Hydroxyethyl starch is superior to lactated Ringer as a replacement fluid in a pig model of acute normovolaemic haemodilution


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Background. Tissue tolerance to oxygen privation during acute normovolaemic haemodilution with different fluids remains unclear. We tested the hypothesis that hydroxyethyl starch (HES) is superior to lactated Ringer’s solution in pigs for preserving tissue perfusion during acute normovolaemic haemodilution.

Methods. Twenty-four animals were randomized into control, lactated Ringer’s solution and HES groups. All groups, except the control, underwent acute normovolaemic haemodilution. Haemodynamics, oxygen parameter indices, global anaerobic metabolic markers, echocardiographic parameters, gastric tonometry and serum osmolarity were monitored at baseline, immediately after (0 min) and 60 and 120 min after the end of haemodilution. Myocardial, liver, stomach and intestine samples were collected for further evaluation.

Results. Cardiac and oxygen parameter index responses to acute normovolaemic haemodilution were comparable. However, the increment in cardiac index, stroke volume index, and left ventricular stroke work index were more sustained in the starch group. In the lactated Ringer’s group, gastric pH decreased significantly and was accompanied by a significant increase in lactate. Myocardial ultrastructure was better preserved in the starch group. The other tissue samples presented no change.

Conclusions. In this model of ANH, the starch group had a superior haemodynamic response. Minor loss of myocardial cellular integrity and preserved gastric pH i reinforce these findings.


Keywords: blood, acute normovolaemic haemodilution; gastric tonometry; HES; lactated Ringer’s; measurement techniques, echocardiography; myocardial ultrastructure; pigs

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Although acute normovolaemic haemodilution (ANH) has been used for more than 30 yr, its characteristics are still under investigation.1 It has been demonstrated mathematically that for ANH to be efficacious, the expected surgical blood loss should be 0.70 or more of the patient’s blood volume and the transfusion ‘trigger’ haematocrit should be 18–21%.2

Compensatory mechanisms of ANH have been well studied, including the influence of depth and type of anaesthesia on haemodynamic response to haemodilution.3 However, studies on the critical limits of lower haemoglobin under haemodilution on the structure and function of different organs are scarce. Because of an increase in stroke volume to compensate acute anaemia, cardiac tolerance to ANH has been the focus of clinical and experimental investigations.4 Besides, conventional monitoring of cardiovascular function, systemic and regional markers of the transition from aerobic to anaerobic metabolism are used to identify critical oxygen delivery.5–8 None of these studies, however, verified the consequences of oxygen privation because of acute anaemia and fluid resuscitation at the cellular level.

Crystalloid and colloid solutions are both used to maintain intravascular volume during haemodilution. However, no agreement has been reached regarding the best choice.
In a model of isovolaemic haemodilution, it was shown that colloids yielded haemodynamic stability and adequate tissue oxygen supply, whereas crystalloids alone jeopardized tissue perfusion and oxygenation. This investigation was designed to verify the hypothesis that hydroxyethyl starch (HES) is superior to lactated Ringer’s solution in relation to the maintenance of cardiovascular function, myocardial ultrastructural morphologic changes, and systemic and regional perfusion during ANH in pigs.

Methods

Experimental preparation

This study was approved by the ethics and animal investigation committees at our institution. The experiments were performed in 24 Landrace×Large White crossbreed pigs [weighing 46.6 (6.0) kg] fasted for 12 h with free access to water. Before the experiments, the animals were sedated with an intramuscular mixture of ketamine (10 mg kg\textsuperscript{-1} H\textsubscript{2}O of positive end-expiratory pressure and respiratory rate 201), fentanyl (5 \mu g kg\textsuperscript{-1}), and pancuronium (5 \mu g kg\textsuperscript{-1} min\textsuperscript{-1}). Anaesthesia was induced with propofol (5 mg kg\textsuperscript{-1}) administered i.v. After endotracheal intubation, anaesthesia was maintained with an infusion of ketamine (5 mg kg\textsuperscript{-1} h\textsuperscript{-1}), fentanyl (100 \mu g kg\textsuperscript{-1} h\textsuperscript{-1}) and pancuronium (5 \mu g kg\textsuperscript{-1} min\textsuperscript{-1}). The lungs were mechanically ventilated with an inspired oxygen fraction of 40\%, tidal volume of 6 ml kg\textsuperscript{-1}, and P\textsubscript{aCO\textsubscript{2}} of 30–35 mm Hg (Galileo, Hamilton Medical, Rhözüns, Switzerland). The pigs’ temperatures were maintained around 38°C using a warming blanket (Medi-therm II, Gaymar Industries, Orchard Park, NY, USA).

