Extracorporeal rewarming from experimental hypothermia: Effects of hydroxyethyl starch vs saline priming on fluid balance and blood flow distribution

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Running title: Colloid vs crystalloid priming in extracorporeal rewarming from experimental hypothermia

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Key words: accidental hypothermia, extracorporeal circulation, blood volume, organ blood flow, cardiac arrest

Total number of words in paper (excluding references and figure legends): 4691

Total number of references: 42

- **What is the central question of this study?**
    - Mortality in accidental hypothermia patients rewarmed by extracorporeal circulation remains high. Knowledge concerning optimal fluid additions for extracorporeal rewarming is lacking, with no apparent consensus. Do colloid vs crystalloid priming have different effects on fluid balance and blood flow distribution during extracorporeal rewarming?
- **What is the main finding and its importance?**
    - In our rat model of extracorporeal rewarming from hypothermic cardiac arrest, hydroxyethyl starch generates less tissue oedema, increases circulating blood volume and organ blood flow, compared to saline. Composition of fluid additions appears important during extracorporeal rewarming from hypothermia.

Abstract

Rewarming by extracorporeal circulation is the recommended treatment of accidental hypothermia patients with cardiac instability. Hypothermia, along with initiation of ECC, introduces major changes to fluid homeostasis and blood flow. Scientific data to recommend best practice use of ECC for rewarming these patients is lacking, and no current guidelines exist concerning the choice of priming fluid for the extracorporeal circuit. The primary aim of this study was to compare effects of different fluid protocols on fluid balance and blood flow distribution during rewarming from deep hypothermic cardiac arrest (DHCA). Sixteen anaesthetized rats were cooled to DHCA, and rewarmed by ECC. During cooling, rats were equally randomized into two groups; extracorporeal circuit primed with saline or primed with hydroxyethyl starch (HES). Calculations of plasma volume (PV), circulating blood volume (CBV), organ blood flow (OBF), total tissue water content (TTW), global O_2 delivery (DO_2) and consumption (VO_2) were made. During and after rewarming, pump flow rate, mean arterial pressure, PV and CBV was significantly higher in HES-treated compared to saline-treated rats. After rewarming, the HES group had significantly increased DO_2, as well as blood flow to brain and kidneys compared to the saline group. Rats in the saline group demonstrated a significantly higher TTW in kidneys, skeletal muscle and lung. Compared to crystalloid priming, the use of an iso-oncotic colloid prime generates less tissue oedema and increases PV, CBV and organ blood flow during ECC rewarming. The composition of fluid additions appears to be an important factor during ECC rewarming from hypothermia.

Introduction

Severe accidental hypothermia (involuntary drop in core body temperature ≤ 28°C) (Zafren et al., 2014) is associated with ventricular arrhythmias and a high risk of cardiac arrest. By reducing cellular oxygen demands, hypothermia protects vital organs during periods of cellular ischemia, and patient case reports have documented successful recovery after prolonged resuscitation (Mark et al., 2012; Romlin et al., 2015; Wollenek, Honarwar, Golej, & Marx, 2002). In accidental hypothermia accompanied by cardiac instability, rewarming by extracorporeal circulation (ECC) has become the gold standard (Brown, Brugger, Boyd, & Paal, 2012). As rewarming increases metabolic rate, ECC offers advantages compared to other rewarming methods by providing rapid circulatory support and adequate tissue perfusion (Wollenek et al., 2002). However, despite the widespread use of ECC for rewarming accidental hypothermia patients, more research data is needed in order to optimize the use of this treatment modality (Mair & Ruttmann, 2014). This notion is substantiated by findings in a recent publication from a Norwegian university hospital reporting that no change in survival rate has taken place over the last 30 years when using ECC for rewarming in these patients (Svendsen, Grong, Andersen, & Husby, 2017).

Initiating ECC with a colloid or crystalloid prime causes haemodilution, which along with impaired capillary integrity due to hypothermia and possible hypoxia/ischemia give rise to an increased fluid extravasation (Farstad et al., 2003). This can lead to fluid overload which is related to organ dysfunction and adverse patient outcome (Farstad et al., 2006). As crystalloid solutions pass freely across the endothelial glycocalyx and underlying endothelial cells, they are evenly distributed within the extracellular fluid compartment, leaving only about one fifth of the administered fluid in the intravascular space (Chappell, Jacob, Hofmann-kiefer, Conzen, & Rehm, 2008; Myers & Wegner, 2017). Administering colloids instead of crystalloids during cardiopulmonary bypass (CPB) has, however, been reported to

reduce fluid leakage and oedema formation in both preclinical (Farstad, Kvalheim, & Husby, 2005) and clinical studies (Himpe, 2003; Kvalheim et al., 2010). Moreover, improved post-operative cardiac function is reported to take place if using colloids rather than crystalloids during CPB (Kvalheim et al., 2010). Still, in general, the use of synthetic colloids in clinical medicine has also been associated with side effects such as coagulopathies, risk of acute kidney failure and allergic reactions, which has led to restricted use in critical care patients (Chappell et al., 2008; Perner et al., 2012).

