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Cardiopulmonary Resuscitation and Rewarming from Accidental Hypothermia

Cardiovascular physiology during hypothermia, cardiopulmonary resuscitation and extracorporeal rewarming

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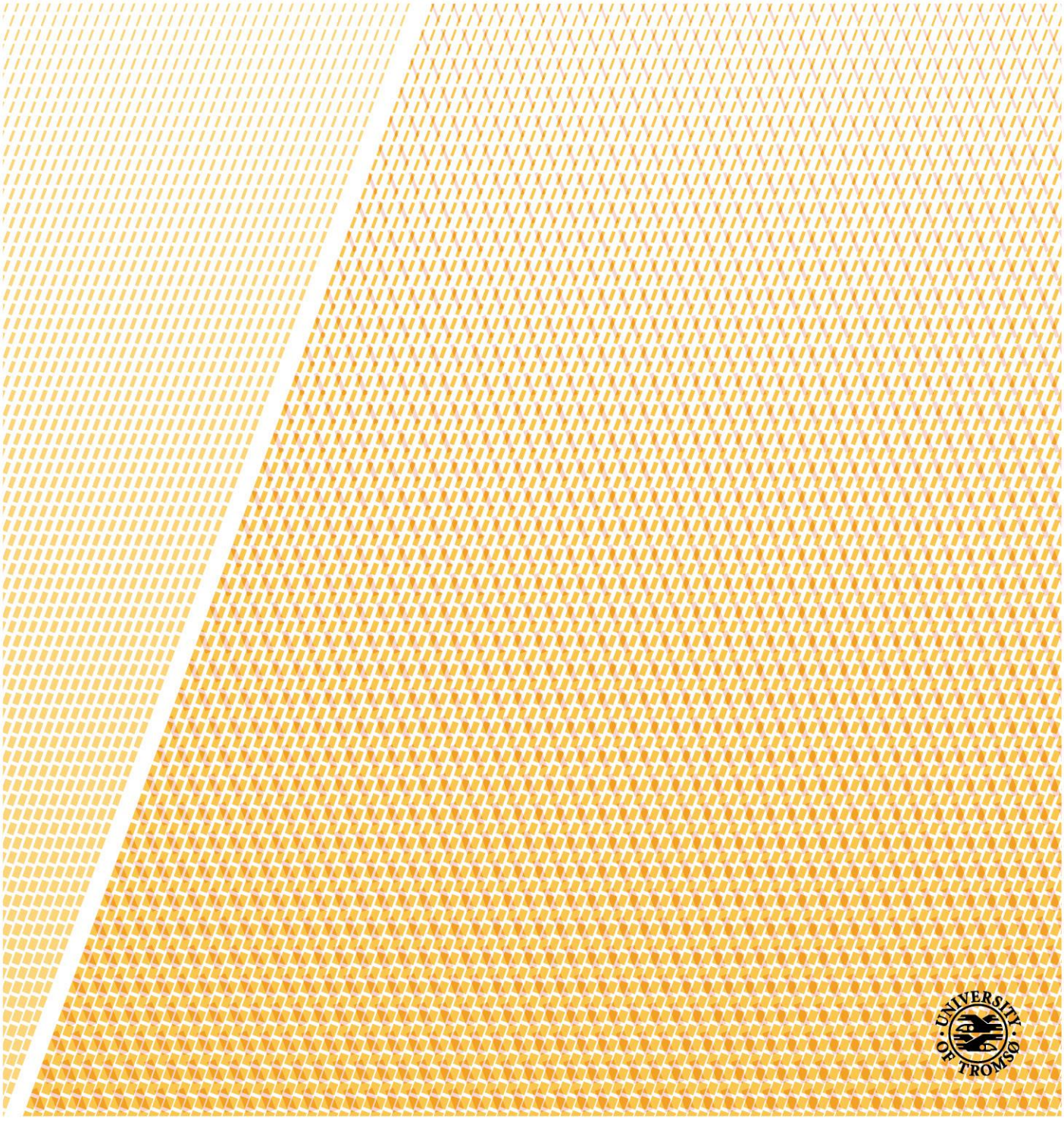

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1 Acknowledgements

This thesis springs from the collaborative efforts of the Anaesthesia and Critical Care Research Group at the University of Tromsø. The start in 2007 was impeded by me leaving Tromsø in 2008 after completing the first series of experiments. I ventured into science again in 2013 and continued where I left off thematically but with different animal species.

From 2013 we continued working on an established pig model that we further evolved to suit our experimental purpose. The beginning saw much trial and error, but persistence gave us an intact pig model for cooling, CPR, and ECMO rewarming, no small feat in itself. My colleague Torvind Næsheim was instrumental in developing this model; his enthusiasm, ideas, and practical help have been invaluable. Perfusionist Jon Kjetil Jensen was of great help in building the ECMO circuit and gave countless practical advice on which equipment to use and how to run the ECMO circuit.

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2 Abbreviations

BE – Base Excess

Ca^{2+} - calcium

$[Ca^{2+}]_i$ – intracellular calcium content

CBV – Circulating Blood Volume

CNS – Central nervous system

CO – Cardiac output (L/min)

C_aO_2 – Oxygen content in arterial blood

$C_{jugular}O_2$ – Oxygen content in blood from the jugular bulb

C_vO_2 – Oxygen content in venous blood CPB – Cardiopulmonary bypass

CPR – Cardiopulmonary resuscitation

CVP – Central venous pressure

DO_2 – Global oxygen delivery (ml/kg/min)

$DO_{2cerebral}$ – Cerebral oxygen delivery (ml/kg/min)

dP/dt_{max} – Maximum rate of pressure change in the left ventricle of the heart

dP/dt_{min} – Minimum rate of pressure change in the left ventricle of the heart

ECLS – Extracorporeal Life Support

ECMO – Extracorporeal membrane oxygenation

ECPR – Extracorporeal cardiopulmonary resuscitation

EDV – End-diastolic volume

F_iO_2 – Fraction of inspired oxygen

Hb – Hemoglobin (g/dl)

HR – Heart rate, beats per minute

HSD – Hypertonic saline dextran

HT - Hypothermia

ICP – Intracerebral pressure

LVP – Left ventricular pressure

MAP – Mean arterial pressure

O_2ER – Oxygen extraction ratio, the fraction of delivered oxygen that is consumed, $\dot{V}O_2/DO_2$

PA-catheter – Pulmonary artery catheter

PAP – Pulmonary artery pressure

$PaCO_2$ – Partial pressure of carbon dioxide in arterial blood

PaO_2 – Partial pressure of oxygen in arterial blood

PEEP – Positive end-expiratory pressure

RBC – Red Blood Cell

ROSC – Return Of spontaneous circulation

S_aO_2 – Oxygen saturation in arterial blood

SD – Standard deviation

SV – Stroke volume, the volume of blood pumped out of the left ventricle of the heart in one contraction

TPR – Total peripheral resistance

VF – Ventricular fibrillation

VO_2 – Oxygen consumption rate (ml/kg/min)

$VO_{2cerebral}$ – Cerebral oxygen consumption rate (ml/kg/min)

3 List of papers

1. Nilsen JH, Schanche T, Kondratiev T V., Hevrøy O, Sieck GC, Tveita T. Maintaining intravenous volume mitigates hypothermia-induced myocardial dysfunction and accumulation of intracellular Ca^{2+}. Exp Physiol. 2021; 1-12.

2. Nilsen JH, Valkov S, Mohyuddin R, Schanche T, Kondratiev T V., Naesheim T, et al. Study of the Effects of 3 h of Continuous Cardiopulmonary Resuscitation at 27°C on Global Oxygen Transport and Organ Blood Flow. Front Physiol. 2020 Apr; 11:213

3. Nilsen JH, Valkov S. Effects of Rewarming with Extracorporeal Membrane Oxygenation to Restore Oxygen Transport and Organ Blood Flow After Hypothermic Cardiac Arrest in a Porcine Model. (Manuscript accepted for publication).

4 Abstract

This thesis springs from 3 different papers:

Paper 1

A randomised controlled study in rats to investigate whether an infusion of crystalloids or colloids during rewarming from hypothermia could alleviate the alterations in cardiovascular function seen in untreated control animals.

Paper 2

A randomised controlled study in pigs, examining normothermic vs hypothermic cardiopulmonary resuscitation (CPR). Our central hypothesis was that the cardiac output (CO) generated by CPR would not be affected by hypothermia, thereby replacing a larger portion of native CO at a reduced temperature. This, in turn, would provide sufficient oxygen delivery to sustain aerobic metabolism in organs during 3 hours of CPR at 27°C.

Paper 3

The effects of extracorporeal membrane oxygenation (ECMO) rewarming to restore oxygen delivery and organ blood flow were explored. Pigs were cooled to 27°C, underwent 3 hours of hypothermic CPR and subsequent rewarming by ECMO.

Main results and conclusions

Volume substitution improved hemodynamic function during rewarming from hypothermia and mitigated post-hypothermic cardiac dysfunction. We saw a sustained hemodynamic effect in rats treated with dextran vs crystalloid solution. The treated animals displayed reduced levels of intracellular calcium (Ca^{2+}) in cardiac tissue compared to controls. Maintaining euvolemia with intravenous fluids during rewarming from hypothermia diminished the cardiac dysfunction seen in untreated controls.

CO and mean arterial pressure (MAP) generated by CPR were not affected by hypothermia. Three hours of continuous resuscitation at 27°C provided limited but sufficient oxygen delivery to allow aerobic metabolism. In contrast, the normothermic animals displayed deteriorated hemodynamic function and significantly reduced organ blood flow after 45 minutes of CPR. These findings encourage early and continuous CPR in victims of accidental hypothermia.

Rewarming pigs by ECMO after 3 hours of CPR at 27°C restored CO, MAP, the oxygen extraction ratios (O_2ER), and blood flow to heart, left temporal lobe, cerebellum, stomach and small intestine. After ECMO rewarming, the favourable prognostic markers of survival after cardiac arrest were present in all animals: moderately increased lactate levels, return of pH to physiologic levels, and a shockable heart rhythm. These factors may be attributed to the protective effects of hypothermia during prolonged CPR, but they also support the critical role of ECMO in rewarming victims of accidental hypothermia.

5 Introduction

Hypothermia can be divided into therapeutic/protective or accidental. This thesis will focus on accidental hypothermia and the cardiovascular physiology associated with cooling, cardiac arrest and rewarming.

5.1 Therapeutic hypothermia

In the tale of sleeping beauty, time is sidestepped, an idea that has fascinated writers and scientists alike. During the 1960s, cryonics came into fashion as a pseudo-science, promising to keep people frozen after their deaths until medical science could catch up and provide a cure (1). The many case reports of victims of accidental hypothermia being successfully rewarmed and reanimated without significant sequelae after prolonged resuscitation (2) and low temperatures (3) fuel the idea that hypothermia provides opportunities.

Therapeutic hypothermia has been practised as long as we have written accounts of medicine; the Romans advocated induced cooling for battle-inflicted trauma and several cerebral disturbances (4). The use of hypothermia has been grounded in the phenomenon that the cold slows down physiologic processes. Therapeutic hypothermia as a treatment regimen in cardiac arrest patients with return of spontaneous circulation (ROSC) gained momentum in the 1980s and showed great promise (5). In 2002, two randomised prospective studies showed that mild therapeutic hypothermia after cardiac arrest increased the rate of favourable neurologic outcomes and reduced mortality (5,6). This prompted a change in the treatment of these patients (7) until a new randomised trial found no differences between resuscitated cardiac arrest patients maintained at 33°C vs 36°C (8), shifting the paradigm towards temperature management to prevent fever. The primary use of therapeutic or protective hypothermia is for surgical procedures requiring cardiopulmonary bypass (CPB) or circulatory arrest (9) and treating neonatal asphyxia (10). Research into applications of therapeutic hypothermia is ongoing.

5.2 Accidental hypothermia

Accidental hypothermia is defined as an involuntary drop in core temperature < 35°C (11). Primary hypothermia refers to healthy individuals overcome by cold stress, whereas secondary hypothermia may be seen in ill persons, even in temperate environments.