Animals were monitored with ECG including ST analysis (Viridia CMS, Hewlett-Packard, Andover, MA, USA). Both femoral arteries were catheterized, one for measuring mean arterial pressure and for arterial blood sampling, and the other for blood withdrawal. Two large-bore venous catheters were inserted into the right femoral and jugular veins for fluid infusion. A pulmonary artery catheter (7.5F, Baxter, Irvine, CA, USA) was introduced through the left femoral vein, positioned in the pulmonary artery, for the measurement of cardiac output (CO), right atrial pressure (RAP), pulmonary artery pressure (PAP), pulmonary capillary wedge pressure (PCWP), central body temperature, and collection of mixed venous blood samples. Pressure transducers were connected to a multiparametric data collection system (Viridia CMS, Hewlett-Packard, Andover, MA, USA) for continuous monitoring of heart rate (HR), pressures and waveforms. CO was determined by thermodilution at end expiration by using 10 ml of iced glucose 5\%, and the mean value of three measurements within ±10\% variation was recorded (Vigilance, Baxter, Irvine, CA, USA). Cardiac index (CI) was calculated according to calculated body surface (\(k\times BW^{0.75}\), where \(k=0.09\)).

Systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), stroke volume index (SVI), left ventricular stroke work index (LVSWI) and right ventricular stroke work index (RVSWI) were calculated using standard formulae. At each measurement point, an arterial and mixed venous sample was taken simultaneously. The arterial blood samples were used to determine serum osmolarity (3300 Micro Osmometer, Advanced Instruments, Norwood, USA), arterial oxygen partial pressure (\(P_{aO_2}\)), pH, base excess, bicarbonate concentration (HCO\textsubscript{3}), haematocrit (H\textsubscript{t}), haemoglobin concentration (Hb), haemoglobin oxygen saturation, blood lactate, sodium and potassium (ABL 555, Radiometer, Copenhagen, Denmark). The mixed venous samples were used to determine venous partial oxygen pressure (\(P_{vO_2}\)) and venous oxygen saturation (\(S_{vO_2}\)). Arterial oxygen content (\(C_{aO_2}\)), mixed venous oxygen content (\(C_{vO_2}\)), arteriovenous oxygen content difference (\(C_{a-vO_2}\)), systemic oxygen delivery index (DO\textsubscript{2I}), systemic oxygen consumption (VO\textsubscript{2}) and systemic oxygen extraction ratio (O\textsubscript{2}ER) were calculated using standard formulae. Veno-arterial carbon dioxide tension difference (\(\Delta P_{CO_2}\)) and \(\Delta P_{CO_2}/C_{a-vO_2}\) ratio were used to detect global anaerobic metabolism.

Gastric intramucosal pH (pHi) was measured using air-automated tonometry. I.V. ranitidine (2.0 mg kg\textsuperscript{-1}) was administered before the insertion of the gastric catheter. A tonometer tube with a silicone rubber balloon (catheter TRIP NGS, Tonometrics, Worcester, MA, USA) was positioned in the stomach. Prior to catheter insertion, the stomach was cleaned with 500 ml of normal, warm saline. Satisfactory placement of the tube was confirmed by auscultation over the epigastrium after injection of 50 ml of air into the lumen. The tonometer and the airway sampling line were connected to an automated gas analyser (Tonocap, Datex, Helsinki, Finland). Arterial blood samples were taken simultaneously with the measurement of gastric carbon dioxide (\(P_{rCO_2}\)) to calculate the regional-to-arterial \(P_{CO_2}\) difference [\(P_{r(a)CO_2}\)]. pH\textsubscript{i} was calculated by the Tonocap method, and the formula \(pH_i=pH_a−log(P_{rCO_2}/P_{aCO_2})\).