Preclinical experiments of severe hypothermia have reported major physiological changes in blood distribution, including reduction in both plasma volume (PV) and circulating blood volume (CBV) (Chen & Chien, 1977; Hammersborg et al., 2005; Kondratiev, Flemming, Myhre, Sovershaev, & Tveita, 2006; Lofstrom, 1957) in response to cooling, and significant alterations in organ blood flow upon rewarming (Tveita et al., 1996). Applying ECC for rewarming accidental hypothermic patients may be particularly challenging as a decreased CBV can lead to an inadequate venous drainage and thereby difficulties in obtaining a sufficient pump flow rate (PFR). Consequently, additional fluids must be given, sometimes large amounts (Romlin et al., 2015; Wollenek et al., 2002), which in turn may exacerbate fluid extravasation and volume overload.

Fluid regimes during ECC have been extensively studied in the setting of normothermia and the use of hypothermic CPB for cardiac surgery. In ECC rewarming from accidental hypothermia, however, the majority of literature originates from retrospective studies and case reports. Extrapolating approaches for ECC rewarming of accidental hypothermia patients from studies in cardiac surgery patients may be unwarranted, as these patient groups differs in both intravascular volume status, circulatory function and duration of hypothermic insult (Valkov et al., 2019). Recommendations concerning choice of fluids in ECC rewarming of accidental hypothermia patients are lacking in both American (Vanden

Hoek et al., 2010) and European (Truhlár et al., 2015) guidelines, and seem to vary between institutions; some reporting sole use of crystalloids (Weuster et al., 2016), others using addition of colloid to the prime (Suominen, Vallila, Hartikainen, Sairanen, & Korpela, 2010), or continuous colloid infusion during CPB (Farstad et al., 2006). Hence, this study aimed to investigate the physiological effects of two different fluid protocols on fluid balance and blood flow distribution during rewarming from severe, accidental hypothermia. For this purpose we used a novel rat model of conduction cooling and subsequent extracorporeal rewarming.

Methods

Ethical approval

Male Wistar rats (258-395 g) provided by Charles River were used for the experiment. The rats had a microbiological status according to the recommendation of the Federation of European Laboratory Animal Science Associations, and were quarantined for 1 week on arrival. Housing during experiment was provided in accordance with guidelines for accommodation and care of animals (article 5 of European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986). Free food and water access were permitted. The experimental protocol was approved by the Norwegian Animal Research Authority (reference no. 01/15, ID no. 7185) and conducted accordingly.

Experimental protocol (figure 1)

After instrumentation, rats were given 500 IU/kg of heparin and were allowed to rest for 30 minutes before baseline measurements and initiation of cooling. Following surface cooling until deep hypothermic circulatory arrest (DHCA), the animals were connected to the extracorporeal circuit and rewarmed. The rats were randomized into two groups, of which the circuit was primed either with 0.9% saline ($n=8$) or HES 130/0.4 (Voluven®) ($n=8$). The experiment was terminated after rewarming to 37°C and following data sampling. Animals were not weaned from the ECC circuit, and were euthanized by an I.V. injection of 1 ml sodium pentobarbital (50 mg/ml). Rats had mean weights of 332.5 (38.8) g and 340.5 (49.0) g, in the HES and saline group, respectively.

Anaesthesia and respiratory support

Anaesthesia was introduced in an induction chamber with isoflurane 4% followed by continuous isoflurane 2% on a facemask. Perioperative analgesia was achieved by a

subcutaneous injection of buprenorphine (0.05mg/kg). Following tracheostomy, isoflurane 2% was given via a 14 G tracheal tube. All rats had spontaneous and sufficient ventilation at core temperatures > 20°C. Below 20°C ventilation was achieved via a respirator (TOPO™, Dual Mode Ventilator, Kent Scientific, CT, USA). During ECC, the isoflurane was connected to the oxygenator, as the majority of blood were excluded from the pulmonary circulation. Anaesthesia level was monitored by toe-pinch for any sign of discomfort in the animal, and adjusted if necessary. No neuromuscular blockers were used at any time during the experiment.