5.2.1 Epidemiology of accidental hypothermia

Accidental hypothermia due to environmental exposure may occur in any season and even in temperate climates. It is a disease historically seen in wars and disasters (12). It is associated with those working or travelling in cold areas. The most considerable risk is exposure to cold and wet environments. It is also associated with poverty and the abuse of alcohol and illegal drugs. Secondary hypothermia is seen in sepsis, trauma, diseases that decrease metabolic rate and conditions that affect thermoregulation (13).

There is a scarcity of epidemiological data on accidental hypothermia. Patient cohorts tend to be heterogeneous, and data is often pooled from several clinics to get more significant numbers. In the United States, between 1995 and 2004, the annual incidence of hypothermia and cold-related morbidity visits to the emergency department was 0,56 per 100 000 persons (14). A Polish survey in

2011 identified a prevalence possibly as high as 5,05 cases per 100 000 residents per year (15). Both of these studies found hypothermia to be associated with poverty. In Ireland, between 1979 and 1985, the annual incidence of hospital admittance due to hypothermia was 5,36 cases per 100 000 (16). The elderly and isolated had an increased risk of hypothermia and death from hypothermia, and nearly 27% of death certificates identified a chronic illness. The annual incidence rates in these studies vary from 0,56 – 5,05 cases per 100 000 residents, a factor of 10. The studies show a considerable heterogeneity between groups studied and a lack of a standardised international registry. Recently, the International Hypothermia Registry was established in Geneva (17).

A Swedish study between 2000 and 2007 found the annual hypothermia incidence rate to be 3,4 per 100 000 inhabitants. In contrast, incidence rates in the northern region of Sweden have been reported at 1,35 per 100 000 inhabitants (18). This can imply that inhabitants of northern regions possess inherent knowledge related to survival in the arctic.

The mortality of accidental hypothermia has decreased over the last decades and is in later studies reported to be 28-35% (19,20). The favourable outcome in most patients is linked to intact spontaneous circulation, as survival rates in hypothermic patients presenting with cardiac arrest are considerably lower. Our university hospital reported a survival rate of 37,5% (9 out of 24 patients) between 1999 and 2013 (2).

5.2.2 Classification of accidental hypothermia

There are different classifications of accidental hypothermia; they are categorised either according to temperature or vital signs.

The hypothermia classification system of the American Heart Association distinguishes between mild (core temperature > 34°C), moderate (core temperature 30-34°C) and severe hypothermia (core temperature < 30°C)(21). The 30°C dividing line between moderate and severe hypothermia reflects that cardioversion of ventricular fibrillation (VF) at temperatures below it is rarely successful, making 30°C a critical decision point regarding clinical strategy.

The European Resuscitation Council's classification of 2010 divides hypothermia according to core temperature into three groups: mild (32-35°C), moderate (28-32°C) or severe (< 28°C) (22). This classification is advantageous when core temperature measurements are available. The Swiss staging system for accidental hypothermia is according to clinical signs; it recognises five different stages (23). Hypothermia (HT) I is a conscious patient who is shivering (core temperature comparable to mild hypothermia), HT II is a patient with impaired consciousness in the absence of shivering (core temperature equal to moderate hypothermia), HT III is an unconscious patient (core temperature 24-28°C), HT IV is an apparently dead patient with asystole or ventricular fibrillation on ECG but with a compressible chest (core temperature < 24°C) and HT V is a dead patient without vital signs and a chest that is non-compressible (core temperature < 13,7°C). This last classification might be the most useful to rescue workers and medical personnel in the field. From 2015 the European Resuscitation Council included the Swiss staging system in their recommendations (24). However, there is evidence that core temperature and the clinical signs in the Swiss staging system only correlates in about 50%

of case reports (25). This emphasises the importance of core temperature measurements, even in the pre-hospital setting.

5.3 Pathophysiology of hypothermia

Thermoregulation involves sophisticated control mechanisms in the hypothalamus and peripheral feedback loops to keep the core temperature at 37°C irrespective of ambient temperature. To optimise organ, tissue and cellular function, the thermoregulatory system needs to balance heat production from metabolism with heat loss from radiation, conduction, convection and evaporation (9). When mild hypothermia ensues, the body responds by generating heat by shivering accompanied by a sympathetic response to fight the lower core temperature. In essence, vasoconstriction and increased oxygen consumption (VO_2), respiratory rate, heart rate (HR), blood pressure, and CO ensue (26). As body temperature lowers, the metabolic rate follows; there is roughly a 6% decrease in VO_2 per 1°C drop in body temperature (27). Below 32°C, thermoregulation is impaired, and the body will need exogenous heat to rewarm. Shivering stops around 30°C. Cognitive functions are affected at a core temperature around 33-34°C; they deteriorate progressively with further cooling, resulting in confusion, apathy, drowsiness and eventually coma. Brain stem auditory responses are absent below 20°C, but usually present at core temperatures above 25°C (28). With temperatures below 30°C bradycardia usually occurs along with a decreased CO. Abnormalities in the heart's electric conduction lead to dysrhythmias, and below 28°C, the heart is susceptible to VF(13). Cooling beyond 30°C will depress respiratory rate; in an experiment on dogs, hypothermia below 30°C resulted in diminished respiration, and at 24°C there was no spontaneous respiration (29). Figure 1 summarises the physiologic changes attributed to hypothermia.

	MILD (32-35°C)	MODERATE (28-32°C)	SEVERE (<28°C)
Central Nervous System	Confusion Dysarthria Hyperreflexia Ataxia Increased sympathetic drive	Decreased consciousness Hyporeflexia Decr. cerebral metabolism Dilated pupils	Coma, Decreased evoked potentials EEG silent < 20°C
Heart	Increased HR Increased CO Increased BP	Decreased HR/CO ECG abnormalities (J-wave) Incr.risk of arrhythmias	Severely decr. CO/HR/BP Increased risk of VF Asystole < 20°C
Respiratory System	Increased respiratory rate Increased minute volume	Decreased RR/MV Respiratory acidosis	Apnoea < 24°C
Metabolism	Shivering Increased VO_2	Decreased VO_2 as shivering subsides	Severely decreased VO_2 (6% per °C)
Muscular	Hypertonia	Rigidity	Rhabdomyolysis
Blood	Hemoconcentration (Hct increases 2% per °C drop in temperature)	Decreased coagulation	Severely decr. coagulation DIC Bleeding
Renal	Cold diuresis	Cold diuresis Hyperkalaemia	Decreased renal perfusion Oliguria Hyperkalaemia
Gastro-Intestinal	Ileus	Pancreatitis Gastric erosions	Pancreatitis Gastric erosions

Figure 1 - *Pathophysiology and clinical signs of hypothermia*

5.3.1 Oxygen delivery and consumption during hypothermia

Cooling with spontaneous circulation slows the metabolic rate, resulting in reduced cardiac output along with reduced oxygen delivery (DO_2) and consumption (VO_2)(30–32). Our research group has shown that in an intact pig model, oxygen transport and tissue blood flow are maintained during 1-3 hours of stable hypothermia at 25-27°C and during rewarming (32,33).

The decreased metabolic rate and VO_2 explain why hypothermic tissues can endure a more extended period of hypoxemia without irreversible injury. Experiments on dogs show that VO_2 is reduced by 50% at 28°C and 75% at 20°C (34,35). VO_2 increases during the initial cooling phase due to the organism's effort to maintain core temperature by shivering, thereby producing heat (36).

DO_2 to tissues is impaired in hypothermia due to a reduced cardiac output (37). Animal studies measuring both DO_2 and VO_2 during experimental hypothermia show VO_2 levels dropping proportionally more than DO_2 levels (32,38), which might act in favour of maintaining tissue oxygenation.

Critical oxygen delivery in hypothermia

In healthy tissues, a modest decrease DO_2 does not lower VO_2 because oxygen extraction increases proportionately. When DO_2 is reduced below a critical threshold, VO_2 will drop because oxygen extraction has reached its limit. The VO_2 is, in this case, delivery dependent, and a drop in DO_2 below the critical threshold will force the body to switch to anaerobic metabolism. As such, critical DO_2 is an essential marker for this transition (39,40). A study on hypothermic pigs showed that critical DO_2 was associated with increased lactate levels, indicating anaerobic metabolism (41).

The oxygen extraction ratio (O_2ER) visualises the concept of delivery dependent oxygen consumption better than delivery and consumption rates as these will vary between species and organs. O_2ER is the fraction of oxygen consumption and oxygen delivery, VO_2/DO_2. This ratio is reported to be 0,6-0,7 for most tissues (42). During hypothermia in animals with spontaneous circulation, the O_2ER during acute cooling is reduced compared to normal values (38,43). The rates of delivery and consumption differ in normothermia compared to hypothermia; the same is true for critical DO_2 values. However, a study on dogs showed comparable O_2ER in normothermia and hypothermia, reflecting the organs' need for oxygen regardless of temperature (44).

5.3.2 Cardiovascular dysfunction in hypothermia

Hypothermia impairs cardiovascular function, leading to decreased CO and increased electrical irritability of the heart. The decreased CO is multifactorial: due to bradycardia, reduced stroke volume (SV), decreased contractility and reduced circulating blood volume (CBV)(33,45,46).

The reduction in CBV is mainly due to plasma volume reduction with an increased haematocrit and blood viscosity (47–50). During hypothermia, blood viscosity increases, giving rise to increased systemic vascular resistance (SVR), which may be further aggravated by increased vascular tone (11). In this hypothermia-induced low-flow state, aggregation of red blood cells (RBCs) in the microcirculation may occur (51,52). Trapping of RBC aggregates in capillaries excludes them from the effective circulation, and compromises flow through the microcirculation (53).

The hypothermia-induced cardiac dysfunction will often complicate the rewarming process, clinically ranging from a minor depression of CO to full circulatory collapse ("rewarming shock")(54). On the cellular level, the pathophysiology remains elusive. Preclinical experiments have shown a significant elevation of intracellular Ca^{2+} during hypothermia and after successful rewarming (55,56). Ca^{2+} plays a crucial role in excitation-contraction coupling in heart muscle. In cardiomyocytes, the action potential triggers the flow of Ca^{2+} into the cell, initiating the release of more Ca^{2+} from the sarcoplasmic reticulum, which in turn binds to troponin C and switches on contraction. Ca^{2+} is then removed from the cytosol to allow for relaxation (57). Cytosolic Ca^{2+} level plays an essential role in the pathophysiology of heart failure and arrhythmias (58). The hypothermia induced cardiac dysfunction is related to Ca^{2+} overload; increased exposure aggravates the Ca^{2+} overload (55). Isolated rat myocytes show increased cytosolic Ca^{2+} content after hypothermia (59). Studies on isolated rat papillary muscle and rat hearts have shown decreased myocardial contractility attributed to reduced Ca^{2+} sensitivity and increased cardiac Troponin I phosphorylation (60,61). This points towards using calcium sensitisers as a pharmacologic treatment option in cardiac dysfunction during

hypothermia/rewarming rather than the use of catecholamines which tend to have adverse effects during hypothermia.(60,62,63)

5.4 Management of accidental hypothermia

A prerequisite for successfully handling hypothermic patients in cardiac arrest is guidelines for treatment, ensuring treatment consistency throughout the chain of survival (64,65). The University Hospital of North Norway was instrumental in developing guidelines for managing hypothermic patients in the northern region of Norway (66). The effects of different modalities available during rescue and transport need to be investigated and documented. These data should be collected from actual hypothermia patients. Although a registry is now in place (17), patient data volume is still limited, which necessitates collecting data from preclinical experiments.

In broad terms, treatment of patients with mild or moderate hypothermia that are conscious and hemodynamically stable will be external rewarming with heated air, blankets and warm fluids (67). The severely hypothermic patients are hemodynamically unstable, presenting without vital signs or with unstable vital signs such as impaired consciousness and cardiac instability. They are challenging to treat both in the pre-hospital (13) and in-hospital settings.

