of saturating amount of ADP. To assess the activity of the mitochondrial respiratory chain complexes II, complex I was then blocked with amital (0.02 mmol l^{-1}) and complex II stimulated with succinate (25 mmol l^{-1}) for selective complexes II-linked substrate state respiratory rate monitoring (CII linked). After the measurements, fibres were dried at room temperature for 24 h and 15 min at 150°C.[16–18] Respiration rates were expressed as μmol O_2 min^{-1} g dry $weight^{-1}$.

Metabolomic measurement

High resolution magic angle spinning (HRMAS) NMR spectroscopy was performed for all the tissue samples of the cohort. Tissue samples were prepared at −20°C as previously described.[19,20] HRMAS NMR spectra were analysed on a Bruker Avance III 500 spectrometer, Bruker, Wissembourg, France operating at a proton frequency of 500.13 MHz. HRMAS NMR data acquisition and spectra processing were performed as previously described.[19,20] A one-dimensional proton spectrum using a Carr–Purcell–Meiboom–Gill pulse sequence[21] and 128 transients was acquired for each sample. Free induction decays were multiplied by an exponential window function of 0.3 Hz prior to Fourier transformation and were corrected for phase and baseline distortions using TopSpin 2.1, Bruker, Wissembourg, France. The chemical shift was referenced to the peak of the methyl proton of L-lactate at 1.33 ppm. The quantification procedure was based on the pulse length-based concentration measurement as previously described.[20] Spectra were normalised according to each sample weight and calibrated using the signal intensity of a 19.3 nmol reference standard external solution of lactate, scanned under the same analytical conditions. Quantification results were expressed as nmol mg^{-1} of tissue.

Electron spin resonance

Containing unpaired electrons, reactive oxygen species can absorb electromagnetic radiation energy and move between energy levels in a magnetic field. Through detecting and recording the electromagnetic radiation energy, the reactive oxygen species concentration could be directly determined with a spin probe [1-hydroxy-3-methoxycarbonyl-2, 2, 5, 5-tetramethylpyrrolidine hydrochloride (CMH)], which is specific for superoxide anion.[22,23] Arterial and venous blood samples were collected in a heparinised syringe and stored on ice at 4°C. After 1 h, 20 μl of blood were mixed with 20 μl of spin probe CMH : heparin solution (400 μmol l^{-1} : 100 IU ml^{-1}) and introduced in a capillary. Detection of reactive oxygen species production was conducted using a Bench-Top EPR spectrometer E-SCAN, Bruker, Wissembourg, France. The EPR signal is proportional to the unpaired electron numbers and can, in turn, be transformed into absolute produced micromoles (μmol min^{-1}).

For tissue superoxide production, muscle biopsies harvested from the gastrocnemius were incubated in a plate at 37°C with the spin probe CMH (200 μmol l^{-1}) for 30 min under 20 mmHg of oxygen partial pressure (mimic physiological conditions) using Gas Controller (Noxygen Sciences Transfer, Elzach, Germany). Detection of reactive oxygen species production in the supernatant was conducted using the BenchTop EPR spectrometer E-SCAN. After the EPR measurement, pieces of muscles were dried at room temperature for 24 h and 15 min at 150°C, and reactive oxygen species production was expressed in μmol l^{-1} min^{-1} mg dry $weight^{-1}$.

Statistical analysis

Analyses were performed using pooled analysis of the 20 animals to study global haemorrhagic shock effects and comparative analysis of the albumin ($n = 10$) and the HES ($n = 10$) groups.

All data are shown as SEM. The Student's *t*-test (substituted by Mann–Whitney test if d'Agostino and Pearson omnibus normality test failed) or analysis of variance (ANOVA) followed by the Dunnett multiple comparison test, when appropriate, were used to calculate *P* values. As pooled analysis aimed at studying the effects of haemorrhagic shock on study parameters we use T0 as the reference value for pooled ANOVA. As comparative analysis aimed at studying the fluid therapy effect on study parameters we used T1 as the reference value for within each group ANOVA. A value of $P < 0.05$ (two sided) was required to reject the null hypothesis. Statistical analyses were performed using the Prism 6 software', Graphpad, La Jolla, USA. As there were no previous data to support an educated sample size calculation, this study also serves as a pilot to better determine the sample size.