Extracorporeal circuit

The extracorporeal circuit (figure 2) was made up of a venous reservoir, a peristaltic pump (Masterflex® L/S®, Cole-Parmer Instrument Company, USA), a hollow fibre miniaturized oxygenator (Sorin, Mirandola, Italy) with oxygenation capacities previously validated (Cresce et al., 2008) and a heat exchanger. All components were connected by silicone tubing with an internal diameter of 3 mm on the venous side (pre-oxygenator) and 2 mm on the arterial side (post-oxygenator) of the circuit. Venous drainage was facilitated via a custom made 4.5 F multi-orifice cannula inserted in the right internal jugular vein and advanced to the right atrium. For arterial inflow, a 20 G cannula was placed in the aortic arch via the right common carotid artery. A 20 cm height difference between the rat and venous reservoir combined with the relatively large venous cannula resulted in excellent venous drainage. Further facilitation of venous drainage was available by applying a pressure of -10 cmH_2O through a vacuum regulator connected to the venous reservoir. Total priming volume of the circuit was 10 ml, gas exchange surface was 450 cm^2, and surface area for heat exchange was 12.8 cm^2.

Hemodynamic measurements

Continuous arterial pressure was obtained by a fluid filled 22 G catheter connected to a pressure transducer, inserted in the left femoral artery and advanced to the aorta. Heart rate

(HR) was derived from ECG monitoring using limb leads (Animal Bio Amp, FE136, ADInstruments, Australia).

Conduction cooling

Animals were cooled by circulating cold water from a water bath (Isotemp® R20, Fisher Scientific, Pittsburgh, USA) through the double-layered operating table, made of hollow aluminium. In addition, a U-shaped hollow polyethylene tube connected to the water bath, was gently inserted into the lower bowel. Core temperature was continuously monitored using a thermocouple wire positioned in the lower part of oesophagus, connected to a Thermalert Th-5 thermocouple controller (Bailey Instruments, UK).

Extracorporeal rewarming

Cardiac arrest was assessed by careful monitoring during late stages of cooling, and confirmed by ECG and a flat arterial pressure line for one minute, after which extracorporeal rewarming was initiated. Target flow rate (TFR) at 37°C was $120ml \cdot kg^{-1} \cdot min^{-1}$. At lower temperatures, TFR were set in accordance with normal values for cardiac output in spontaneous circulating rats at corresponding temperatures (Dietrichs, Håheim, Kondratiev, Sieck, & Tveita, 2014; Dietrichs, Kondratiev, & Tveita, 2014). To increase flow in accordance to the TFR spontaneously, fluids needed to be added to the extracorporeal circuit in some experiments, each group received the same fluid as was used for priming. Rewarming was assisted by adjusting temperature of water circulating the hollow operating board to 37°C. Difference between core body temperature and water circulating the heat exchanger never exceeded 10°C.

Determination of plasma volume (PV) and circulating blood volume (CBV)

PV was determined by dye dilution, using a solution of Evans blue (EB) (Sigma-Aldrich, Norway) (1mg/ml) injected in the arterial inflow cannula of the ECC circuit. Ten minutes

after injection, blood (0.2 ml) was sampled from the femoral artery and centrifuged (10min, 4°C, 4400 rpm) (Centrifuge 5702R, Eppendorf AG, Hamburg, Germany). Plasma dye concentration was determined by spectrophotometry (Wallac 1420, Victor2™, PerkinElmer Life and Analytical Sciences, Finland), and PV was calculated as: Dye injected (mg) / plasma dye concentration (mg/ml). Haematocrit (Hct) vials were collected in duplicates and measured after 3 min centrifugation in a micro-centrifuge (StatSpin III, StatSpin Technologies, MA, USA). Subsequently, CBV was determined by the following equation: CBV = PV/(1 – (0.96 x Hct/100)) (Sandel, Hubbard, & Schehl-Geiger, 1982). The factor 0.96 corrects for the fraction of plasma entrapped between erythrocytes (Gregersen & Rawson, 1959). To quantify the pre-existing dye concentration prior to repeated measurements, a reference blood sample was taken immediately before new injection of EB, and were corrected for when calculating consecutive PV. During ECC, intracorporeal volumes were calculated by excluding the volumes in the extracorporeal circuit, the following equations was used: Intracorporeal PV = calculated PV – extracorporeal PV, and: Intracorporeal CBV = calculated CBV – extracorporeal blood volume.

Organ blood flow measurements

At pre-determined core temperatures, stable isotope-labelled microspheres (BioPAL Inc., Worcester, USA) were injected into the arterial line of the ECC circuit. Simultaneously, a reference arterial blood sample with a constant rate of 0.5 ml · min^{-1} was drawn from the abdominal aorta. After experiment, tissue samples were collected and along with reference blood samples sent for neutron activation analysis (BioPAL Inc., Worcester, USA) for measurements of microsphere radioactivity in decays per minute (dpm). Organ blood flow (OBF) in ml·min^{-1}·g^{-1} was calculated by the following equation: OBF = tissue sample activity (dpm) / total activity injected (dpm) / tissue sample weight (g) x PFR (ml · min^{-1}).