5.4.1 Treatment algorithms

There are several different treatment algorithms for victims of accidental hypothermia. Figure 2 is a schematic of the treatment algorithm proposed by the International Commission for Mountain Emergency Medicine (ICAR MEDCOM)(67), and Figure 3 is the algorithm developed by the University Hospital of North Norway (66). These algorithms are similar, differing concerning the triage point for body temperature where extracorporeal rewarming should be considered. ICAR MEDCOM advocates cessation of CPR with body temperatures > 30°C and avalanche burial > 60 minutes. It presupposes that cooling to 30°C will take at least 60 minutes. The North Norwegian experience is that the population of avalanche victims is heterogeneous, varying from scantily clad children in avalanche house burials to snowmobilers with heavy warm clothing. In these circumstances, it does not make sense strictly adhere to a 60-minute timeframe during which cooling to 30°C takes place. Our regional algorithm advocates early measurement of core temperature and extracorporeal rewarming of patients with body temperatures up to 32°C and serum potassium levels up to 12 mmol/L. The reason for this is that accidental hypothermia is rare even in our region, and this over triage is acceptable.

During the rescue and pre-hospital care, the circumstances are often complicated, and a shortage of complete information about the patient and events is often present. A recent survey among mountain rescue teams in 27 countries showed that adherence to hypothermia guidelines was low and that equipment such as automated CPR devices, temperature probes and devices to measure serum potassium was scarce (68). This study emphasises the need for clear guidelines as well as knowledge of these guidelines amongst pre-hospital care providers.

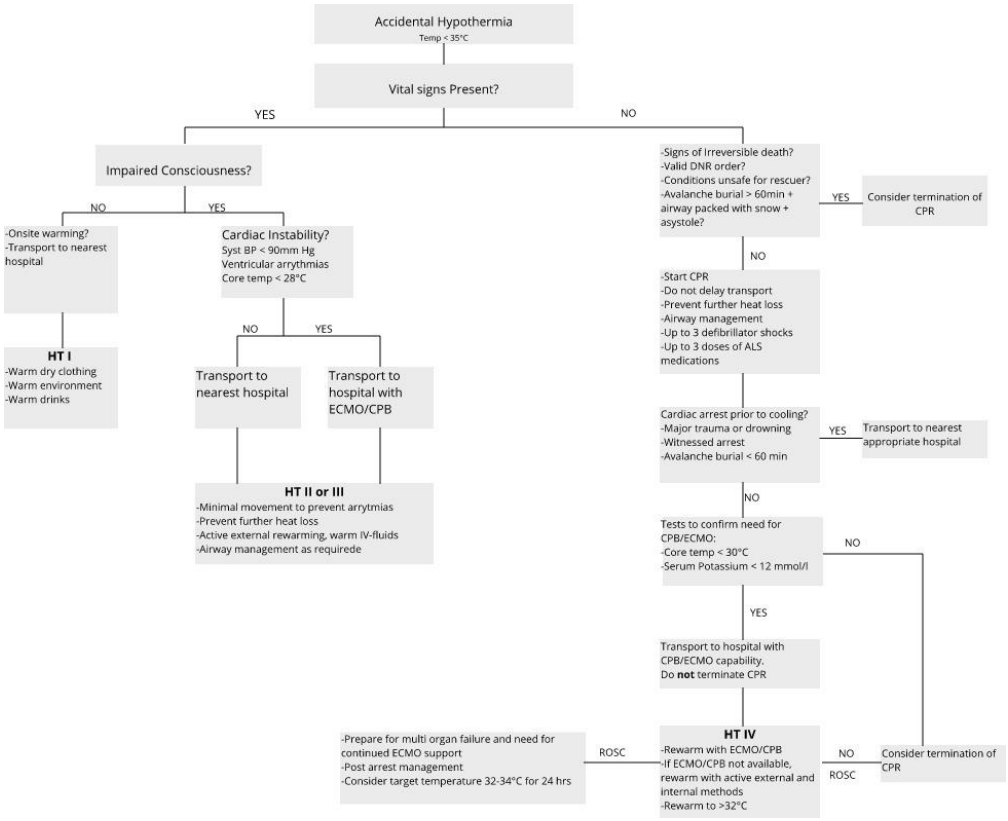

Figure 2 *- Treatment algorithm for accidental hypothermia proposed by ICAR MEDCOM*

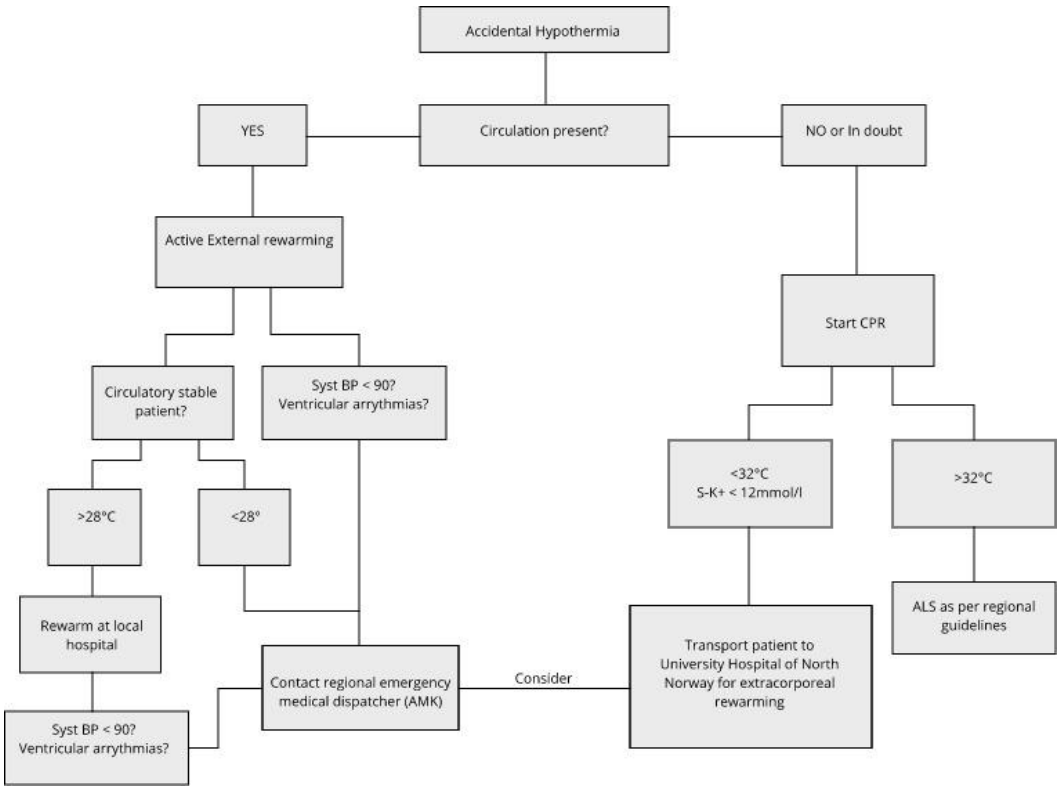

Figure 3 *- Regional algorithm for accidental hypothermia in northern Norway*

5.4.1 Cardiovascular drugs in hypothermia

Different cardiovascular drugs have been examined under experimental hypothermic conditions. Our research group has done several studies to elucidate the role of cardiovascular drugs during hypothermia and rewarming. Kondratiev and Tveita showed increased peripheral resistance without an elevation in CO after administering epinephrine during rewarming of hypothermic rats (69) and showed adverse effects of epinephrine during cooling and rewarming in rats (70). Dietrichs showed hypothermia-induced β-adrenoceptor sensitivity in vitro and in vivo, thereby dispelling notions of β-adrenoceptor dysfunction during hypothermia. This effect is more likely due to severely elevated total peripheral resistance (TPR)(63). Filseth investigated dopamine in pigs 25°C and found altered pharmacokinetics of dop

amine and an unchanged cardiac index despite increased HR (71).

Unlike β-agonists like epinephrine that stimulate cyclic AMP formation, the phosphodiesterase 3 (PDE3) inhibitors work by preventing cAMP breakdown in cardiomyocytes. Dietrichs and Tveita studied the effects of levosimendan (62) (calcium sensitiser and PDE3-inhibitor) and milrinone (72) (PDE3-inhibitor) during rewarming from hypothermia in rats. They found that both drugs ameliorated cardiac dysfunction during rewarming, restoring SV and CO. They concluded that preventing cAMP breakdown seems a more viable strategy during rewarming than β-stimulation.

Studies on hypothermic cardiac arrest in pigs have reported increased coronary perfusion pressure after administration of epinephrine and vasopressin (73). Still, the chance of return of spontaneous circulation (ROSC) was not higher with epinephrine (74). Another study reported no improved outcome from amiodarone or vasopressin in hypothermic cardiac arrest in pigs (75). The European Resuscitation Council recommends withholding CPR drugs until the patient has a body temperature > 30°C, and doubling the intervals between doses between 30-35°C(24).

5.4.2 Intravenous fluids in hypothermic patients

Hypothermia induces cardiac dysfunction through both a reduction in CBV and decreased mechanical function of the heart. Researchers have advocated against administering large volumes of intravenous fluids to treat hypothermic patients as the CBV tends to correct itself after rewarming (76). Preclinical experiments have shown that reversal of fluid lost to the interstitium is impaired after prolonged exposure to the cold (31,77). Current opinion focuses on avoiding hypovolemia during rewarming; alas, liberal fluid administration when needed is advocated (11,24,67,78).

Intravenous fluid administration increases CBV, thereby increasing CO by two main mechanisms: intrinsic factors and nervous stimulation (79). Intrinsic factors cause the heart to pump with increased force in response to increased filling, elevating the CO as much as 2½-3-fold. The Frank-Starling mechanism is the most important of the intrinsic mechanisms, allowing the heart to adapt to increased preload rapidly. Allen and Kurihara showed that increased length of cardiac myocytes does not correspond with increased intracellular calcium (80). The Frank-Starling response is on the cellular level partly due to increased myofilament sensitivity to calcium, and therefore calcium-independent (81). Second, nervous stimulation can almost double CO by increasing HR and heart muscle strength (79).

Fluids are usually not recommended in the pre-hospital setting as they tend to cool quickly in this environment. Warm crystalloids are recommended for in-hospital use, and it is advisable to avoid saline as it can aggravate metabolic acidosis (11). During rewarming, vasodilation will gradually expand the vascular bed, and patients may need large volumes of crystalloid infusions (24).

There are no recommendations on the use of colloid solutions during rewarming from accidental hypothermia. In recent years, synthetic colloids have gained some notoriety after studies in critically ill patients showed increased mortality (82). The use of colloids in trauma patients has been discontinued as they decrease coagulation (83). However, a recent review informs that both crystalloids and colloids induce coagulopathy when the hemodilution exceeds 40% and advocates the use of colloids when the infused volume of crystalloids exceeds 3-4 litres, if blood transfusion is not indicated (84).

Synthetic colloids have regained some popularity as additives in hypertonic saline solutions. Hypertonic saline dextran (HSD) increases survival in patients with traumatic brain injury and hypotension (85) and in patients with penetrating injuries to the chest (86). Limiting the volume of fluid and thereby limiting oedema might be beneficial in hypothermic patients. The role of HSD in resuscitating hypothermic patients with cardiac arrest or severe hypotension is not clear. Kaakinen et al. did a study on juvenile pigs, cooled to 18°C on cardiopulmonary bypass and then subjected to 75 minutes of cardiac arrest with infusions of either HSD or saline (87). The animals that received HSD had a better neurologic recovery, lower intracranial pressure, higher cerebral perfusion pressure and brain metabolism was better preserved. Miclescu et al. did a study on pigs subjected to 12 minutes of cardiac arrest before starting CPR. The animals were cooled with either 30 ml/kg cold saline or 3 ml/kg HSD to reach a core temperature of 34°C (88). They found no statistical differences in the time to reach hypothermia and no advantage of HSD over saline with regards to neurologic damage.

Our research group recently published a work where rats were rewarmed on an ECMO-circuit primed with crystalloids or colloids (89). CBV was found to be significantly higher in rats rewarmed on the colloid-primed ECMO circuit. Blood flow to vital organs was also improved compared to rats rewarmed on a crystalloid-primed ECMO circuit.

5.4.3 Hypothermic cardiac arrest

Due to the instability of vital functions that accompany moderate and severe hypothermia, a recent commentary suggested hypothermia for all practical purposes can be divided into mild (32-36°C) and everything else (65). The hypothermic patients with a core temperature < 32°C need to be handled carefully as physical manipulation may precipitate malignant arrhythmias (90). Treating patients with hypothermic cardiac arrest is demanding with regards to treatment options, rescue, transport and en-route care.