Transoesophageal echocardiography measurements were performed with a 7.5/5.0 MHz transducer (Omniplane II T6210, Agilent Technologies, Andover, MA, USA) advanced approximately 35 cm into the oesophagus. Echocardiographic measurements were made on the long axis, 2-chamber views, allowing for repetitive frame-by-frame analyses. Left ventricular systolic and diastolic volumes, stroke volume and ejection fraction were computed using the simplified Simpson’s rule.

Experimental design

Following instrumentation, animals were allowed to stabilize for 30 min and were then randomized into three groups. All animals, regardless of the group, received 5 ml kg\textsuperscript{-1} h\textsuperscript{-1}...
of lactated Ringer’s solution as a maintenance fluid. Animals in the control group (n=8) were anaesthetized and ventilated without haemodilution. In the HES group (n=8), blood withdrawn was simultaneously replaced with 6% HES 200 000/0.5 (HAES-steril, Fresenius-Kabi, Germany) at a 1:1 ratio (1 ml of HES to 1 ml of blood). In the lactated Ringer’s group (LR, n=8), lactated Ringer’s solution was used at a 3:1 ratio (3 ml of lactated Ringer’s to replace 1 ml of removed blood). Haemodilution was performed for 30 min. The solutions were warmed to 38°C before infusion. The volume of blood removed (V) was previously calculated according to the formula:

\[ V = \text{EBV} \times \left[ (H_0 - H_1)/H_{av} \right] \]

where EBV is the estimated blood volume, \( H_0 \) the initial haematocrit, \( H_1 \) the target haematocrit and \( H_{av} \) the average haematocrit \( [(H_0+H_1)/2] \). A graduated cylinder was used to measure the correct amount of blood withdrawn. Target haematocrit was checked every 10 min to determine blood withdrawn.

**Target haematocrit**

Target haematocrit was established at 15%, which represents approximately 50% of the initial. The normal pigs haematocrit and haemoglobin range between 23.5–33.0 and 7.3–10.2 gr dl\(^{-1}\) respectively,\(^{14}\) which corresponds to adult human values of haemoglobin of 12.0–16.0 gr dl\(^{-1}\) for women and 14.4–17.0 gr dl\(^{-1}\) for men. Following ANH, episodes of diminution in mean systemic arterial pressure >20% of baseline were treated with additional fluid (lactated Ringer’s or starch) according to the respective group.

Data were collected before blood withdrawal (baseline), immediately after (zero), and 60 and 120 min after the end of haemodilution.

**Tissue samples and analysis**

After final data collection at 120 min, with the animals still anaesthetized, the abdominal and thoracic cavity were opened. A cardioplegic dose of potassium chloride was immediately injected into the aortic root; the heart was quickly removed and washed in physiological saline. Five samples were collected from each animal from the subendocardial area of the left ventricle (midportion of anterior wall) for optical and electron microscopy analyses (JEM-1010 electron microscope, JEOL, Peabody, MA, USA) independently carried out by one pathologist who was blinded to the protocol. Five sections were observed in each sample. Liver, small intestine and stomach tissue samples were also obtained, fixed in 10% formalaldehyde and stained with haematoxylin–eosin stain.

**Statistical analysis**

Removed blood, and replacement fluid were analysed using the Student’s \( t \)-test. Body weight, urinary volume, haemodynamic and other parametric data were analysed within groups and between groups by using analysis of variance (ANOVA) for repeated measurements (InStat 3.01, GraphPad, San Diego, USA). When appropriate, post hoc analysis was performed with the Tukey test. \( P<0.05 \) was considered statistically significant. Values are presented as means (sd).

**Results**

Body weight and body surface area did not vary. Blood withdrawn and final haematocrit were similar between the two study groups. Haematocrit observed after ANH was 14.4 (1.7)% in the HES group and 15.4 (0.8)% in the LR group (no significant differences). Urine output and the volume of fluids administered during and post ANH was significantly higher in the LR group (Table 1).