Blood gases and tissue oxygenation

Arteriovenous blood gases were analysed (ABL800 FLEX; Radiometer Medical, Copenhagen, Denmark) for quantification of acid-base parameters, electrolytes and determination of blood O_2-content, which was calculated using the formula: $SO_2 \cdot Hb \cdot 1{,}34 + (\alpha \cdot PO_2)$ (ml O_2/100ml blood). The solubility coefficient of O_2 (α), was corrected for temperature and haemoglobin (Hb) levels according to values obtained by Christoforides and Hedley-Whyte (Christoforides & Hedley-Whyte, 1969). During ECC, global O_2 delivery (DO_2) was calculated as: PFR · arterial O_2-content. Global O_2 consumption (VO_2) was calculated as: PFR · (arterial O_2-content – venous O_2-content). O_2 extraction ratio (ER) was determined as the ratio of VO_2 / DO_2.

Total tissue water content (TTW)

After experiment, tissue samples were collected from brain, cerebellum, heart, lung, liver, skeletal (psoas) muscle and both kidneys. Samples were immediately weighed and subsequently placed in a drying chamber at 80°C until reaching stable weight. TTW was determined as weight reduction relative to dry weight, and is given as g/g dry weight.

Statistical analyses

Results are presented as mean (SD). Data were analysed and presented using SigmaPlot version 13.0 (Systat Software, San Jose, CA, US). Data distribution was tested for normality (Shapiro-Wilk) and equal variance (Brown-Forsythe). For analysis of repeated measurements, a two-way factorial ANOVA for repeated measures was used. When significant differences were found, a post-hoc Dunnett's test was used to analyse differences between the two groups, and changes within group from pre-hypothermic (37°C) baseline. If normality test failed, the Friedman test was used to analyse within-group changes from baseline. Single measurements were analysed using a two-tailed, unpaired Student's t-test. For assessment of

between-group differences, Mann-Whitney rank sum test was used whenever normality test or equal variance test failed. Differences were considered significant at $p < 0.05$.

Results

All rats undergoing the experimental protocol survived cooling and rewarming. Average temperature for DHCA was 11.6°C in the HES group and 11.9°C in the saline group. Cardiac rhythm at DHCA was asystole in all animals. Rewarming time was 95.4 (12.9) min in the saline group and 81.7 (19.1) min in the HES group ($p = 0.12$).

Hemodynamic variables

Cooling to 15°C significantly reduced mean arterial pressure (MAP) and heart rate (HR) in both groups compared to baseline values (table 1). After rewarming, HR returned to within pre-hypothermic values in the HES group, but remained significantly reduced compared to pre-hypothermic baseline in the saline group. In both groups, MAP during ECC rewarming was significantly reduced compared to baseline values. Compared to the saline group, HES treated rats had higher MAP at all temperatures during rewarming, and a significantly higher HR at 37°C after rewarming. Systemic vascular resistance (SVR) was significantly increased in the saline group at 32°C, while no differences between groups were found after rewarming to 37°C. During rewarming, sinus rhythm was restored in all animals. However, as the majority of blood was excluded from the pulmonary circulation with a consequent reduction in left ventricular preload, contribution of the left ventricle to cardiac output was considered negligible.

Plasma- and circulating blood volume

Compared to baseline, a non-significant reduction of CBV by 17% and 21% when cooling to 15°C was found in the saline and HES group, respectively (figure 3). PV was reduced in a similar manner, and no differences were found between groups. In the HES group, PV and

CBV was significantly increased during (24°C) and after (37°C) ECC rewarming compared to baseline values. Contrary, no changes in PV or CBV from baseline values was observed throughout ECC rewarming in the saline group. Comparison of the two groups revealed a significantly higher PV and CBV in HES treated rats at both 24°C and 37°C, during and after rewarming.

Hct and fluid addition during ECC (figure 4)

Both groups demonstrated a significant reduction in Hct upon start of ECC. In the HES-group, no supplemental fluids were added to the circuit at temperatures < 28°C; > 28°C, mean volume of fluid added was 1 (1.3) ml. This was significantly lower than the saline group, where rats received an average of 35 (7.2) ml during the entire course of ECC. Hct remained indifferent between groups throughout rewarming.

Blood gases during ECC (table 2)

Compared to the HES group, pH was significantly reduced in the saline group at 32°C; 7.37 (0.07) vs 7.28 (0.10) and 37°C; 7.31 (0.08) vs 7.14 (0.15). At all temperatures during ECC, HES treated rats demonstrated a significantly increased bicarbonate (HCO_3^-) and reduced base excess compared to the saline group. Arterial partial pressure of carbon dioxide ($PaCO_2$), arterial oxygen saturation (SaO_2), and concentration of lactate, Na^+, Cl^- and Hb was similar between groups. At 24°C, arterial partial pressure of oxygen (PaO_2) was significantly increased in the saline group, whereas central venous oxygen saturation (SvO_2) was significantly higher in the HES group. Further rewarming neutralized these differences.