The hypothermic heart is prone to arrhythmias at temperatures < 30°C, minor movements of the patient can result in VF. If VF occurs, the hypothermic heart may not respond to therapies other than rewarming before successful cardioversion (11,76,91). Asystole in hypothermic patients is related to profoundly low core temperature, prolonged arrest and asphyxia. However, the presenting cardiac

rhythm does not significantly influence survival in patients rewarmed by extracorporeal circulation(92).

The Ischemia/reperfusion injury seen after prolonged normothermic resuscitation seems to be diminished or suppressed in hypothermia. In low-flow or no-flow situations when the tissue becomes ischemic, reperfusion triggers different plasma cascade systems, including complement-, coagulation-, kinin- and fibrinolytic systems, which in turn play a role in mediating the inflammatory process (93). A study on dogs undergoing prolonged resuscitation in a refractory cardiac arrest scenario with 40 minutes total arrest showed better neurologic survival in 2 groups treated with either mild (34°C) or moderate (27°C) hypothermia during the last 20 minutes of CPR compared to normothermic controls (94). Another animal study confirmed these findings, showing that intra-arrest hypothermia improved survival and neurological outcome compared to normothermia (95). Clinical trials have failed to reproduce these results: a randomised controlled study on pre-hospital intra-arrest cooling vs in-hospital therapeutic hypothermia found no differences between groups with regards to increased survival, cerebral performance and levels of inflammatory biomarkers (96). Two other randomised controlled trials on intra-arrest cooling confirm these findings (97,98).

Several case reports of hypothermic cardiac arrest show favourable neurologic outcomes after in-hospital rewarming (2,3,99–102). This seems linked to the use of extracorporeal rewarming techniques as well as the quality of pre-hospital treatment. Patients have made full recoveries with little or no sequelae with body temperatures as low as 13,7°C (3) and resuscitation times exceeding 6 hours (102). However, the maximum time of hypothermic CRP remains elusive, as there are no animal or human studies to advise on this.

The hypothermia case reports are in stark contrast to resuscitation of patients with cardiac arrest under normothermic conditions, where CPR is usually terminated after 20-30 minutes if ROSC is unsuccessful. Normothermic CPR provides about 30% of pre-arrest cardiac output (103–105) and is generally discontinued after 20-30 minutes if ROSC is not achieved as these patients have poor clinical outcomes (106–108). The European Resuscitation Council recommends cessation of CPR after 20 minutes of asystole (109). Preclinical studies support this clinical notion (103,110–112), but the maximum duration of CPR in normothermic patients remains undetermined (113). New resuscitation strategies, such as extracorporeal cardiopulmonary resuscitation (ECPR), continuously challenge what we can achieve clinically. Guidelines are vague concerning the role of ECPR, but it is recommended in selected patients when conventional CPR is failing (114).

5.4.4 Cardiopulmonary resuscitation devices

The European Resuscitation Council recommends immediate start of CPR on hypothermia victims with cardiac arrest. Continuous CPR and using a mechanical CPR device should be considered during evacuation and transport (24). The LINC randomised trial investigated 4-hour survival in out of hospital cardiac arrest treated with either mechanical compressions with the LUCAS device or standard CPR. They found no differences between groups (115). A review article confirmed that data from several randomised trials showed no superiority in CPR with mechanical devices, but pooled observational data seemed to favour mechanical devices (116). However, the main reason to use an automated CPR device is to reduce rescuer fatigue, especially when dealing with small teams, remote

locations and long transport times. A study that evaluated rescuers' performance in hospitals in real CPR situations showed that CPR rate was consistent during 2-minute rotations, but compression depth was reduced after 90 seconds of CPR, indicating rescuer fatigue (117). A study comparing compressions only vs standard 30:2 CPR in a manikin scenario showed signs of rescuer fatigue after 3 minutes in the compression-only group (118). A study comparing a mechanical CPR device to manual compressions during helicopter rescue reported increased CPR quality and reduced hands-off time with the automatic device, but longer time to the first defibrillation attempt (119). From the referred studies, it is reasonable to advocate using mechanical CPR devices in hypothermia patients, especially in rescue scenarios and during pre-hospital transportation.

5.4.5 Extracorporeal rewarming

The recommended treatment for victims of accidental hypothermia with cardiac arrest is rapid transfer to a hospital capable of cardiopulmonary bypass (CPB)- or ECMO rewarming (11). These recommendations are based on encouraging clinical reports favouring extracorporeal rewarming. The 2015 European Resuscitation Council recommends extracorporeal rewarming in cardiac arrest patients with core temperatures < 32°C and potassium levels < 8 mmol/L (120).

The CPB circuit consists of an oxygenator, pump, venous reservoir, arterial filter, arterial and venous cannulas. Figure 4 shows a schematic of the bypass circuit.

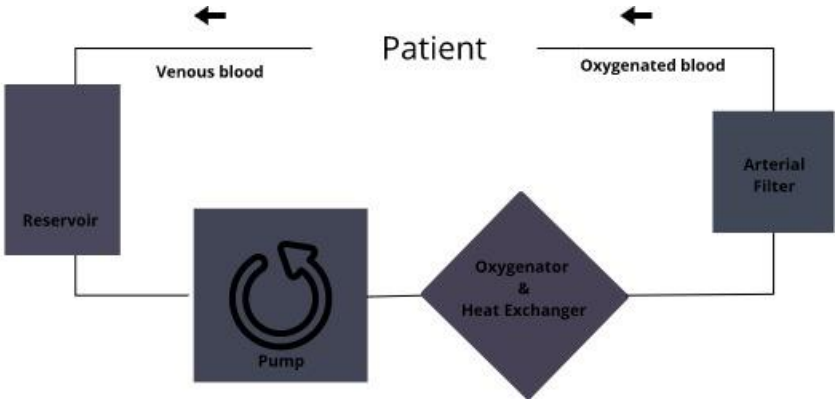

Figure 4 *- Schematic of the CPB circuit*

Cannulation is usually performed through a thoracotomy incision. The venous cannula is generally placed in the right atrium, and blood drains to the venous reservoir by gravity. A pump drives the circuit where blood is pumped from the reservoir through an oxygenator/heat exchanger, through an arterial filter and back into the patient's aorta (121). Extracorporeal bypass facilitates open heart surgery for several hours.

The term extracorporeal membrane oxygenation describes long-term therapy (days to weeks) directed towards oxygenation. The term extracorporeal life support (ECLS) encompasses all the different cannulation strategies and therapeutic indications for long term tissue oxygenation in patients with life-threatening cardiac or respiratory failure, or both (122). The cannulas are placed in major veins/arteries but peripheral to cannulation sites for CPB (122). The ECMO circuit is more straightforward than the CPB circuit. A standard ECMO circuit consists of a mechanical pump

coupled with an oxygenator/heat exchanger connected with tubing between the venous access cannula and the arterial cannula (Veno-Arterial ECMO) or a venous infusion cannula (Veno-venous ECMO), as shown in figure 5 (123).

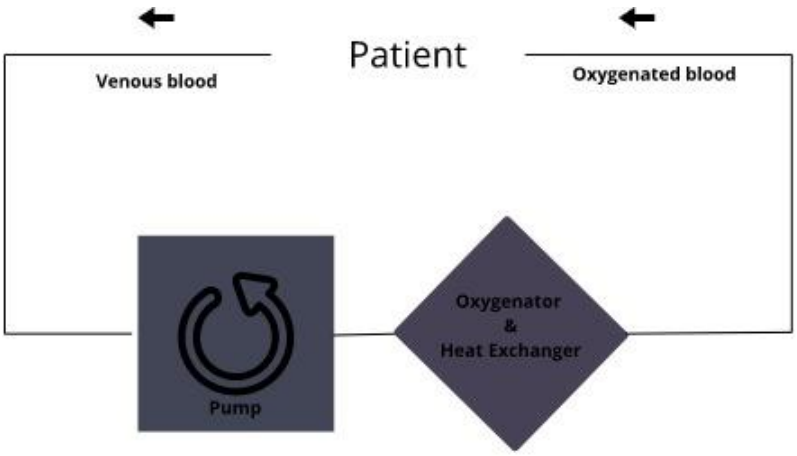

Figure 5 *- Schematic of the ECMO circuit*

The preferred treatment for hypothermic patients with absent vital signs and no signs of life is extracorporeal rewarming on CPB or ECMO (21,100,124–128). Extracorporeal rewarming yields excellent rewarming rates, estimated at 6-9°C/hr (11). Survival rates without neurologic impairment differ between studies, between 47 and 63% (92,100,124,128). A Japanese study showed increased survival in hypothermic patients rewarmed on ECMO compared to patients rewarmed with conventional rewarming methods with a survival rate of 84 to 47% (127). Cases from our region include successful rewarming from a body temperature of 13,7°C and successful rewarming after 4,5 hours of CPR (2,3).

ECMO is preferred over CPB as it can provide circulatory support after the rewarming phase, which these patients usually require. ECMO is portable and does not need an operating room to set up. It makes for easy in-hospital patient transfer, requires lower anticoagulation levels, and allows percutaneous cannulation techniques (129). A study from Austria on 59 hypothermia patients treated with CPB or ECMO showed a 6,6-fold higher chance of survival in patients rewarmed on ECMO (92). 65% of deaths in the CPB group was due to pulmonary oedema vs 0% in the ECMO group. A study from the university hospital in Bergen describes the extracorporeal rewarming of 69 patients from 1987 to 2015 (130). They found survival rates of 26,5%, comparable to 37,5% reported from north Norway (2). The standard rewarming technique was CPB; only 4 of 69 patients were admitted to the ICU on V-A ECMO.

The use of percutaneous techniques also favours ECMO, especially in patients with extended transfer times, as percutaneous cannulation of the femoral vessels is feasible during transport. This saves time for in-hospital vascular access. Consequently, vascular sheaths for venous or arterial use are standard equipment on the helicopter emergency services at the University Hospital of North Norway.

6 Aims of the thesis

This thesis is based on studies initiated with the rat model and concluded with the pig model. The clinical experiences of the University Hospital of North Norway inspired the protocols for these studies. Among these is the successful resuscitation of a woman with a core temperature of 13,7°C (3) and survival without sequelae after 6 hours 52 minutes of CPR (102).

Study 1 stems from the research done in our group on the efficacy of cardioactive drugs during hypothermia. Epinephrine (70) and dopamine (71) do not positively alleviate hypothermia-induced cardiac dysfunction, but levosimendan (62) and milrinone (72) showed positive results. Knowing that CBV is reduced during hypothermia, we wanted to see if we could improve cardiac function with intravenous fluids and if there would be a difference between crystalloids or colloids.

The inspiration for the models and experimental setup for studies 2 and 3 are regional case histories, as well as the workings of our aeromedical evacuation system (2,3,66). The scenario we imagined was a case of hypothermic cardiac arrest at the far reaches of our catchment area. With an ambulance aeroplane, the flight times to our north-eastern and south-western borders are about 1,5 hours, implying a 3-hour hypothermic arrest period before rewarming could start in the University Hospital of North Norway.

For studies 2 and 3, we needed to refine further the pig model for rewarming on ECMO. We wanted an intact model for 3-hour CPR as well as subsequent rewarming. The use of percutaneous techniques and ultrasound was instrumental in developing this model. We used isotope-labelled microspheres at different time points to explore cardiac output distribution during cooling, CPR and rewarming.

Specifically, the aims of the papers are:

Study 1.

The aim of study 1 was to examine the effects of different strategies to achieve euvolemia during rewarming from hypothermia. This was achieved through intravenous infusions of crystalloids or colloids during rewarming of hypothermic rats. We hypothesized that maintaining euvolemia during rewarming would mitigate the post-hypothermic myocardial dysfunction and that colloids could have beneficial effects surpassing crystalloid solutions.

Study 2.