Results

One animal did not survive throughout the entire study period (5% global mortality rate). All other animals completed the study. Total body weight, amount of shed blood and colloid resuscitation volumes did not significantly differ between the two treatment groups. The experimental vascular volume management characteristics did not differ between the albumin and HES groups (Table 1). Table 2 shows standard monitoring values and

Table 1 Volume characterisation of the haemorrhage and resuscitation

	HES ($n=10$)	Albumin ($n=10$)	P
Body weight (kg)	25.9 ± 1.4	26.1 ± 1.0	0.94
Shed blood (ml kg^{-1})	31.5 ± 1.9	31.5 ± 2.2	0.98
Colloid resuscitation (ml kg^{-1})	22.3 ± 1.9	24.0 ± 3.1	0.63

Mean ± SEM. HES, hydroxyethyl starch.

main biological findings. As per protocol, haemorrhage markedly reduced MAP to the target value at T1 and similarly increased heart rates in both groups. Blood lactate concentrations were increased to a similar value in both groups at T1.

The animals' core temperature and SpO_2 did not differ between the study groups at any time point.

Electron spin resonance superoxide anion production assessment

Figure 1 shows arterial and venous EPR superoxide production. Pooled analysis including all animals showed a significant decrease in arterial blood superoxide anion production between T0 (baseline) and T2 (90 min after colloid resuscitation initiation, 0.46 ± 0.04 and 0.33 ± 0.03 μmol min^{-1}, respectively, $P<0.05$, $n=20$). Venous blood superoxide anion production showed a similar trend between T0 and T2 without reaching statistical significance (0.34 ± 0.04 and 0.23 ± 0.02 μmol min^{-1}, respectively, $P=0.09$, $n=18$).

Comparative analysis of the arterial and venous blood EPR superoxide production in treatment groups showed a statistical difference between the albumin and HES group only at T1 in the arterial blood.

Figure 2 shows tissue EPR superoxide production in gastrocnemius skeletal muscle samples. Compared with baseline values, there was no significant change in pooled analysis values at T1, T2 and T3. Comparative analysis of albumin and HES groups at T1 and T2 showed no difference between the groups.

Gastrocnemius muscle mitochondrial respiratory chain functional assessment

In pooled analysis of gastrocnemius muscle mitochondrial respiratory chain oxidative capacity, combining T1, T2 and T3 compared with T0, there was no statistically change in oxidative capacity as measured by CI-linked substrate state (2.85 ± 0.25, 2.71 ± 0.18, 2.68 ± 0.21 and 3.1 ± 0.24 μmol O_2 min^{-1} g dry weight^{-1}, respectively, $P>0.05$), and by CII-linked substrate state (3.02 ± 0.24, 2.84 ± 0.16, 2.87 ± 0.17 and 3.20 ± 0.23 μmol O_2 min^{-1} g dry weight^{-1}, respectively, $P>0.05$).

Comparative analysis of the gastrocnemius skeletal muscle mitochondrial respiratory chain oxidative capacity after HES or albumin volume restoration is shown in Fig. 3. After HES or albumin resuscitation, CI-linked and CII-linked substrate state oxidative capacity did not differ between the groups at T2 and T3.

Gastrocnemius muscle metabolomics screening

Gastrocnemius muscle lactate content measured with metabolomics screening is shown in Fig. 4. In pooled analysis, there was no statistically significant variation at T1, T2 and T3 as compared with T0. Comparative analysis between albumin and HES groups did not show any difference at T2 and T3.

Gastrocnemius muscle creatine and phosphorylcreatine content, as assessed with metabolomic screening, is shown in Fig. 4.

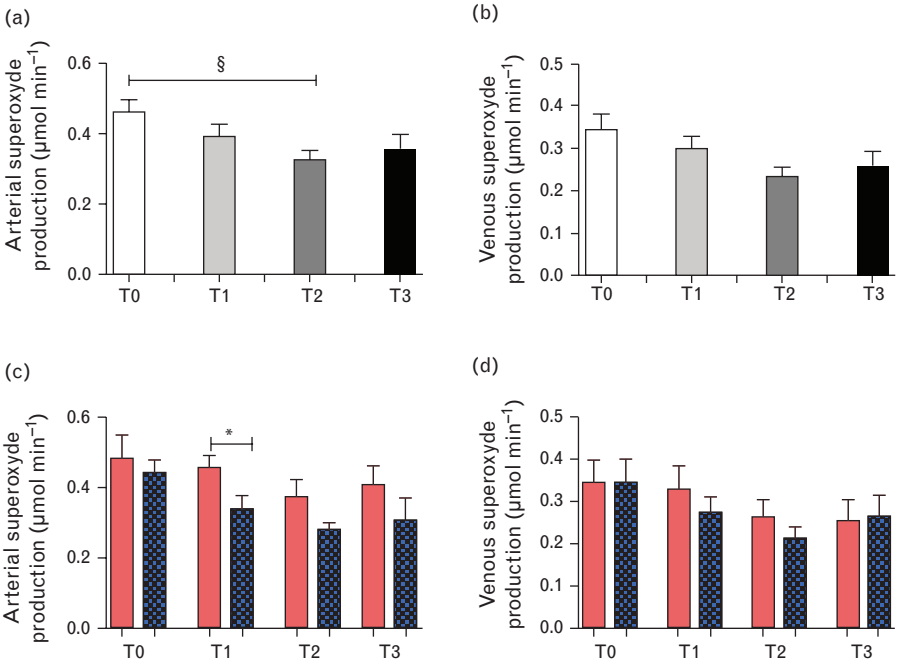
In pooled analysis, there was a statistically significant decrease in gastrocnemius muscle creatine content from T0 (12.19 ± 0.57 nmol mg^{-1}) to T2 (9.93 ± 0.73 nmol mg^{-1}, $P<0.05$) and T3 (9.35 ± 0.78 nmol.mg^{-1},