Baseline values were similar among the three groups with regard to all parameters measured. During the observation period, no changes were seen in the control group (Tables 2 and 3). After ANH in the experimental groups, HR, MAP and PCWP did not change. In contrast, RAP and PAP increased significantly in the LR group 60 and 120 min after ANH. CI increased significantly in both groups just after haemodilution. However, this increment was significantly greater in the HES group compared with the LR group. Interestingly, SVI and LVSWI increased significantly only in starch-treated animals. RVSWI increased significantly in HES animals immediately after ANH, but increased significantly only in the LR group 60 and 120 min after the end of ANH. SVRI significantly decreased in both haemodiluted groups after haemodilution; this was more sustained in the HES group (Table 2).

Ejection fraction, obtained through transoesophageal echocardiography, did not present significant changes after haemodilution in both groups when compared with that at baseline and in controls (Table 2).

### Table 1 Mean weight of pigs, blood withdrawn, volume of 6% hydroxyethyl starch and lactated Ringer’s infused during and after acute normovolaemic haemodilution (ANH), and urine output. Values are means (sd). *\( P<0.05 \) from Control; †\( P<0.05 \) from HES. ANH, acute normovolaemic haemodilution; HES, hydroxyethyl starch; LR, lactated Ringer’s solution.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (kg)</th>
<th>BSA (m²)</th>
<th>Urine output (ml)</th>
<th>Blood withdrawn ANH (ml)</th>
<th>Volume during ANH (ml)</th>
<th>Volume post ANH (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>43.7 (4.1)</td>
<td>1.09 (0.10)</td>
<td>293 (222)</td>
<td>2150 (307)</td>
<td>2150 (307)</td>
<td>1263 (296)</td>
</tr>
<tr>
<td>HES</td>
<td>37.9 (6.4)</td>
<td>1.00 (0.12)</td>
<td>1063 (847)</td>
<td>6575 (2442)†</td>
<td>4363 (852)†</td>
<td></td>
</tr>
<tr>
<td>LR</td>
<td>38.1 (12.1)</td>
<td>1.00 (0.20)</td>
<td>2609 (1484)* †</td>
<td>2200 (823)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
As expected, $\text{SV}_O_2$ and $\text{DO}_2I$ decreased significantly after ANH in both groups. At the same time, the $\text{O}_2\text{ER}$ increased significantly in both groups after ANH; however, this increment was significant only in the LR group (Table 3).

Gastric pH decreased significantly 60 and 120 min after blood withdrawal only in the LR group (Fig. 1).

Both experimental groups had a significant decrease in base excess after haemodilution (T1). However, arterial pH did not decrease significantly. In the LR haemodiluted group, a significant decrease occurred in serum sodium concentration and serum sodium bicarbonate, just after haemodilution and at 60 min. Serum lactate increased significantly only in the LR group after haemodilution.

$\Delta P_{\text{CO}_2}$ and the $\Delta P_{\text{CO}_2}/C_{a-v}$ ratio remained unaltered during haemodilution (Fig. 1).

Serum osmolarity decreased significantly in the LR group after haemodilution (Fig. 2).

### Discussion

The primary end-point of this prospective, randomized study was, in a model of ANH, to compare the effects of...
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Table 3 Oxygenation and electrolyte measurements during acute normovolaemic haemodilution (ANH). Values are means (SD). HES, hydroxyethyl starch group; LR, lactated Ringer’s solution group; Ht, haematocrit; PaO2, mixed venous partial pressure of oxygen; DO2I, systemic oxygen delivery index; VO2I, systemic oxygen consumption index; O2ER, oxygen extraction ratio; PVO2, mixed venous oxygen saturation; HCO3−, bicarbonate; Na+, sodium; K+, potassium; BE, base excess. *P<0.05 from baseline; †P<0.01 from baseline; ‡P<0.05 from Control; §P<0.05 from HES