The aim of study 2 was to mimic a clinical scenario of hypothermic cardiac arrest and to determine central hemodynamics, global DO_2, VO_2 and organ blood flow during 3 hours of CPR at 27°C. It is documented that CPR reproduces a proportion of native CO; we hypothesised that the reproduced level is unaffected by temperature, implying that CPR at a specific low temperature could completely replace a diminished CO. We compared CPR in normothermic and hypothermic pigs with the ambition to answer the following questions:

- Can CPR replicate all of the native CO during hypothermia?
- Is oxygen delivery and consumption different between hypothermic and normothermic CPR?
- Is oxygen delivery sufficient during hypothermic CPR to sustain aerobic metabolism?
- Is the CO distributed differently during hypothermic CPR compared to normothermic CPR?

Study 3.

The promising results from study 2, indicating partially sustained aerobic metabolism during 3 hours of CPR at 27°C, led to study 3. The aim of study 3 was to evaluate if spontaneous cardiac activity and oxygen delivery to critical organs could be re-established following 3 hours of CPR at 27°C and subsequent ECMO rewarming. Our ambition was to describe the following variables during ECMO rewarming:

- Global and cerebral oxygen delivery and consumption
- Organ blood flow and metabolism

7 Methods and methodological considerations

This thesis consists of 3 studies conducted with two different experimental models of accidental hypothermia: the first study is in a rat model, the last two are in a pig model.

7.1 Ethics and the three Rs

Working with animals and using them for scientific research is a privilege earned by treating them humanely in the pens and the lab. The Arctic University of Norway in Tromsø has excellent animal facilities with 24/7/365 care of animals. Animal care followed the Norwegian Animal Welfare Act.

Wistar rats (males, 250-350 g) were used for the rat experiments, age around 60 days. The rats had microbiological status under the Federation of European Laboratory Animal Science Associations' recommendations and were provided by Harlan UK. On arrival, the rats were quarantined for one week. Housing during experiments was as per 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). Unlimited food and water were accessible. The Norwegian Animal Research Authority approved the experimental protocol, and all experiments were conducted accordingly.

The pigs were allowed to acclimatise for 2-5 days in the pens prior to experiments; they had access to water at all times and were fed twice daily. The local steering committee of the Norwegian Animals Experiments Authority approved all experiments.

During all experiments, the animals were under deep anaesthesia. The anaesthesia protocols were the same for all animals in the rat series and for all animals in the two pig series of experiments. After concluding the experiments, the animals were euthanised with an iv bolus of pentobarbital followed by a bolus of potassium chloride. No neuromuscular blockers were used at any time in the experiments.

When researching a topic with a minimal number of patients, alternatives to clinical trials must be sought. Our research focuses on physiology and ways to ameliorate pathological processes during hypothermia and rewarming. For this reason, we are unable to Replace animals with other models.

We have taken precautions when detailing the experiments and the statistics needed to Reduce the number of animals to a minimum. In studies 2 and 3, we wanted to use the same animals for both papers, as study 3 can be seen as a logical continuation of study 2. However, many pigs died during ECMO cannulation, most due to ruptured vessels during cannulation. These pigs constitute the ones included in study 2. As such, our efforts in planning to reduce the number of animals was unsuccessful.

The rat and the pig models have been used for years in our research group. We Refined the pig model further, making a more intact model, thereby limiting confounding factors.

7.2 Animals

We chose the rat for the first study as it is well adapted to hypothermia with maintained circulation even at low temperatures < 20°C. In contrast, larger animals such as pigs are susceptible to arrhythmias at these temperatures. Our research group has utilised a rat model for several years, an established and reliable model (55,56,77,132). Also, the anaesthetised rats require very little surgical intervention but yield a wide range of data.

We refined a pig model of accidental hypothermic cardiac arrest that could be rewarmed on ECMO for the second and third studies. Our group has used this pig model in several experimental studies (33,71). Small animals such as rats have some shortcomings in translational research due to their physiology differing from human physiology during hypothermia. Dogs have been used to study hypothermia on larger animals, but the use of porcine models has been advocated as pigs have more similarities to man regarding both anatomy and physiology (132–134). The pig is a well-suited model animal for conducting experiments on cardiac arrest (135). They are responsive to CPR, electric cardioversion, and pharmacological interventions. And they are large enough to tolerate the drawing of several blood samples.

7.3 Study design

All studies were performed as acute, prospective, controlled experimental studies. Animals in studies 1 and 2 were randomised between study and control groups, whereas study 3 only has one group of animals serving as their own controls.

7.3.1 Study 1.

Wistar rats were randomised into three different groups:

Group 1 (n=7) is the hypothermic non-intervention control group. The animals were cooled, kept at 15°C for 4 hours, rewarmed and euthanised. No intravenous fluids except the fluids accompanying the anaesthesia and during thermodilution measurement of CO were infused.

Group 2 (n=7) is the hypothermic dextran treated group. The animals were cooled, kept at 15°C for 4 hours, rewarmed and euthanised. During the first 30 minutes of the rewarming period, 12 mL/kg of dextran 70 (60 mg/ml in 0,9 % saline) was infused intravenously. In addition, the rats received fluid accompanying the anaesthesia and during thermodilution measurement of CO.

Group 3 (n=7) is the hypothermic crystalloid treated group. The animals were cooled, kept at 15°C for 4 hours, rewarmed and euthanised. During the first 30 minutes of the rewarming period, 25 mL/kg of saline (9 mg/ml) was infused intravenously. In addition, the rats received fluid accompanying the anaesthesia and during thermodilution measurement of CO.

Figure 6 shows a schematic diagram of the experimental protocol

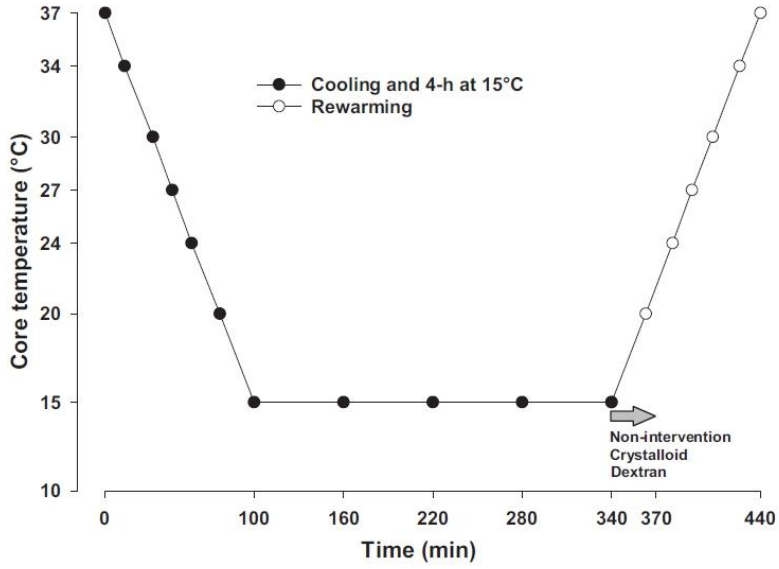

***Figure 6** – Experimental protocol, study 1*

7.3.2 Study 2

This study compares normothermic CPR (n=4) to hypothermic CPR (n=7). The animals were randomised into two groups:

Group 1 – Hypothermic cardiac arrest. The animals were surface cooled to 27°C, cardiac arrest induced, and CPR started and continued for 3 hours before conclusion and euthanasia.

Group 2 – Normothermic cardiac arrest. Cardiac arrest was induced, CPR started and continued for 45 minutes before the experiments were concluded and the animals euthanised.

Hemodynamic measurements, blood sampling and administration of microspheres were performed at baseline 38°C, during cooling at 32°C and 27°C, during CPR at 15, 60, 120 and 180 minutes in the hypothermia group. In the normothermia group, hemodynamic measurements, blood samples and administration of microspheres were performed at baseline 38°C, after 15 minutes of CPR and 45 minutes of CPR (Figure 7).

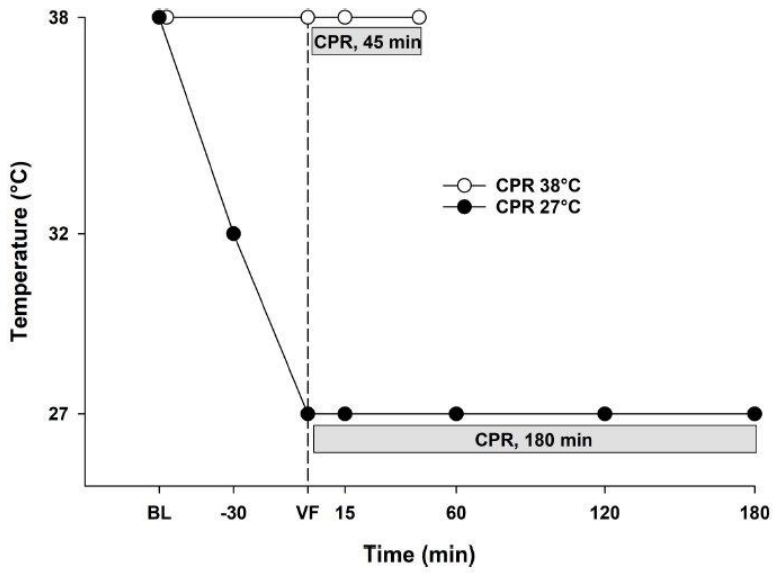

Figure 7 *– Experimental protocol, study 2. 38°C Baseline (BL); Cardiopulmonary resuscitation (CPR); Ventricular fibrillation (VF)*

7.3.3 Study 3

This study includes one group (n=8) of pigs that were surface cooled to 27°C before cardiac arrest was induced and CPR started. After 3 hours of CPR, the animals were rewarmed on ECMO before the experiment was concluded and the animals euthanised.

Hemodynamic measurements, blood samples and administration of microspheres were performed at 38°C baseline, during cooling at 32°C and 27°C, during CPR at 15, 60, 120 and 180 minutes, and during rewarming at 32°C and 38°C (Figure 8)

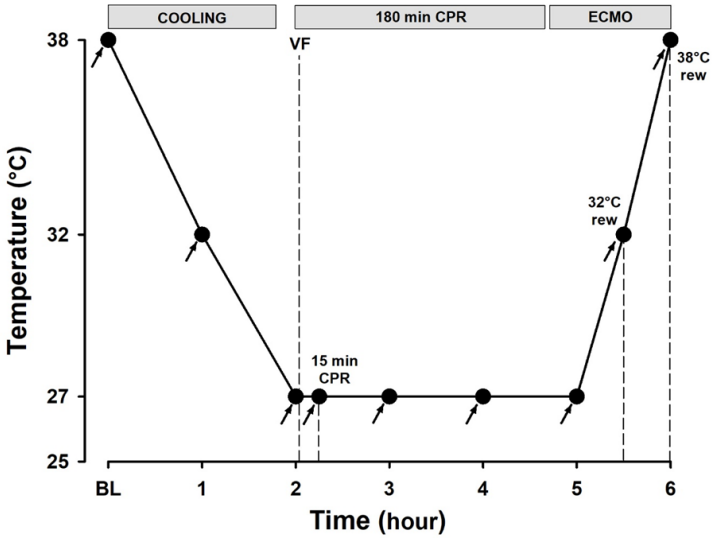

Figure 8 *– Experimental protocol, study 3. Baseline (BL); Cardiopulmonary resuscitation (CPR); Extracorporeal membrane oxygenation (ECMO); Rewarming (rew).*

7.4 Study 1 – the rat model

7.4.1 Experimental setup

In the first study, we used an established rat model of accidental hypothermia. The experimental setup is shown in figure 9. The experiments had the following structure:

1. **The rats were anaesthetised.**
   Anaesthesia was induced with an intraperitoneal injection of pentobarbital sodium 50 mg/kg, followed by a continuous infusion of pentobarbital 7.5 mg/kg/h through an intravenous line through the right jugular vein into the right auricle. Anaesthesia was discontinued at 30°C due to hypothermia-induced anaesthesia and reduced drug metabolism, and reintroduced at 30°C during rewarming. The animals showed no signs of discomfort during the experiments.

2. **Respiratory support.**
   The animals were placed on an operating table in the supine position, the trachea incised, and a 14 G tracheal tube inserted. At core temperatures > 20°C, all animals had spontaneous and sufficient ventilation. At core temperatures < 20°C, normoventilation was achieved by a volume-controlled small-animal respirator (New England rodent ventilator, model 141, New England Instruments, Medway, MA) using room air.