Table 2 Haemodynamic variables and biochemical data at different experimental time points

		T0	T1	T2	T3
Heart rate (bpm)	HES	91 ± 6[a]	152 ± 17	122 ± 7	132 ± 6
	Albumin	78 ± 6[a]	153 ± 16	137 ± 14	126 ± 9
MAP (mmHg)	HES	66 ± 3[a]	40 ± 2	55 ± 3[a]	52 ± 3[a]
	Albumin	63 ± 2[a]	42 ± 2	51 ± 2[a]	52 ± 2[a]
SpO_2 (%)	HES	94 ± 2	92 ± 7	89 ± 5	95 ± 3
	Albumin	95 ± 3	98 ± 1	97 ± 1	97 ± 2
Temperature (°C)	HES	36.9 ± 0.5	38.4 ± 0.7	38.0 ± 0.7	38.0 ± 0.8
	Albumin	36.7 ± 0.2[a]	38.0 ± 0.3	38.0 ± 0.3	37.8 ± 0.4
HCO_3^- (mmol l^{-1})	HES	32.0 ± 1.5	27.9 ± 0.5	27.4 ± 1.9	30.6 ± 0.85
	Albumin	32.4 ± 0.6[a]	29.0 ± 1.0	30.5 ± 0.7	30.7 ± 0.7
Lactate (mmol l^{-1})	HES	1.5 ± 0.4[a]	3.5 ± 0.7	2.2 ± 0.3	1.5 ± 0.2[a]
	Albumin	1.7 ± 0.2[a]	3.0 ± 0.3	1.5 ± 0.3[a]	1.3 ± 0.2[a]
Hb (g dl^{-1})	HES	8.6 ± 0.3	7.5 ± 0.4	5.0 ± 0.3[a]	5.5 ± 0.3[a]
	Albumin	8.1 ± 0.3	7.7 ± 0.4	5.0 ± 0.4[a]	4.8 ± 0.3[a]

Mean ± SEM. T0 = baseline, T1 = end of bleeding, T2 = 90 min after volume expansion, T3 = 180 min after volume expansion. HES group $n=10$, albumin group $n=10$. Variations in SpO2 values observed at T2 may be explained by artefacted signals. Blood gas and lactate measurement were performed only on 13 animals for technical reason. Hb, arterial haemoglobin; HES, hydroxyethyl starch; MAP, mean arterial blood pressure; SpO_2, oxygen saturation by pulse oximetry. [a] $P<0.05$ vs. T1 within each group.

Fig. 1



Effect of haemorrhagic shock (T1), 90 min (T2) and 180 min (T3) colloid resuscitation on arterial ($n = 20$) and venous ($n = 18$) superoxide anion production compared with baseline (T0) by electron paramagnetic resonance. Pooled albumin and HES group analysis on arterial (a) and central venous (b) blood superoxide anion productions. Comparative analysis of albumin (no pattern) and HES (black box pattern) groups' arterial (c) and central venous (d) blood superoxide anion productions. Values are means (SEM). $P > 0.05$ for within each group ANOVA. Venous blood sample could not be analysed for technical reasons in animals 6 and 7. ANOVA, analysis of variance; HES, hydroxyethyl starch.

$P < 0.05$), whereas phosphorylcreatine content did not show any significant variation.