Tissue oxygenation during ECC

In the HES group, rewarming to 37°C led to a significant increase in DO_2 compared to at 24°C (table 3). In the saline group, no changes in DO_2 was found during rewarming from 24°C to 37°C. At 37°C, DO_2 was significantly increased in the HES group compared to the saline group. No between-group differences were found in VO_2. ER was significantly lower in

the HES group than the saline group at 24°C, but no differences were found at higher temperatures.

Blood flow during ECC

PFR was significantly higher in the HES-group at all temperatures above 20°C (figure 5). Compared to the saline group, OBF to the heart and skeletal muscle was significantly higher in the HES group at 32°C, and to the brain, cerebellum, heart and both kidneys at 37°C (table 4).

Total tissue water content (TTW)

Compared to HES-treated animals, TTW in the saline group was significantly higher in skeletal muscle, lung and both kidneys (table 5).

Discussion

This study demonstrates that priming the extracorporeal circuit for rewarming with 6 % HES 130/04 is superior to isotonic saline with respect to maintaining CBV at low temperatures, supporting venous drainage and increasing pump flow rate. Also, improved blood flow to vital organs was seen in the HES-group.

Numerous studies have documented reduced PV during cooling and stable hypothermia (Kondratiev et al., 2006; Lofstrom, 1957; Tveita et al., 1996), and rewarming does not restore PV to pre-hypothermic values (Tveita et al., 1996). The causal mechanism for volume reduction during hypothermia is not fully understood, but is likely multifactorial. Both increased extravasation of plasma from intravascular to interstitial space (Farstad et al., 2005; Hammersborg et al., 2005), as well as intravascular erythrocyte aggregation in the peripheral vasculature (Lofstrom, 1959) are found to take place in the hypothermic circulation. Hammersborg et al. demonstrated microvascular fluid and plasma volume responses to

surface cooling in pigs (Hammersborg et al., 2005). In accordance with our findings, no changes in PV or Hct-levels were observed during cooling. Stable, prolonged hypothermia did, however, significantly reduce PV and increase Hct, suggesting that microvascular changes during surface cooling depends on the duration of hypothermic exposure.

Various methods of cooling seem to affect fluid homeostasis differently; a simultaneous decline of PV and plasma proteins in surface cooled animals indicates loss of whole plasma (Chen & Chien, 1977; Hammersborg et al., 2005). In contrast, CPB-cooled animals exhibits an instant increase in fluid extravasation with stable plasma protein concentrations (Farstad et al., 2003), implying pathophysiological differences between fluid leakage in surface and CPB-induced cooling. Consequently, the current model of surface cooling is likely more suitable for studying the effects of accidental hypothermia on fluid balance than models using CPB-assisted cooling and rewarming.

Compared to the HES group, rats in the saline group received considerably higher amounts of fluid during rewarming in an attempt to reach target flow rate. Still, no between-group differences were observed in Hct and Hb levels, suggesting that the majority of administered saline escapes the intravascular compartment. The volume effect of fluid additions, i.e. the fraction of infused fluids remaining in the intravascular compartment, seem to depend on the context of which it is given. Normovolemic haemodilution with 6% HES results in a volume effect of almost 100 % (Jacob et al., 2003). Conversely, after infusing an acute bolus loading of either 5% Albumin or 6% HES in normovolemic patients, only about 40 % of dose given is present in the intravascular space after 30 minutes (Rehm et al., 2001). This outcome has been related to a deterioration of the endothelial glycocalyx, caused by hypervolemia and subsequent release of atrial natriuretic peptide from the cardiac atria (Chappell et al., 2014). To compensate for acute blood loss, use of the crystalloid Ringer's lactate is found to give a volume effect below 20 % in normothermic subjects (Jacob et al., 2012). In line with our

findings, the part of administered crystalloid solutions escaping the effective circulation seems even more pronounced during hypothermia; when given to hypothermic dogs, a saline infusion corresponding to 20 % of PV gave no reduction in Hct compared to dogs receiving no fluid supplementations (Roberts, Barr, Kerr, Murray, & Harris, 1985). Contrary to saline priming, HES priming led to an immediate increase in PV during ECC, and PV remained significantly higher than pre-hypothermic baseline values throughout rewarming, a finding that may be explained by the ability of HES to recruit fractions of PV normally retained within the endothelial glycocalyx (Jacob et al., 2003).

In the present study, global DO_2 was significantly elevated in the HES group compared to the saline group after rewarming to 37°C, while global VO_2 remained similar between groups. Furthermore, O_2 extraction ratio remained below 50 % in both groups throughout ECC rewarming, lower than the reported critical value of 60-70 %, at which VO_2 becomes dependent on DO_2 (Valkov et al., 2019). A significantly increased TTW of the lung, skeletal muscle and both kidneys in the rats receiving saline implies a higher degree of tissue oedema in this group. Tissue oedema may impair oxygen diffusion via compression of microvessels (Stolker et al., 2013), and between-group differences in microvascular oxygenation to isolated organs can be found even in the absence of global differences in DO_2 and VO_2 (Stolker et al., 2013). As microvascular oxygenation was not determined in our experiment, no conclusions can be drawn concerning this variable.