3. **Placement of catheters.**
   A 22 G catheter was placed through the right jugular vein and into the right auricle to allow for continuous anaesthesia and for injecting saline to measure CO. A 22 G fluid-filled catheter was placed in the left femoral artery for continuous recording of arterial pressure. A thermocouple was placed through the right femoral artery and positioned in the aortic arch to measure CO by thermodilution. A 22 G catheter was placed through the right carotid artery and into the left ventricle for continuous recording of left ventricular pressure. The animals were left to rest for 45 minutes before the start of the experiment.

4. **Core cooling and rewarming**
   The animals in the hypothermic groups were cooled and rewarmed by circulation of cold or warm water (Thermostatic water bath type RTE-110, Neslab Instruments, Newington, NH) through U-shaped polyethylene tubes placed in the oesophagus and the lower bowels. They were placed on a double-layered table made of hollow aluminium circulated by temperature-adjusted water. Thermocouple wires positioned in the aortic arch via the right femoral artery, connected to a thermocouple controller (Thermoalert TH- 5, Columbus Instruments, Columbus, OH), provided continuous monitoring of core temperature. This cooling method is well established in our lab, and we have used it in many experiments (56,69,72,131). This method allows for rapid (around 1,5 hours) cooling of rats and enables us to keep them within the narrow range of 13-15°C where they are not susceptible to arrhythmias. The rats were euthanised at the end of the experiments with a 1 ml iv bolus of pentothal 50 mg/ml.

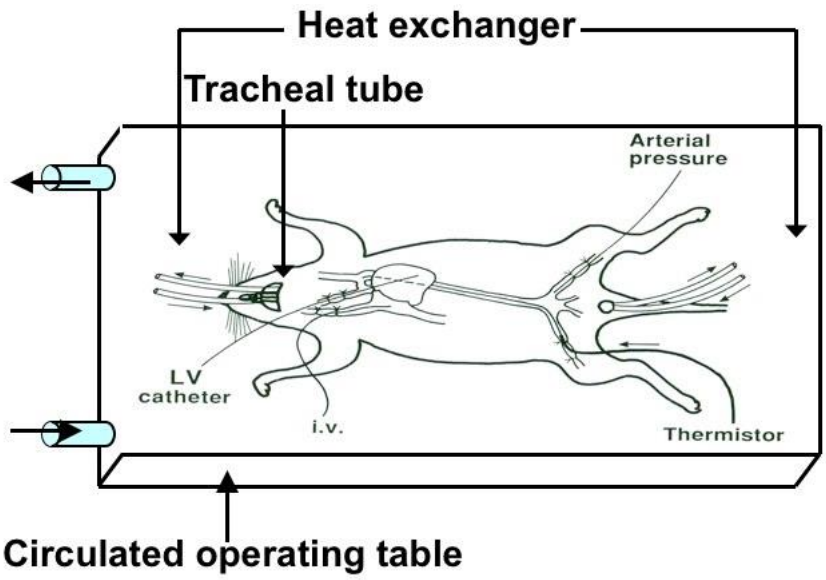

Figure 9 *- Experimental setup rat model.*

7.4.2 Measurements

Signal processing

The catheters in the left femoral artery and the left ventricle of the heart were connected to pressure transducers. The signals from the pressure transducer were amplified to 0–10 V and passed to a 12-bit analogue-to-digital converter (BNC 2090, National Instruments, Austin, TX). Signal processing and analysis were performed with a unique computer program developed at our department using a software package (LabVIEW version 6.0, National Instruments, Austin, TX).

CO measurements

In our research group's established rat model, the measurement of CO and assessment of left ventricular function has evolved from using thermodilution to pressure-volume conductance catheters (72,136). Conductance catheters give real-time pressure-volume loop readings, which mean left ventricular function can be closely monitored. In this experiment, however, the animals were given intravenous infusions of crystalloids and dextran, which would change the blood's conductance, rendering results from conductance catheters invalid. This resulted in the choice of thermodilution as a reliable method for measuring CO.

CO was measured by injecting saline (0.1–0.15 ml) precooled in ice water through the intravenous line positioned in the right auricle. The temperature change was recorded from the thermocouple placed in the aortic arch. Thermodilution curves were recorded on a Linearcorder (type Mark II, WR3101, Watanabe Instruments) and digitised on a Calcomp digitising table (model 23180, Calcomp Digitzer Products Division, Anaheim, CA). CO was calculated with a program designed with the LabView package. We calculated CO as the mean of three consecutive measurements.

The thermodilution technique was first described by Fegler (137). It was validated in rats by Hanwell in 1972 (138). Reliability of the method using different volumes of injectate (0,1-0,5 ml) was investigated. Of note here is that the lowest volume of 0,1 ml underestimated CO in the rats. This

impacts our work as we used 0,1-0,15 ml of injectate to measure CO. This could be a source of error and explain why we had technical difficulties measuring CO < 20°C. With 17 different measuring points, each point yielding 0,3 ml over 3 injections, every animal ended up getting a minimum of 5,1 ml of saline to measure CO. In the crystalloid intervention group, a rat weighing 300 grams would get an infusion of 7,5 ml of saline during the rewarming treatment period. If we had increased the injectate volumes, the animals would be getting more fluids from CO measurements alone than from interventions, which is why injectate volume was kept to a minimum.

The thermodilution technique has been studied under hypothermic conditions. Merrick investigated thermodilution in dogs during cooling to 20°C and found it to be valid. However, baseline drifts during cooling and rewarming substantially altered measured values and needed to be corrected for. Some reservations on using the method in very low temperatures and very low CO are warranted (139). Kissling evaluated the technique in rats and found that thermodilution overestimates CO compared to direct and electromagnetic flowmetry done simultaneously (140).

Blood gases

Blood gases, oxygen saturation, pH, and base excess (BE) were measured in 0.15 ml arterial blood samples taken from the femoral artery at the start of the experiment, at 15°C, and after rewarming to 37°C. Samples were analysed by a RapidLab 800 blood-gas analyser (Chiron Diagnostics).

Measurement of intracellular Ca^{2+} content

The method of radiolabelled calcium ($^{45}Ca^{2+}$) was used to measure total myocardial $[Ca^{2+}]_i$. This isotope has been used on Langendorff-perfused hearts to measure calcium (141), and Kondratiev adapted this method for in vivo use (56). He found that the time needed for $^{45}Ca^{2+}$ to equilibrate in an intact rat model was 2 hours.

All rats were injected with 20 µCi of $^{45}Ca^{2+}$ (ARX-102 Calcium-45, American Radiolabelled Chemicals Inc., St.Louis, MO, USA) at the start of the experiments to allow sufficient time for tissue equilibration. After the experiments were concluded, the rats' hearts were excised and perfused for 3 minutes on a Langendorff system to wash out extracellular $^{45}Ca^{2+}$. Kondratiev found the time needed to wash out extracellular calcium to be 1 minute (56); we chose a 3 minute washout time in our experiments.

After calcium washout, the hearts were freeze clamped with a Wollenberger clamp using liquid nitrogen and vacuum dry frosted (Christ Alpha 1-4; Medizinischer Apparatebau, Osterode, Harz, Germany) before being pulverised by a micro-dismembrator (Braun Messungen AG, Germany).

80-90 mg of the homogenate (HW) was extracted in perchloric acid for 10 minutes and centrifuged at 3000 rpm for 10 minutes (Kubota 1700 centrifuge, Kubota Corp., Tokyo, Japan). The residual intracellular radioactivity ($[RR]_{heart}$) was counted by a liquid scintillation spectrometer (Model 1900 TR, Packard Instrument Company, IL, USA). An arterial blood sample was drawn just before terminating the experiment, centrifuged, and the specific radioactivity in plasma ($[SpR]_{plasma}$) was determined using a liquid scintillation spectrometer. The calcium concentration in plasma ($[Ca^{2+}]_{plasma}$) was determined using a blood-gas analyser (RapidLab 800, Chiron Diagnostic Corp., USA).

Intracellular calcium content was calculated as follows:

$[Ca^{2+}]_i = [RR]_{heart} \times [Ca^{2+}]_{plasma} / HW \times [SpR]_{plasma}$

7.5 Study 2 & 3 - the pig model

7.5.1 Experimental setup

For studies 2 and 3, the pig model previously used by our group was refined. The setup was the same for both studies, but study 3 has two additional steps, as these animals were rewarmed on ECMO and not euthanised after the CPR period.

The structure is as follows:

1. **Premedication in pen (Study 2 and 3)**
   The animals were injected with an intramuscular bolus of ketamine 20 mg/kg, midazolam 30 mg and atropine 1 mg. When the pigs were sedated after 2-3 minutes, they were put in a bed and transported to the research lab.

2. **Anaesthesia and respiratory support (Study 2 and 3)**
   The pigs were transferred to an operating table in the research lab, and iv-access was obtained in an ear vein. Anaesthesia was induced by a bolus of fentanyl 10 µg/kg and pentobarbital sodium 10 mg/kg intravenously. An 8,5 Fr introducer was placed in the left femoral vein. The side port was used for continuous infusion of anaesthetics: fentanyl 20 µg/kg/h, midazolam 0,3 µg/kg/h and pentobarbital sodium 4 mg/kg/h.
   A tracheostomy was performed, and the airway was secured with a #7 endotracheal tube. A ventilator was connected (Siemens Servo 900D, Solna, Sweden), and the animals were ventilated with positive end-expiratory pressure (PEEP) of 0 cm H_20. The fraction of inspired oxygen (FiO_2) was adjusted to maintain the partial pressure of oxygen in arterial blood (PaO_2) > 10 kPa, and alveolar ventilation was adjusted to keep the partial pressure of carbon dioxide in arterial blood ($PaCO_2$) at 4,5-6 kPa uncorrected for temperature (α-stat).

3. **Instrumentation (Study 2 and 3)**
   The following catheters were placed:
   - An 8 Fr sheath (Edwards Lifesciences, Irvine, CA, USA) was placed into the left femoral vein to ensure the rapid establishment of continuous intravenous anaesthesia.
     - A 7,5 Fr thermodilution catheter (Edwards Lifesciences, Irvine, CA, USA) was inserted through the sheath in the left femoral vein and into the pulmonary artery.
   - An 8 Fr Super Arrowflex (Arrow International Inc., Reading, PA, USA) sheath was placed in the left femoral artery.
     - A 7,5 Fr catheter (Edwards Lifesciences, Irvine, CA, USA) was inserted through the sheath and advanced to the aortic arch for continuous blood pressure measurement.
   - A 10 Fr Super Arrowflex sheath (Arrow International Inc., Reading, USA) was placed in the right carotid artery.

    - A 6 Fr pigtail catheter (Cordis Corporation, Miami, FL, USA) was inserted through the sheath and into the left ventricle of the heart. We used this catheter to inject microspheres.
- A 3 mm flow probe (Cardiomed AS, Norway) was placed on the left carotid artery.
- An 18 G central venous catheter Arrow (Arrow international Inc., Reading, PA, USA) was placed retrograde into the left jugular bulb.
- Three 6 Fr sheaths (Cordis Corporation, Miami, FL, USA) were placed in the right jugular vein, right femoral vein and right femoral artery, respectively, to allow for placement of ECMO cannulas during the last 30 minutes of CPR. (Only study 3)
- A 3,5 Fr pressure catheter (SPR-524, Millar Instruments Inc., Houston, TX, USA) was introduced into the left hemisphere of the brain through a burr-hole in the skull.
- A 14 Fr urinary catheter was introduced into the bladder through a small incision in the abdomen.

After instrumentation, 5000 IU of heparin was given intravenously, and the animals were allowed to stabilise for 45 minutes. Figure 10 shows the placement of catheters.

4. Cooling (Study 2 and 3)

The pigs were immersion-cooled in ice water until they reached a blood temperature of 27°C. Anaesthesia was discontinued at this temperature. If shivering was observed during cooling, repeated boluses of fentanyl were administered to minimise it. The animals in the control group in study 2 were not cooled.