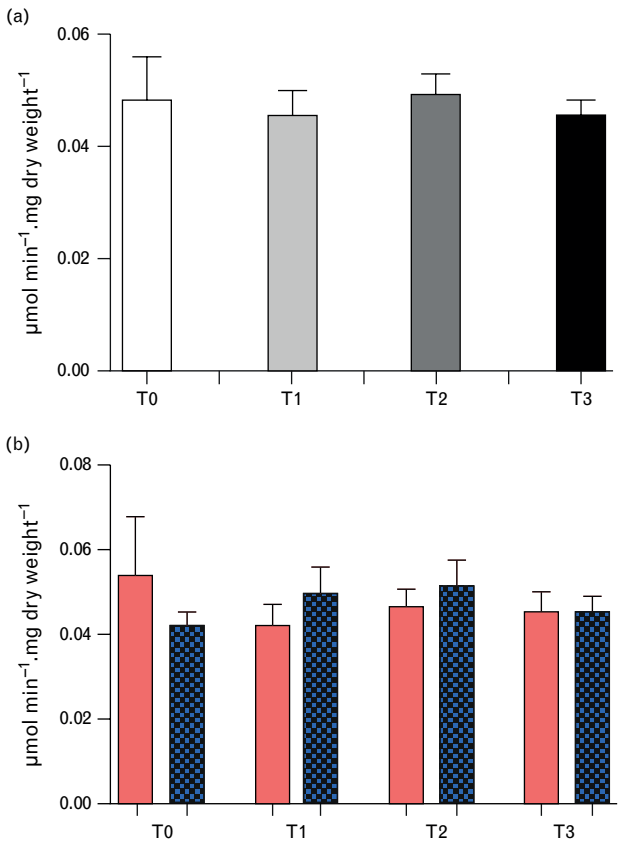
Comparative analysis at T2 and T3 showed no difference between albumin and HES groups in gastrocnemius muscle creatine and phosphorylcreatine content.

Discussion

Our experimental study of haemorrhagic shock in swine demonstrated a systemic reduction in superoxide anion production during the fluid therapy period. This coincided with unaltered skeletal muscle metabolic profile in both HES and albumin-resuscitated groups (evidenced by unaltered mitochondrial oxidative capacity, confined tissue superoxide anion production, a preserved lactate and phosphorylcreatine muscular contents). To our knowledge, this is the first experimental study comparing the effects of albumin with HES fluid therapy on oxidative stress and muscular metabolism during experimental haemorrhagic shock. Albumin is expected to have free radical-trapping properties because of its cysteine residue representing the main thiol circulating pool[24] and its six methionine residues. However, these biochemical properties have to be confirmed *in vivo* during standardised haemorrhagic shock resuscitation.

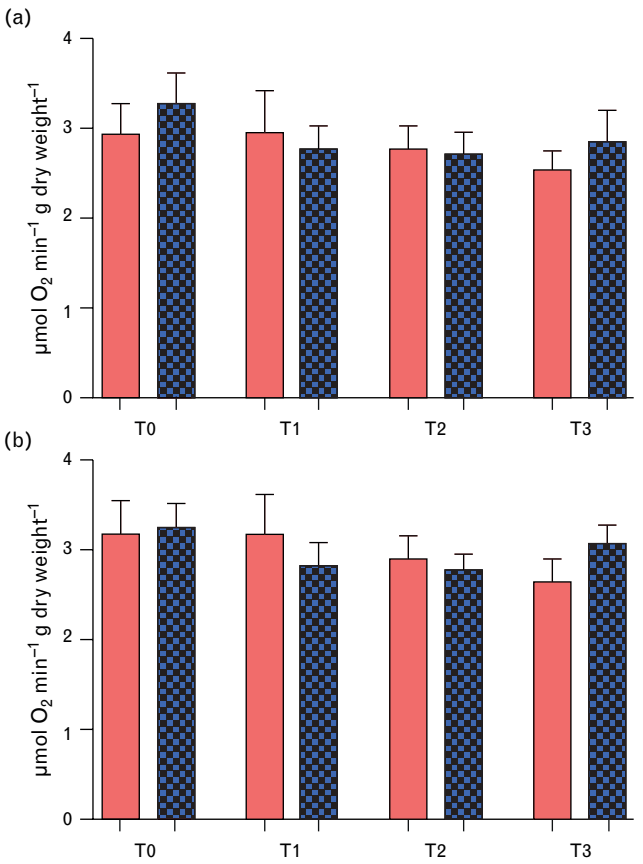
Our findings on oxidative stress are in line with some indirect experimental evidence of decreased reactive oxygen species production during haemorrhagic shock resuscitation with 130/0.4 HES. In a rodent volume-targeted haemorrhagic shock model, intestine, lung, liver and brain lipid peroxidation levels were decreased in the 130/0.4 HES-treated groups compared with the 200/0.5 HES groups and succinylated gelatin groups.[25] The same findings were observed in a pressure-controlled haemorrhagic shock model focusing on ileal levels of lipid peroxidation and comparing 130/0.4 HES, Ringer's lactate and shed blood.[26] On the contrary, an increase in systemic reactive oxygen species production has been suspected during experimental haemorrhagic shock resuscitation with Ringer's lactate.[7] Reactive oxygen species production seems to be alleviated but not abolished by balanced crystalloid resuscitation.[27] There could be several reasons for the absence of an increase in reactive oxygen species at the end of the low MAP time period. Reactive oxygen species overproduction could be expected mainly after correction of oxygen delivery, that is, during resuscitation.[8] Isoflurane-based anaesthesia could alter the reactive oxygen species production[28] inducing a lower reactive oxygen species response to haemorrhagic shock. The amount of haemorrhagic shock-driven tissue insult may have been insufficient to trigger massive reactive oxygen species release. Haemodilution could also be a reason for the decrease in systemic reactive oxygen species assessment after initiation of fluid therapy.