After rewarming, the saline group demonstrated a significantly lower pH and HCO_3^-, while BE was increased compared to the HES group. Lactate levels were elevated in both groups, but with no between-group differences. Thus, the difference in pH between groups are not solely due to lactacidosis. Some level of hyperchloraemic acidosis may also have been present, as chloride levels above normal for rats were measured in both groups. This is supported by an anion gap just slightly above normal values when correcting for lactate, and

may have been less pronounced if a balanced salt solution was used instead of saline. Elevated TTW in both kidneys and significantly reduced renal blood flow in the saline group may have affected renal function with a subsequent loss of HCO_3^- or diminished renal acid excretion. Also, extreme haemodilution and expansion of extracellular volume with alkali-free isotonic fluids can lead to a fall in serum bicarbonate causing metabolic acidosis (Garella, Chang, & Kahn, 1975).

Blood flow to the brain, cerebellum and kidneys were significantly higher in the HES treated rats after rewarming. Several factors are likely to contribute to these differences; reduced MAP in the saline group may have been below the lower limit for autoregulation. Furthermore, a higher total blood flow was present in the HES group during rewarming. Autoregulation of blood flow to vital organs during ECC has however been found to be intact over a wide range of temperatures and pump flow rates (Slater, Orszulak, & Cook, 2001). Hyperventilation resulting in hypocapnia was found in both groups during ECC, likely causing cerebral vasoconstriction and subsequent reduction in cerebral blood flow. There were however no significant differences in $PaCO_2$ between groups. Another contributing factor may lie within the inherent differences in rheological properties of the prime solutions used. Priming with HES will give a higher blood viscosity than saline (Eckmann, Bowers, Stecker, & Cheung, 2000). In the context of haemodilution, increased viscosity has been found to increase the volume of perfused capillaries by enhancing the shear stress dependent release of nitric oxide (Tsai et al., 2005), giving rise to improved OBF through improved microcirculation with a higher fraction of perfused capillaries.

Considering the potential adverse effects of HES on renal function, the significantly increased renal perfusion in the HES group was an interesting finding. In the present study, however, potential renal or haematological effects of HES were not a subject for investigation, and no measures of renal function, injury or morphology were made. Rather, HES served as a model

drug to evaluate physiological differences in priming the extracorporeal circuit with a high viscosity, iso-oncotic colloid compared to a crystalloid solution.

Limitations of study

As previously stated, this study aimed to investigate physiological effects of two different prime solutions during ECC rewarming from hypothermia. In our opinion this new knowledge is needed for making new guidelines, as scientific data concerning this subject are scarce. A more clinically relevant approach may have been to include correction of blood gases and use vasoactive drugs to correct low MAP, particularly in the saline group. We decided not to apply such interventions because they would have confounded the interpretation of results due to their effects on blood flow distribution (Slater et al., 2001). Also, to increase clinical relevance, balanced salt solutions could have been used rather than saline, as large volumes of saline may induce hyperchloraemic acidosis. Because of technical limitations to our model, we were not able to investigate DO_2 and VO_2 to individual organs, which would certainly have been of high interest, and as a result no conclusions concerning effects on organ tissue oxygenation could be made. Another limitation was the lack of measures for fluid losses to bodily cavities such as the peritoneum and pleura, in which fluids was observed during autopsies in several rats.

Conclusions

Compared to a crystalloid priming solution, the use of an iso-oncotic colloid prime significantly increases PV and CBV, as well as blood flow to vital organs during and after ECC rewarming from experimental hypothermia. Animals receiving HES also demonstrated a more favourable fluid balance and improved haemodynamics compared to saline priming.

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Legends to figures and tables

Figure 1: Experimental protocol

Overall protocol layout. Rats were surface cooled until cardiac arrest, then rewarmed by extracorporeal circulation. The circuit was primed with either HES (n=8) or saline (n=8).

Figure 2. Extracorporeal circuit

The extracorporeal circuit consisted of A: Venous reservoir, B: Syringe for priming and fluid additions, C: Peristaltic pump, D: Hollow fiber membrane oxygenator, E: Heat exchanger.

Figure 3: Plasma volume (PV) and circulating blood volume (CBV)

PV and CBV during cooling and extracorporeal rewarming. #Significantly different from pre-hypothermic baseline ($p < 0.05$), *Significant differences between groups ($p < 0.05$).