5. Induced cardiac arrest and CPR (Study 2 and 3)

At a core temperature of 27°C, the water was drained, and the pigs were immobilised on a vacuum mattress. A 25 G needle was inserted under the xiphoid process and into the left ventricle of the heart; an electrical current (5-20 mA, 6 Hz and 30 V) was run through the needle to induce ventricular fibrillation. After 90 seconds of cardiac arrest, an automated chest compression device (LUCAS chest compression system, Physio-Control Inc., Lund, Sweden) was started asynchronously to ventilation at 100 compressions per minute. CPR was continued for 3 hours. For the normothermic animals in the control group in study 2, CPR was discontinued after 45 minutes. For the animals in study 2, this step concludes the experiments, and they were euthanised.

6. Establishing ECMO (Study 3)

The ECMO circuit was built with 3/8″ tubes for the main circuit and 1/4″ tubing for the cannulas. The circuit was primed with 1000 ml ± 100 ml Ringers Acetate solution.
The 6 Fr sheaths in the femoral artery, femoral vein and internal jugular vein were used as ports to ensure rapid percutaneous placement of the ECMO cannulas. A guidewire was inserted through the sheath, which was subsequently removed. The flexible guidewire was replaced with a rigid guidewire (Amplatz super-stiff, Boston Scientific, Marlborough, MA, USA) via a guiding sheath. The vessels were dilated in several steps up to 16 Fr. A 15 Fr x 18 cm ECMO cannula (Bio-Medicus, Medtronic Inc., Minneapolis, MN, USA) was inserted into

the right jugular vein and another into the right femoral artery. A 15 Fr x 50 cm venous ECMO cannula (Bio-Medicus, Medtronic Inc., Minneapolis, MN, USA) was inserted into the right femoral vein and advanced to the right atrium. The placement of guidewires and cannulas was verified by x-ray. Venous blood was drawn through the dual venous cannulas into the oxygenator and heat/exchanger (Quadrox-I Adult, Maquet Cardiopulmonary AG, Hirrlingen, Germany), and the oxygenated blood was then pumped into the artery by a centrifugal pump (Rotaflow, Maquet Cardiopulmonary AG, Hirrlingen, Germany).

7. Rewarming (Study 3)

Flow on the ECMO circuit was adjusted to mimic the hemodynamic characteristics of the individual animal during cooling. The ECMO circuit was started at a flow of 0,8-1 l/min at 27°C, gradually increased to 2-2,5 l/min at 32°C and 3-3,5 l/min at 38°C. Core temperature was elevated by increasing the water temperature in the heater/cooler coupled to the oxygenator on the ECMO circuit. Water temperature was increased gradually and kept 2°C above the animals' core temperature, with a maximum allowed temperature of 38°C. Negative access pressure was measured during rewarming, and volume therapy with Ringer's acetate solution was instituted when access pressure was less than -100 mmHg. The total volume of Ringer's added to the circuit was 2000-6000 ml (mean 3750 ml). The pigs were first rewarmed up to a blood temperature of 32°C and then stabilised there for 10 minutes while hemodynamic measurements, blood sampling and administration of microspheres were performed. Electric cardioversion at 100 joules was attempted up to three times. The pigs were then rewarmed to a blood temperature of 38°C, and cardioversion was attempted again. If ROSC was unsuccessful after three shocks, a sternotomy and pericardiotomy were performed, followed by internal defibrillation with a current of 5-15 joules. The experiment was then concluded, and the animals euthanised.

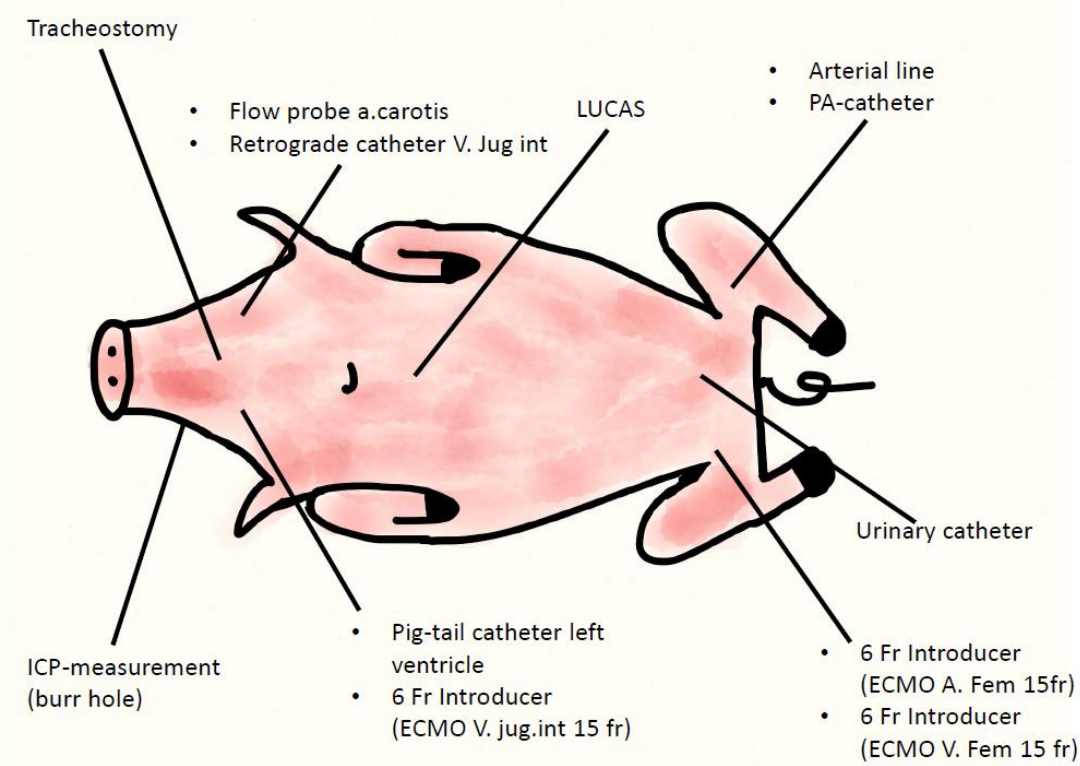

Figure 10 *– Catheter placement, pig model*

7.5.2 Measurements

Hemodynamic measurements

MAP, HR, intracranial pressure (ICP), central venous pressure (CVP), left ventricular pressure (LVP), pulmonary arterial pressure (PAP), left carotid artery flow, and urinary output was measured and registered on LabChart software (PowerLAB 16/35, ADInstruments, Dunedin, New Zealand).

CO was measured by thermodilution using 10 ml cold saline injected into the pulmonary artery catheter (PA-catheter) and registered on a Vigilance monitor (Edwards Lifesciences, Irvine, CA, USA).

Blood gases, oxygen delivery and consumption

Blood gases were sampled from arterial blood, central venous blood, mixed venous blood and from the jugular bulb and analysed on ABL800 FLEX (Radiometer Medical, Copenhagen, Denmark).

Oxygen content (C_aO_2) in all blood gases was calculated as follows:

$C_aO_2 = 1{,}34 \times Hb\ (g/dl) \times S_aO_2 \times 0{,}01 + 0{,}0031 \times P_aO_2\ (kPa) \times 7{,}5$, where

Hb is the haemoglobin level in g/dl,

S_aO_2 is the oxygen saturation in the arterial blood, and

P_aO_2 is the partial pressure of oxygen in the arterial sample.

Oxygen delivery was calculated as DO_2 = CO x C_aO_2 and oxygen consumption as VO_2 = CO x (C_aO_2 – C_vO_2). Mixed venous blood gases were used during cooling and CPR. When rewarming on the ECMO circuit was started, drawing blood from the pulmonary artery was impossible due to venous drainage into the ECMO circuit. Therefore, central venous blood gases were used to calculate VO_2 during rewarming.

Cerebral oxygen delivery was calculated as $D_{O2cerebral}$ = Mean cerebral flow x C_aO_2. Cerebral oxygen consumption was calculated as $V_{O2cerebral}$ = Mean cerebral flow x (C_aO_2 – $C_{jugular}O_2$). Cerebral flow values were calculated as means of pooled data from left and right temporal lobes and cerebellum.

Use of microspheres for regional blood flow measurements

Microspheres were used to investigate organ blood flow in the animals in studies 2 and 3. Stable isotopically labelled microspheres (BioPAL Inc., Worcester, MA, USA) were chosen due to high sensitivity and the absence of radioactive waste. Study samples containing these microspheres are neutron activated, and the emitted radiation is measured. Stable isotopically labelled microspheres have been validated over a wide range of flow (142).

At all sampling points, approximately 10 million stable isotope-labelled 15 µm microspheres of different specificity were injected through the pigtail catheter placed into the left ventricle of the heart. However, during rewarming, the microspheres were injected through the injection port on the arterial ECMO cannula. Simultaneously a 10 ml reference blood sample was drawn from the aortic arch at 5 ml/min using a NE-1000 syringe pump (New Era Pump Systems Inc., Farmingdale, NY, USA). The blood samples were washed with saline-free buffer (sanSaLine, BioPAL Inc., Worcester, MA, USA) and centrifuged twice to remove sodium and chlorine to improve the signal-to-noise ratio. Post mortem tissue samples were collected from the brain (temporal lobes and cerebellum), kidneys, liver, heart, small bowel, stomach and spleen to determine flow in these tissues. All tissues samples were rinsed with saline-free buffer. Blood- and tissue samples were analysed at BioPAL using a neutron activation technique to analyse microsphere specific activity and microsphere content (142).

Assessment of regional blood flow (Q) by microsphere activity in each organ was calculated as follows:

$Q\ (ml/min) = (A/A_{ref}) \times R\ (ml/min)$, where

A is the activity in the tissue sample,

A_{ref} is the activity of the reference blood flow sample, and

R is the withdrawal rate of the reference blood flow sample in ml/min.

7.6 Statistics

Statistical analysis was performed on SigmaPlot statistical software, version 14 (Systat Software Inc., Richmond, CA, USA). Results are presented as means and standard deviations (SD). Differences were considered to be significant at $p < 0.05$.

In study 1, one-way ANOVA was used for between-group comparisons of hemodynamic variables and intracellular calcium content. Scheffe's test was used to obtain p-values in hypothermic groups where significant differences were found. For within-group comparisons of baseline vs post-hypothermic end-point, a paired t-test was used. Two-way repeated-measures ANOVA with Dunnett's post hoc test was used to compare plasma variables.

In studies 2 and 3, normal distribution was checked using the Shapiro-Wilk test. In study 2, a two-way repeated-measures ANOVA was used for comparisons between normothermia and hypothermia groups, as well as intragroup comparisons at 38°C baseline, 15 min and 45/60 min of CPR. Pairwise multiple comparison procedures were performed using the Holm-Sidak test when significant differences were found.

One-way repeated measures ANOVA on normal distributed variables and Friedman repeated-measures ANOVA on ranks for non-normal distributed variables were used to compare values within the hypothermia groups in studies 2 and 3. Where significant differences were found, Dunnett's test was used to compare all values vs baseline values.

7.7 Methodological considerations and limitations

7.7.1 The use of large fluid volumes in rats

For laboratory animals such as the rat, it is not recommended to infuse fluid boluses exceeding 5 ml/kg (1,5 ml bolus in a 300 g rat) or continuous infusions exceeding 4 mL/kg/h (143). In study 1, 12 mL/kg of dextran and 25 ml/kg of saline was infused over 30 minutes. In an average rat weighing 300 grams, this comes to 3,6 mL dextran and 7,5ml saline. As such, more fluids than recommended was administered.

The blood volume (BV) in the rat can be calculated by BV (mL) = 0,06 x BW (g) + 0,77, where BV is blood volume in millilitres and BW is body weight in grams (144). A rat weighing 300 grams should, according to this formula, have a blood volume of 18,77 mL. Saline is the largest volume of the infusions given in the experiments, but this is well below half of the rat's circulating volume. Researchers studied rapid infusion (25 mL/min) of volumes corresponding to between 0,5 and 3 times blood volume in rats (145). They found that infusing 1,5 times the blood volume led to no significant changes in heart rate, oxygen saturation or respiratory rate, but a short (<1 min) respiratory arrest in 50% of the animals. The volume infused in the rats in study 1, although high, falls well below the volumes tested and found to be tolerated.