Fig. 2



Effect of haemorrhagic shock (T1), 90 min (T2) and 180 min (T3) colloid resuscitation on gastrocnemius skeletal muscle electron spin resonance superoxide anion production assessment compared with baseline (T0). (a) Pooled ($n = 20$) albumin and HES group analysis on gastrocnemius skeletal muscle. (b) Comparative analysis of albumin (no pattern, $n = 10$) and HES (black box pattern, $n = 10$) groups on gastrocnemius skeletal muscle superoxide anion productions. Values are means (SEM). $P > 0.05$ between albumin and HES group at each time point. $P > 0.05$ for pooled analysis ANOVA and within each group ANOVA. ANOVA, analysis of variance; HES, hydroxyethyl starch.

Fig. 3

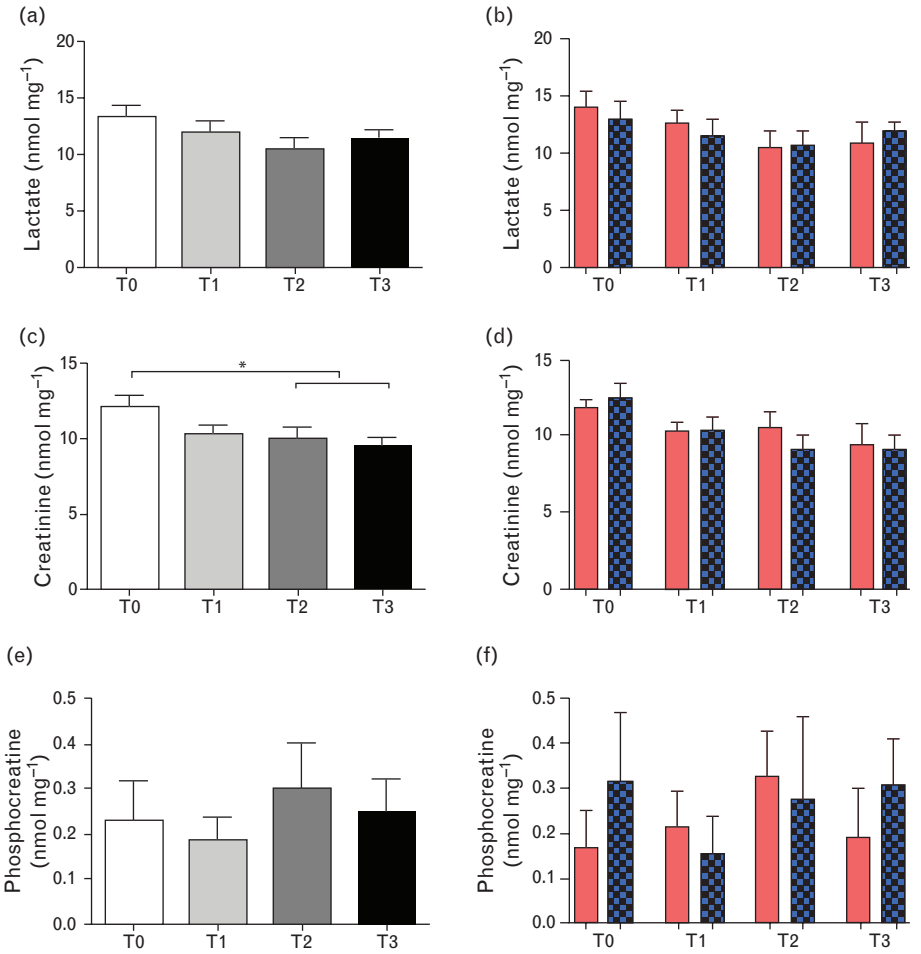


Gastrocnemius skeletal muscle mitochondrial respiratory chain oxidative capacity in albumin (no pattern, $n = 10$) and HES (black box pattern, $n = 10$) groups. (a) Complex I-linked oxidative capacity, (b) complex II-linked oxidative capacity. Values are means (SEM). $P > 0.05$ between albumin and HES group at each time point. $P > 0.05$ for within each group ANOVA (T1 as reference value). ANOVA, analysis of variance; HES, hydroxyethyl starch.