Figure 4: Fluid additions and hematocrit (Hct)

A: Total fluid additions given during extracorporeal rewarming in the two groups. B: Hct during cooling and extracorporeal rewarming. #Significantly different from pre-hypothermic baseline ($p < 0.05$). *Significant differences between groups ($p < 0.05$)

Figure 5: Pump flow rate (PFR)

PFR during extracorporeal rewarming in the two groups. Dotted line marks target flow rate. *Significant differences between groups ($p < 0.05$). #Significantly different from 15°C

Table 1: Hemodynamics

Heart rate (HR) and mean arterial pressure during cooling and extracorporeal rewarming. Systemic vascular resistance (SVR) during extracorporeal rewarming. #Significantly different from pre-hypothermic baseline ($p < 0.05$). *Significant differences between groups ($p < 0.05$). §Significantly different from 24°C$_{ECC}$

Table 2: Blood gases and acid-base parameters during extracorporeal rewarming.

pH, arterial partial pressure of carbon dioxide ($PaCO_2$); arterial partial pressure of oxygen (PaO_2); hemoglobin (Hb); oxygen saturation in arterial blood (SaO_2); oxygen saturation in central venous blood (SvO_2); lactate; bicarbonate (HCO_3^-); Base excess (BE); concentration of chloride (Cl^-); concentration of sodium (Na^+).

*Significant differences between groups ($p < 0.05$). #Significantly different from $24°C_{ECC}$

Table 3: Oxygen delivery, consumption and extraction ratio.

Global oxygen delivery (DO_2), global oxygen consumption (VO_2) and oxygen extraction ratio (ER) during extracorporeal rewarming. *Significant differences between groups ($p < 0.05$). #Significantly different from $24°C_{ECC}$

Table 4: Organ blood flow during extracorporeal rewarming

Blood flow in $ml \cdot min^{-1} \cdot g^{-1}$ to brain, cerebellum, heart, liver, stomach, both kidneys and skeletal muscle. *Significant differences between groups ($p < 0.05$). #Significantly different from $24°C_{ECC}$

Table 5: Total tissue water content (TTW)

TTW in brain, cerebellum, heart, liver, stomach, both kidneys and skeletal muscle. Given as $g \cdot g^{-1}$ dry weight. *Significant differences between groups ($p < 0.05$).

Table 1: Hemodynamics

Parameter		$37°C_{BL}$	15°C	$24°C_{ECC}$	$32°C_{ECC}$	$37°C_{ECC}$
HR (Beats·min^{-1})	*HES*	421 (27.6)	45 (4.2) #	166 (22.3) #	344 (45.0) #	396 (41.5) *
	Saline	418 (16.4)	44 (8.0) #	147 (24.9) #	327 (55.8) #	323 (87.5) #
MAP (mmHg)	*HES*	101 (12.9)	22 (3.0) #	56 (10.1) #*	67 (22.0) #*	71 (13.5) #*
	Saline	99 (17.4)	22 (3.8) #	33 (15.6) #	44 (9.9)	36 (18.0) #
SVR (mmHg·ml^{-1}·min^{-1})	*HES*			2.1 (0.6)	2.0 (0.8) *	1.8 (0.3)
	Saline			2.5 (1.5)	4.1 (2.3) §	2.2 (1.1)

Table 2: Blood gases and electrolytes

Parameter	Group	24C ECC	32C ECC	37C ECC
pH	*HES*	7.40 (0.10)	7.37 (0.07) *	7.31 (0.08) *
	Saline	7.38 (0.15)	7.28 (0.10)	7.14 (0.15) #
$PaCO_2$ *(kPa)*	*HES*	3.6 (1.3)	3.0 (0.9)	2.5 (1.2)
	Saline	2.8 (1.9)	2.4 (1.2)	1.6 (0.6)
PaO_2 *(kPa)*	*HES*	34.5 (8.9)	38.8 (10.7)	37.5 (12.3)
	Saline	53.2 (5.8) *	43.7 (5.8) #	47.1 (9.0)
Hb *($g \cdot dl^{-1}$)*	*HES*	5.0 (0.7)	4.6 (0.7)	4.4 (0.8) #
	Saline	5.9 (1.4)	5.2 (1.5) #	5.0 (1.4) #
SaO_2 *(%)*	*HES*	99.2 (0.4)	98.4 (1.8)	97.1 (1.2) #
	Saline	98.5 (1.7)	98.4 (2.0)	96.2 (2.5) #
SvO_2 (%)	*HES*	69.9 (7.2) *	54.1 (6.5) #	53.9 (8.2) #
	Saline	57.5 (5.5)	56.2 (7.8)	49.3 (8.7)
Lactate *($mmol \cdot L^{-1}$)*	*HES*	3.3 (1.0)	5.1 (1.6) #	8.4 (2.8) #
	Saline	3.9 (2.6)	5.3 (2.4)	9.6 (4.7) #
HCO_3^- *($mmol \cdot L^{-1}$)*	*HES*	17.5 (1.7) *	14.5 (2.1) #*	11.3 (2.2) #*
	Saline	13.5 (2.4)	10.4 (2.3) #	7.82 (2.9) #
BE *($mmol \cdot L^{-1}$)*	*HES*	- 8.5 (2.1) *	-12.1 (2.6) #*	-16.6 (3.1) #*
	Saline	-13.7 (2.9)	-18.0 (3.0) #	-22.1 (4.2) #
Anion gap *($mmol \cdot L^{-1}$)*	*HES*	16.0 (6.4)	18.7 (8.2)	23.6 (4.0)
	Saline	24.1 (11.1)	22.8 (4.9)	25.2 (5.3)
Na^+ *($mmol \cdot L^{-1}$)*	*HES*	141 (5.6)	139 (12.1)	142 (3.5)
	Saline	147 (10.1)	145 (11.5)	147 (7.4)
Cl^- *($mmol \cdot L^{-1}$)*	*HES*	105 (3.1)	106 (3.9)	112 (4.7) #
	Saline	109 (3.7)	110 (6.9) #	116 (5.3) #