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Baseline</th>
<th>Minutes post ANH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>Control</td>
<td>32 (3)</td>
<td>33 (3)</td>
</tr>
<tr>
<td></td>
<td>HES</td>
<td>34 (4)</td>
<td>14 (2)†</td>
</tr>
<tr>
<td></td>
<td>LR</td>
<td>33 (4)</td>
<td>15 (1)†</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>Control</td>
<td>146.4 (16.1)</td>
<td>140.3 (21.9)</td>
</tr>
<tr>
<td></td>
<td>HES</td>
<td>142.0 (36.2)</td>
<td>134.4 (34.6)</td>
</tr>
<tr>
<td></td>
<td>LR</td>
<td>159.7 (31.2)</td>
<td>131.9 (29.5)</td>
</tr>
<tr>
<td>PVO2 (mm Hg)</td>
<td>Control</td>
<td>51.1 (1.5)</td>
<td>49.0 (2.3)</td>
</tr>
<tr>
<td></td>
<td>HES</td>
<td>48.8 (6.8)</td>
<td>41.5 (4.3)*</td>
</tr>
<tr>
<td></td>
<td>LR</td>
<td>49.5 (7.4)</td>
<td>37.2 (2.8)†</td>
</tr>
<tr>
<td>DO2I (ml min⁻¹ m⁻²)</td>
<td>Control</td>
<td>967 (120)</td>
<td>944 (113)</td>
</tr>
<tr>
<td></td>
<td>HES</td>
<td>909 (289)</td>
<td>679 (162)*</td>
</tr>
<tr>
<td></td>
<td>LR</td>
<td>811 (182)</td>
<td>558 (161)*</td>
</tr>
<tr>
<td>VO2I (ml min⁻¹ m⁻²)</td>
<td>Control</td>
<td>191 (35)</td>
<td>184 (33)</td>
</tr>
<tr>
<td></td>
<td>HES</td>
<td>173 (36)</td>
<td>171 (23)</td>
</tr>
<tr>
<td></td>
<td>LR</td>
<td>177 (53)</td>
<td>196 (59)</td>
</tr>
<tr>
<td>O2ER (%)</td>
<td>Control</td>
<td>21 (9)</td>
<td>21 (9)</td>
</tr>
<tr>
<td></td>
<td>HES</td>
<td>40.4 (0.4)</td>
<td>37.0 (0.3)</td>
</tr>
<tr>
<td></td>
<td>LR</td>
<td>4.0 (1.9)</td>
<td>0.5 (1.5)†</td>
</tr>
</tbody>
</table>

HES 200/0.5 and lactated Ringer’s solution on cardiovascular and tissue perfusion. Haemodilution elicited the expected haemodynamic changes in both experimental groups. The HES group presented a more sustained compensatory response because the CI increase lasted longer and was accompanied by an increase in SVI and LVSWI. In the LR group, the CI increment was attributable to a substantial increase in filling pressures. Cardiac function assessed by transoesophageal echocardiography was also similar in both groups. Evaluation of myocyte ultrastructure presented important changes, demonstrating the poor performance of routine haemodynamic monitoring to indicate changes at the cellular level. Regional perfusion index, such as pH, was also better maintained in the HES group, demonstrating the superiority of HES for maintaining regional splanchnic perfusion, although light microscopy of tissue samples, obtained from this region, did not show any cellular alteration among the groups.