In our study, we also focused on the metabolic consequences of haemorrhagic shock resuscitation on skeletal muscle. Impairment of heart mitochondrial respiratory chain function has been reported during rodent haemorrhagic shock resuscitation combining Ringer's lactate solution and shed blood.[29] Mitochondrial respiratory chain dysfunction is a major cell insult leading to intense redox signalling, reduced ATP production and impaired cell viability. We have previously demonstrated mitochondrial respiratory chain involvement in severe pathological situations such as acute lower limb and mesenteric ischemia.[13,30] Protection against mitochondrial respiratory chain dysfunction is a major therapeutic goal of acute resuscitation. In our study, mitochondrial respiratory chain function was preserved in both groups during the shock state and the resuscitation period. The absence of major mitochondrial alteration could have been mediated by the absence of reactive oxygen species overproduction.[31]

Metabolomic screening of skeletal[32] muscle during the shock and resuscitation time course revealed no major metabolic alteration (decrease in muscular creatine and stable lactate content). Reduced serum content of creatine has been observed in hypothermic resuscitation of haemorrhagic shock and was associated with improved survival.[9] Intramuscular variation of phosphorylcreatine/creatine content has also been described in left ventricular muscle during experimental ischaemic preconditioning procedures.[32] Phosphorylcreatine/creatine cycle act as a shuttle for high-energy phosphate produced in the mitochondrial intermembrane space[33] and as an energy supply source for anaerobic metabolism. Creatine is synthesised in the liver and targeted to the muscle

Fig. 4



Effect of haemorrhagic shock (T1), 90 min (T2) and 180 min (T3) colloid resuscitation on gastrocnemius skeletal muscle metabolomic screening for lactate, creatine and phosphocreatine. Pooled albumin and HES group analysis on gastrocnemius skeletal muscle lactate ($n = 20$; a), creatine ($n = 19$; c) and phosphocreatine ($n = 19$; e) content. Comparative analysis of albumin and HES groups on gastrocnemius skeletal muscle lactate (b), creatine (d) and phosphocreatine (f) content. $P > 0.05$ between albumin and HES group at each time point. $P > 0.05$ for within each group ANOVA. Missing tissular analysis could not be performed in animal 4 for technical reasons. ANOVA, analysis of variance; HES, hydroxyethyl starch.

where it is phosphorylated to phosphorylcreatine. Our data show preserved mitochondrial respiratory rate and stable phosphorylcreatine level, ruling out alteration of high-energy phosphate production. The mechanistic relationship between skeletal muscle creatine content and the protective effect observed in some resuscitation strategies has yet to be determined. Metabolic switch to a hibernating-like condition is one of the suggested theories[32] for the metabolic shift in the creatinine metabolic pathway.

The stable muscle lactate content is associated with the absence of muscular mitochondrial respiratory chain dysfunction at the resuscitation time point. At the end of the shock period, the lack of muscular lactate increase despite haemorrhagic was somewhat surprising. However, this is consistent with microdialysis skeletal muscle interstitial lactate monitoring in another experimental pretransfusionnal haemorrhagic shock model.[34,35]

This experiment has some limitations. This is a pilot experimental study with a limited number of study participants. Additional experimental studies should be conducted with larger numbers of animals to confirm these results, increase the power of the conclusion and address questions about the specific effect of colloids vs. crystalloid resuscitation, the specific role of haemodilution on systemic reactive oxygen species assessment and the precise level of mean arterial pressure needed to trigger reactive oxygen species in this experimental model. Owing to the limited number of study participants, the results are subject to experimental variability as observed with the end of shock difference in arterial blood superoxide production between albumin and HES

groups (Fig. 1). This difference may have precluded interpretation of ANOVA based on T0 as the reference measurement in this group: T2 and T0 would have been statistically different in the HES group but without being able to identify the respective effect of the pre and postfluid therapy period. To avoid this issue, we used T1 as the reference value for ANOVA. Furthermore, as male pigs are known to be more susceptible to stress, imbalances of male/female distribution in the study groups may have influenced the results. The administration of nondepolarising neuromuscular blocking drugs in our model may also have interfered with the skeletal muscle metabolism. However, we did not investigate survival associated with this skeletal muscle metabolism profile. We used the MAP endpoint for the resuscitation of the haemorrhagic shock but did not record the rate of MAP stabilisation across groups, thus our experimental design does not allow for the comparison of the haemodynamic aspects of both strategies. We observed only a moderate increase in systemic lactate levels, thus our haemorrhagic shock models may have been insufficient to trigger significant oxidative stress. There may be differences between pigs and humans in the effects of human albumin. This was an exploratory pilot study on acute antioxidative and muscular effects of two colloid resuscitation strategies associated with crystalloid perfusion, but there was no crystalloid control group in this study, thus our results do not allow the strict comparison of crystalloid vs. colloid properties.