Table 3: Oxygenation

Parameter	Group	24°C$_{ECC}$	32°C$_{ECC}$	37°C$_{ECC}$
DO_2 *(ml·min^{-1}·kg^{-1})*	*HES*	5.8 (1.0)	7.2 (2.1)	8.3 (2.4) #*
	Saline	4.2 (1.9)	5.0 (3.5)	5.2 (3.7)
VO_2 *(ml·min^{-1}·kg^{-1})*	*HES*	2.1 (0.4)	3.6 (1.0) #	4.1 (1.1) #
	Saline	2.0 (0.9)	2.4 (1.7)	2.8 (2.0) #
ER *(%)*	*HES*	29.6 (7.5) *	45.1 (6.1) #	44.5 (8.7) #
	Saline	41.6 (5.5)	43.0 (7.6)	48.8 (8.4)

Table 4: OBF

Organ	Group	24°C$_{ECC}$	32°C$_{ECC}$	37°C$_{ECC}$
Brain	*HES*	0.24 (0.16)	0.41 (0.39) #	0.47 (0.26) #*
	Saline	0.22 (0.15)	0.22 (0.12)	0.16 (0.15)
Cerebellum	*HES*	0.20 (0.09)	0.41 (0.13)	0.72 (0.19) #*
	Saline	0.27 (0.10)	0.35 (0.18)	0.26 (0.32)
Heart	*HES*	2.71 (1.04)	4.41 (2.16) *	5.29 (2.75) #*
	Saline	1.96 (1.35)	2.02 (1.19)	3.26 (1.42)
Liver	*HES*	0.07 (0.04)	0.16 (0.13)	0.09 (0.10)
	Saline	0.04 (0.03)	0.07 (0.06)	0.05 (0.07)
Stomach	*HES*	0.21 (0.23)	0.19 (0.18)	0.26 (0.13)
	Saline	0.11 (0.11)	0.11 (0.05)	0.11 (0.09)
Kidney (left)	*HES*	0.61 (0.48)	0.63 (0.32)	0.82 (0.34) *
	Saline	0.29 (0.23)	0.36 (0.38)	0.36 (0.20)
Kidney (right)	*HES*	0.55 (0.49)	0.67 (0.36)	0.89 (0.44) #*
	Saline	0.28 (0.25)	0.37 (0.42)	0.32 (0.22)
Skeletal muscle	*HES*	0.01 (0.01)	0.05 (0.04) *	0.03 (0.05)
	Saline	0.01 (0.01)	0.02 (0.01) #	0.0004 (0.001)

Table 5: TTW

Tissue	HES	Saline
Brain	3.41 (0.23)	3.52 (0.16)
Cerebellum	3.06 (0.18)	3.25 (0.21)
Heart	3.31 (0.30)	3.50 (0.19)
Liver	2.56 (0.94)	2.72 (0.81)
Stomach	3.47 (0.43)	3.73 (0.50)
Left Kidney	3.59 (0.15) *	4.04 (0.42)
Right kidney	3.54 (0.25) *	4.16 (0.46)
Skeletal muscle	3.01 (0.13) *	3.62 (0.33)
Lung	5.60 (0.62) *	6.51 (1.02)

Additional information:

Competing interests: None of the authors have any competing interests to declare

Author contributions:

Conception and design of the work: T.S., T.K. and T.T. Completion of experiment and collection of data: T.S. and T.K. Data analysis and interpretation: T.S., T.K. and TT. Drafting the manuscript for intellectual content: T.S. and T.T. Revision of the manuscript: T.S., T.K. and T.T. All authors approved the final version of the manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding: This work was generously supported by grants from The Norwegian Research Council (Petromax2) and Ministry of Foreign Affairs (Barents 2020)