The main question while performing ANH in a clinical setting, is to detect early signs of inadequate oxygen delivery to different organs. ANH causes a reduction in arterial oxygen content, and the primary compensatory mechanism is an augmentation in stroke volume and HR to maintain systemic oxygen delivery. Therefore, the lower limit of a permissive decrease in haemoglobin concentration is related to how low haemoglobin can become without interfering with the ability of the heart to sustain an increased pumping requirement. During acute normovolaemic haemodilution, conventional haemodynamic monitoring does not detect cellular oxygen deprivation. In healthy human volunteers, ECG ST-segment changes, observed during an acute reduction in haemoglobin (5–7 g dl⁻¹), were suggestive of, but not conclusive for, myocardial ischaemia. Tolerance to normovolaemic anaemia is still controversial. Van Bommel and colleagues, working with pigs, found critical levels of haemoglobin during ANH at corresponding levels of 10–15% haematocrit, values in agreement with those.
obtained in our study. The experimental model allowed us to perform haemodilution and simultaneously, indirectly assess the heart and splanchnic perfusion with monitors used in clinical practice. At the same time, serum lactate, \( \Delta P_{CO_2}/C_{(a-v)}O_2 \) ratio were used as markers of global anaerobic metabolism.\(^7\) Our haemodynamic data, represented by CI, LVSWI and RVSWI, showed slight differences between groups after haemodilution. LR animals had higher values of PAP and RAP at 60 and 120 min after ANH, while PCWP remained unchanged in both groups. After haemodilution, fluid administration was based on the maintenance of MAP; therefore, LR animals needed greater volumes, which may explain the increased values of RAP and PAP.

Ejection fraction measured by echocardiography tended to increase after ANH in both groups, interpreted as a
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hyperdynamic status attributable to haemodilution. We have difficulties utilizing conventional means of monitoring the decline in cardiac function connected to the histological changes. Conversely, in a previous study in dogs haemodiluted to lower levels of haematocrit compared with this study, we were able to demonstrate a deterioration in cardiac contractility using echocardiography in the last period of LR haemodiluted dogs. It is difficult to ascertain if RAP alone could identify cardiac dysfunction or volaemic overload without any other signals. However, the theoretically more specific echocardiography that we have performed did not reveal cardiac dysfunction.

Concerning the myocardial cellular effects of ANH achieved with different fluids, the literature is scarce. Old studies verified an increase in myocardial extravascular water content after haemodilution associated with extracorporeal circulation. Laks and colleagues studied adult dog hearts on cardiopulmonary bypass while diluting the haematocrit with crystalloid from a baseline of 28.6–9.5 for 1 h and showed a 30.3% increase in wet-dry weight ratios when comparing normal and crystalloid haemodiluted myocardial tissue. Mavroudis and Ebert studied puppies and adult dogs haemodiluted with normal saline and demonstrated that newborn hearts are more susceptible to oedema formation, probably related to a type of collagen and connective tissue deficit. These studies confirm that low osmolarity is the only factor responsible for the observed myocardial defects. The large amount of water in association with Ringer could have caused additional effects.

Global or regional tissue CO₂ production and exchange derived parameters are markers of tissue dysoxia. Mekontso-Dessap reviewed the haemodynamic records of 89 critically ill patients and found that the ΔPCO₂/C(a-v)O₂ ratio is a reliable marker of global anaerobic metabolism, being superior in specificity and sensitivity to ΔPO₂ and C(a-v)O₂ and SWO₂. In our study, ΔPCO₂ and the ΔPCO₂/C(a-v)O₂ ratio were not different among the three groups, probably because of the compensatory mechanism of haemodilution by increasing CO and microcirculatory blood flow. Using a regional ischaemic or hypoxic model in dogs, Vallet and colleagues found that lowering DO₂ by decreasing flow results in an increased ΔPCO₂, whereas lowering DO₂ by decreasing blood oxygenation does not affect ΔPCO₂.

During periods of low haemoglobin, as observed in our study, the increased flow in the microcirculation may be enough to avoid anaerobic metabolism, which could be expressed as increased ΔPCO₂/C(a-v)O₂ gradient. Gastric pH and intramucosal-arterial PCO₂ gradient may also have some limitations in describing intestinal oxygen delivery during haemodilution with preserved blood flow. During ANH states, an increase in flow occurs, but with low oxygen content. This means that the increase in flow should compensate the decrease in oxygen content. In the RL group, the flow was probably not enough to compensate for the decrease in oxygen delivery.

Experimental data comparing hypovolaemia versus haemodilution showed that intramucosal-arterial PCO₂ gradient was significantly higher in hypovolaemia than in haemodilution at the same level of systemic and intestinal oxygen transport.