Conclusion

In this pilot experimental study, during a pressure-controlled haemorrhagic shock model treated with albumin or 130/0.4 HES in conjunction with crystalloid perfusion, skeletal muscle metabolic profile was unaltered.

Acknowledgements relating to this article

Assistance with the study: none.

Financial support and sponsorship: the study was funded in part with a grant from LFB Biomedicament (Courtaboeuf, France) to the Strasbourg University Fundation and the study drugs (Vialbex) were provided by LFB Biomedicament (Courtaboeuf, France). LFB Biomedicaments was not otherwise involved in the study design, data processing, manuscript redaction and submitting process.

Conflicts of interest: EN and JP gave a paid lecture in 2013 for LFB Biomedicament (Courtaboeuf, France).

Presentation: none.

References

1 Minei JP, Cuschieri J, Sperry J, *et al.* The changing pattern and implications of multiple organ failure after blunt injury with haemorrhagic shock. *Crit Care Med* 2012; **40**:1129–1135.
2 Dutton RP, Lee LA, Stephens LS, *et al.* Massive Hemorrhage: a report from the anesthesia closed claims project. *Anesthesiology* 2014; **121**:450–458.
3 Moore FA, McKinley BA, Moore EE, *et al.* Inflammation and the Host Response to Injury, a large-scale collaborative project: patient-oriented research core: standard operating procedures for clinical care. III. Guidelines for shock resuscitation. *J Trauma* 2006; **61**:82–89.
4 Spahn DR, Bouillon B, Cerny V, *et al.* Management of bleeding and coagulopathy following major trauma: an updated European guideline. *Crit Care Lond Engl* 2013; **17**:R76.
5 Kozek-Langenecker SA, Afshari A, Albaladejo P, *et al.* Management of severe perioperative bleeding: guidelines from the European Society of Anaesthesiology. *Eur J Anaesthesiol* 2013; **30**:270–382.
6 Kehlet H. Multimodal approach to control postoperative pathophysiology and rehabilitation. *Br J Anaesth* 1997; **78**:606–617.
7 Takasu A, Shibata M, Uchino S, *et al.* Effects of arterial oxygen content on oxidative stress during resuscitation in a rat haemorrhagic shock model. *Resuscitation* 2011; **82**:110–114.
8 Lejay A, Meyer A, Schlagowski AI, *et al.* Mitochondria: mitochondrial participation in ischemia-reperfusion injury in skeletal muscle. *Int J Biochem Cell Biol* 2014; **50**:101–105.
9 Lusczek ER, Lexcen DR, Witowski NE, *et al.* Prolonged induced hypothermia in haemorrhagic shock is associated with decreased muscle metabolism: a nuclear magnetic resonance-based metabolomics study. *Shock* 2014; **41**:79–84.
10 Hancock HM, Stannard A, Burkhardt GE, *et al.* Haemorrhagic shock worsens neuromuscular recovery in a porcine model of hind limb vascular injury and ischemia-reperfusion. *J Vasc Surg* 2011; **53**:1052–1062.
11 Tsai MC, Chen WJ, Ching CH, *et al.* Resuscitation with hydroxyethyl starch solution prevents nuclear factor kappaB activation and oxidative stress after haemorrhagic shock and resuscitation in rats. *Shock* 2007; **27**:527–533.
12 Inan N, Iltar S, Surer H, *et al.* Effect of hydroxyethyl starch 130/0.4 on ischaemia/reperfusion in rabbit skeletal muscle. *Eur J Anaesthesiol* 2009; **26**:160–165.
13 Diana M, Noll E, Diemunsch P, *et al.* Enhanced-reality video fluorescence: a real-time assessment of intestinal viability. *Ann Surg* 2014; **259**:700–707.
14 Noll E, Schaeffer R, Joshi G, *et al.* Heat loss during carbon dioxide insufflation: comparison of a nebulization based humidification device with a humidification and heating system. *Surg Endosc* 2012; **26**:3622–3625.
15 Régnier MA, Raux M, Le Manach Y, *et al.* Prognostic significance of blood lactate and lactate clearance in trauma patients. *Anesthesiology* 2012; **117**:1276–1288.
16 Charles AL, Guibert AS, Geny B. Effect of post-conditioning on mitochondrial dysfunction in experimental aortic cross-clamping. *Brit J Surg* 2010; **98**:511–516.
17 Bouitbir J, Charles AL, Echaniz-Laguna A, *et al.* Opposite effects of statins on mitochondria of cardiac and skeletal muscles: a 'mitohormesis' mechanism involving reactive oxygen species and PGC-1. *Eur Heart J* 2012; **33**:1397–1407.
18 Pottecher J, Santelmo N, Noll E, *et al.* Cold ischemia with selective anterograde in situ pulmonary perfusion preserves gas exchange and mitochondrial homeostasis and curbs inflammation in an experimental model of donation after cardiac death. *Transpl Int* 2013; **26**:1027–1037.
19 Piotto M, Moussallieh FM, Dillmann B. Metabolic characterization of primary human colorectal cancers using high resolution magic angle spinning 1H magnetic resonance spectroscopy. *Metabolomics* 2009; **5**:291–301.
20 Piotto M, Moussallieh FM, Imperiale A, *et al.* Reproducible sample preparation and spectrum acquisition techniques for metabolic profiling of human tissues by 1H HRMAS NMR. Methodologies in metabolomics: Experimental strategies and techniques for metabolomics research, 2013, vol. 496524.
21 Meiboom S, Gill D. Modified spin echo method for measuring nuclear relaxation times. *Rev Sci Instrum* 1958; **29**:688–691.
22 Dikalov S, Griendling KK, Harrison DG. Measurement of reactive oxygen species in cardiovascular studies. *Hypertension* 2007; **49**:717–727.
23 Dikalov SI, Kirilyuk IA, Voinov M, *et al.* EPR detection of cellular and mitochondrial superoxide using cyclic hydroxylamines. *Free Radic Res* 2011; **45**:417–430.
24 Oettl K, Stauber RE. Physiological and pathological changes in the redox state of human serum albumin critically influence its binding properties. *Br J Pharmacol* 2007; **151**:580–590.
25 Chen G, You G, Wang Y, *et al.* Effects of synthetic colloids on oxidative stress and inflammatory response in haemorrhagic shock: comparison of hydroxyethyl starch 130/0.4, hydroxyethyl starch 200/0.5, and succinylated gelatin. *Crit Care* 2013; **17**:R141.
26 Wang P, Li Y, Li J. Protective roles of hydroxyethyl starch 130/0.4 in intestinal inflammatory response and oxidative stress after haemorrhagic shock and resuscitation in rats. *Inflammation* 2009; **32**:71–82.
27 Aksu U, Bezemer R, Yavuz B, *et al.* Balanced vs unbalanced crystalloid resuscitation in a near-fatal model of haemorrhagic shock and the effects on renal oxygenation, oxidative stress, and inflammation. *Resuscitation* 2012; **83**:767–773.
28 Hirata N, Shim YH, Pravdic D, *et al.* Isoflurane differentially modulates mitochondrial reactive oxygen species production via forward versus reverse electron transport flow: implications for preconditioning. *Anesthesiology* 2011; **115**:531–540.

29 Karamercan MA, Weiss SL, Villarroel JPP, *et al.* Can peripheral blood mononuclear cells be used as a proxy for mitochondrial dysfunction in vital organs during haemorrhagic shock and resuscitation? *Shock* 2013; **40**:476–484.
30 Thaveau F, Zoll J, Rouyer O, *et al.* Ischemic preconditioning specifically restores complexes I and II activities of the mitochondrial respiratory chain in ischemic skeletal muscle. *J Vasc Surg* 2007; **46**:5 41–547.
31 Bonnard C, Durand A, Peyrol S, *et al.* Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. *J Clin Invest* 2008; **118**:789–800.
32 Bravo C, Kudej RK, Yuan C, *et al.* Metabolomic analysis of two different models of delayed preconditioning. *J Mol Cell Cardiol* 2013; **55**:19–26.
33 Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. *Physiol Rev* 2000; **80**:1107–1213.
34 Audonnet-Blaise S, Krafft MP, Smani Y, *et al.* Resuscitation of severe but brief haemorrhagic shock with PFC in rabbits restores skeletal muscle oxygen delivery and does not alter skeletal muscle metabolism. *Resuscitation* 2006; **70**:124–132.
35 Leong B, Reynolds PS, Tiba MH, *et al.* Effects of a combination hemoglobin based oxygen carrier-hypertonic saline solution on oxygen transport in the treatment of traumatic shock. *Resuscitation* 2011; **82**:937–943.