Regarding the increase in serum lactate in LR group, it is difficult to interpret if this was caused by endogenous lactate production, excess infusion or both. An augmentation of endogenous lactate production could be explained by an increase in anaerobic metabolism. However, the indices that express tissue oxygenation such as oxygen consumption, arterial pH, DO₂, ΔPCO₂, BE did not differ significantly between both haemodiluted groups. According to some experimental studies, there is an increase in hepatic flow during haemodilution. Nöldge and colleagues showed in haemodiluted pigs, that hepatic uptake of lactate and pyruvate remained unchanged. Unfortunately, both studies utilized only starch for haemodilution not Ringer. The main

respective. HES 200/0.5 presents sodium concentration and osmolarity of 154 mmol litre⁻¹ and 308 mOsm litre⁻¹ respectively. This means that large amounts of hyposmolar lactated Ringer’s (compared with the swine plasma baseline levels of sodium and osmolarity (140–142 mmol litre⁻¹ and 290–300 mOsm litre⁻¹) may have contributed to a decrease in serum sodium and osmolarity. Indeed, it is difficult to confirm that low osmolarity is the only factor responsible for the observed myocardial defects. The large amount of water in association with Ringer could have caused additional effects.
reason for the lactate elevation in our study was probably because of an excess of exogenous lactate.

In our study, the DO2I response was the same in both groups. In contrast, gastrointestinal oxygenation assessed by gastric tonometry was impaired in the LR group. Our results are in accordance with those observed by Van Bommel and colleagues, who demonstrated that during progressive haemodilution with 6% HES pigs had systemic and intestinal oxygenation impairment at haematocrit of 10–15%. In the Van Bommel and colleagues’ study, regional and systemic lactate concentration remained constant until this level of haemodilution.

Our possible explanation for the VO2I increase at 120 min in LR group is the large variability in relation to a small sample causing discrepancies among the two groups. Indeed, there was no significant increase in oxygen consumption in both groups along with ANH. Only the last values of VO2I in LR group [229 (55) ml min⁻¹ m⁻²] differed significantly from the basal values [177 (53) ml min⁻¹ m⁻²], but there was no overall difference among the groups.

The main limitation of our study may be attributed to the short observation time and the volume of plasma replacement derived from conventional formulae. Plasma volume expansion measurements may be more rigorously determined by using labelled red cells or indocyanine green pulse spectrophotometry. However, these approaches are relatively complex and not easy to perform in daily clinical routine.

If the observation period had been longer than 120 min in LR group is the large variability in relation to a small sample causing discrepancies among the two groups. Indeed, there was no significant increase in oxygen consumption in both groups along with ANH. Only the last values of VO2I in LR group [229 (55) ml min⁻¹ m⁻²] differed significantly from the basal values [177 (53) ml min⁻¹ m⁻²], but there was no overall difference among the groups.

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If the observation period had been longer than 120 min, differences in cardiovascular function between groups may have been more evident, resulting in deterioration of cardiovascular compensatory mechanisms. This study demonstrated that maintenance of haemodynamic parameters during low levels of haemoglobin did not prevent cellular damage. In this study, we focused mainly on the myocardium as, during ANH or severe anaemia, the heart is forced to pump harder in an attempt to maintain oxygen delivery to the periphery. The unanswered question is the specificity and sensitivity of echocardiography and conventional haemodynamics to detect the point at which cardiac compensatory mechanisms may be broken or turned up to serve as a warning to critical haemoglobin levels during anaesthesia. Because of methodological difficulties, we did not measure lactate and oxygen saturation directly from coronary venous sinus. These data could give more precise information about myocardial metabolism with the two fluids used for acute normovolaemic haemodilution. Considering the animal species, another unanswered question in this study is whether this kind of myocardial ultrastructural alteration might be partially or totally reversed with haemoglobin normalization.

In conclusion, in anaesthetized pigs haemodiluted to moderate values of haematocrit, better maintenance of haemodynamic parameters, ventricular systolic function, gastric pH, and oxygen transport and extraction ratio suggest a superiority of HES for replacing removed blood compared with lactated Ringer’s solution. Furthermore, myocardial ultrastructure changes in the lactated Ringer’s solution group reinforces this assumption.

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