7.7.2 A porcine model for CPR

To study the influence of exposure to extreme physiologic conditions on the cardiovascular and respiratory system, the pig is a favoured model as a bridge for potential translation of new research data to human medicine. Studies 2 and 3 were done using an automated chest compression device designed and applied in clinical medicine. Similarities make this model close to a human patient under CPR, but obvious differences exist. The size of the animal and its chest anatomy are fundamentally different from those of an adult human patient, making it challenging to use an automated chest compression device. However, we chose the pig as a model because of its abundant use in several other experiments. Others have thoroughly investigated the effects of using the actual compression device on pigs (146,147).

The pig, while being an adequate model with similarities in physiology and anatomy to man (132), is not necessarily a good model when it comes to examining the effects of prolonged CPR. The reason for this is primarily anatomical; the shape of the porcine sternum and chest is quite different from the human torso. The pig has a chest shaped like the keel of a boat. Figure 11 illustrates this difference.

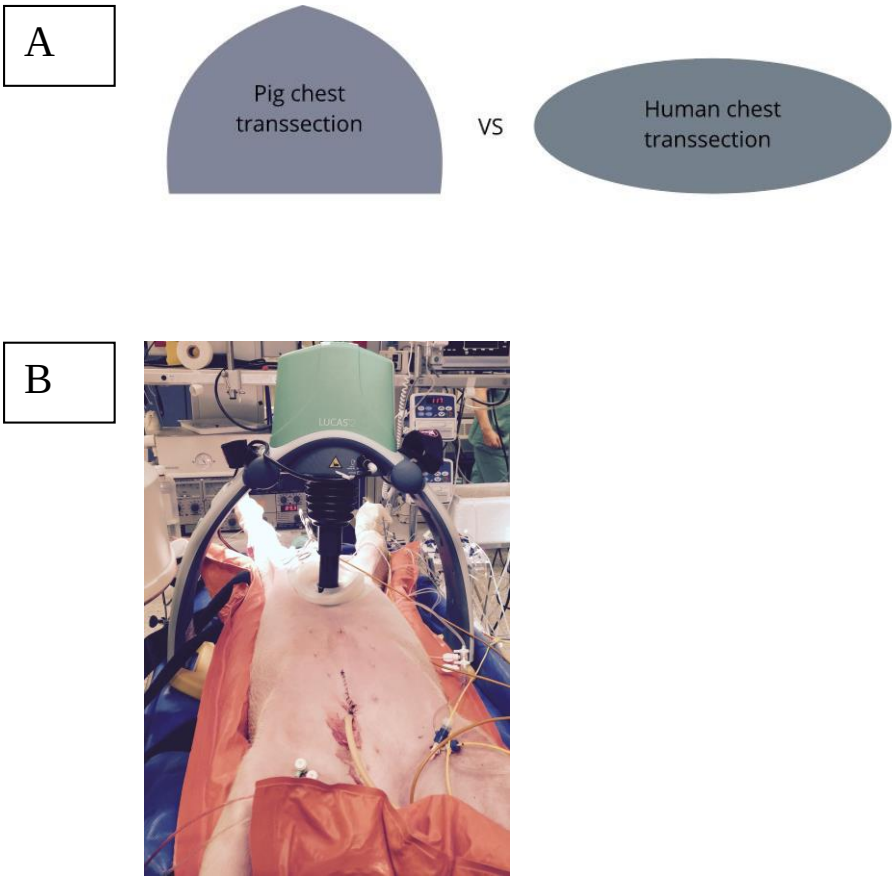

Figure 11 *- Challenges when using the LUCAS on pigs.* ***A)*** *Schematic of the transected chest in pig and man, note the pig's chest looks like a boat laying with the keel upwards.* ***B)*** *CPR in pig with the LUCAS automated CPR device. The pig is immobilised on a vacuum mattress.*

These anatomical differences make it hard to position the pig inside the automated CPR device. A vacuum mattress was used to immobilise them. It was our experience that you get one chance of placing the pig in the correct position inside the CPR device, and it needs to stay in the exact place for

the duration of the CPR period. During the first pilot experiments, some pigs died if repositioned in the LUCAS device. Our theory is that the initial compressions produced rib or sternal fractures and that if repositioned, the edges exerted too much pressure on intrathoracic vessels, resulting in catastrophic bleeds. There are reports on adverse events in humans with automated CPR devices but to a lesser extent than our experience. The CIRC trial used the AutoPulse machine on 2359 persons and found injuries in 12% with the AutoPulse compared to 11% in manual CPR, rib fractures, and subcutaneous emphysema was more prevalent in the AutoPulse group (148). The LINC trial used the LUCAS device on 1300 persons and found seven serious adverse events (115). A study on victims of unsuccessful resuscitation found CPR related injuries in 91,4% of patients resuscitated with an automated device vs 75,9% in patients resuscitated with manual CPR; none of the injuries was deemed the cause of death (149). We attribute the adverse events seen in our experiments to the extended duration of CPR and to the fact that the LUCAS was designed for humans and not pigs.

However, the LUCAS device has been used on pigs yielding a 100% ROSC rate after 15 minutes in fibrillating pigs under normothermic conditions (146). The investigators also found that surface cooling the pigs to 34°C improved ROSC rates after 60 minutes of CPR. Steen et al. found that the pig's heart, placed centrally in the chest, does not compress against the spine(146). The driving force seems to be increased intra-thoracic pressure, a theory known as "the thoracic pump theory". Studies using ultrasound during CPR in humans show that the increased intra-thoracic pressure seems to be the driving force behind CPR driven cardiac output in humans as well (150).

Using the LUCAS device over 3 hours is of concern in that it alters the pig's chest. The chests started out keel-shaped as in Figure 10 but were quite flat after 3 hours of CPR; Steen et al. also observed a softening and flattening of the pigs' chests after 20 minutes of CPR(146). In study 3, several of the animals were refractory to electric cardioversion after rewarming. A sternotomy and pericardiotomy were performed on these refractory pigs to release anatomical pressure on the heart from the chest wall. Sinus rhythm was achieved in some of the pigs simply by removing the chest wall pressure from the heart. It is difficult to speculate on how much this anatomical alteration has affected our results. What argues against this being a substantial source of error is the fact that the hearts did not perform any of the work needed to produce cardiac output during the CPR or rewarming phases of the experiments, and sinus rhythm was achieved in all animals.

7.7.3 Extracorporeal rewarming of pigs

The pig model of accidental hypothermia is well known in our lab and has been used with CPB with central cannulation (33). We used ECMO with peripheral, ultrasound-guided cannulation to keep the model more intact and allow 3 hours of CPR with the LUCAS. We sought the help of a cardiothoracic anesthesiologist and a clinical perfusionist to help design the circuit and establish it in the model.

The circuit was built as simple as possible using ⅜" tubing for the main circuit and ¼" tubing for connections to the cannula. This allowed the use of 15 fr cannulas in the pigs. Venous access pressure was measured to monitor the circuit; fluid was added if access pressure exceeded -100mm Hg. In pilot experiments, a veno-arterial ECMO circuit on the neck of the pig (Figure 12) was tested, but this yielded too little venous drainage to run the circuit with a CO higher than 1-1,5 l/min at the most. This led to a veno-veno-arterial setup with the venous cannulas in the jugular and femoral vein and the

arterial cannula in the femoral artery (Figure 13). The veno-veno-arterial design proved to be much more successful, with sufficient venous return to allow the circuit to run > 3 l/min.

During the first trials with percutaneous cannulation for ECMO, there were vessel injuries during dilation; the femoral vessels proved frail. Several of the pigs died during this critical phase of the experiment. The solution was apparent once identified. The pigs delivered to us weighed around 25 kgs. When the size of the pigs was increased to about 30 kgs, cannulations were successful in all pigs, and there were no more issues with ruptured vessels.

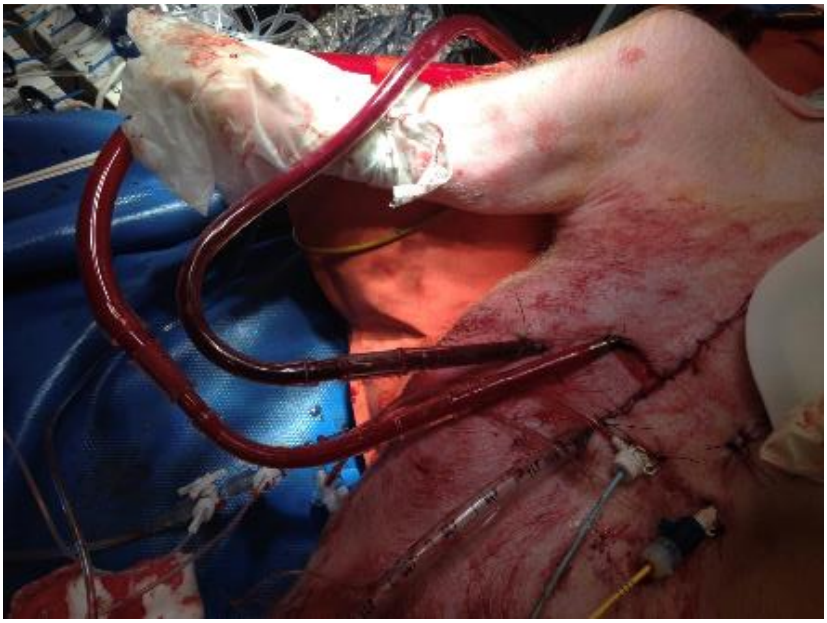

Figure 12 *- Veno-arterial ECMO circuit in the pig model. Here showing venous access in the jugular vein and arterial access in the carotid artery*

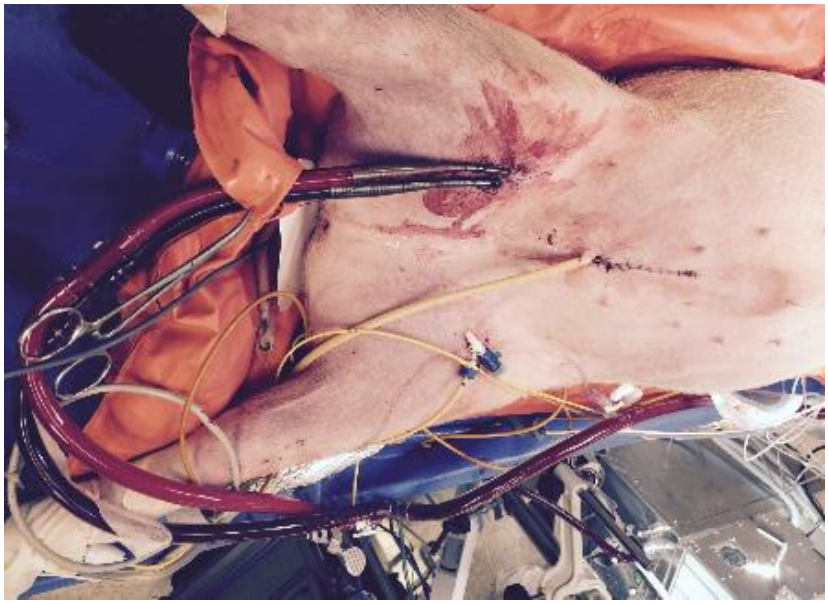

Figure 13 *- Veno-veno-arterial ECMO setup in the pig model. Here showing only cannulas in the femoral vessels*

7.7.4 The use of microspheres during rewarming

Some troubling aspects of the experimental setup became evident during experiments. A major concern was that microspheres were injected into the left ventricle of the heart during cooling and CPR, whereas during ECMO, this was not possible. During CPR, the left ventricle acts as a reservoir for the microspheres, releasing a portion of them through the aortic valve with every compression of the mechanical CPR device. During ECMO, the flow from the arterial cannula closes the aortic valve, making it futile to inject microspheres into the left ventricle. Instead, we opted to inject the microspheres through a side port of the arterial cannula. The methodological implications are that in injecting microspheres into the left ventricle, the microspheres are distributed over time as the heart does not empty entirely on every stroke. This is not possible to do on ECMO apart from injecting the microspheres by syringe pump over time. However, this has its own set of problems and has not been validated that we know. The concern with microspheres is that injecting them directly into the arterial ECMO cannula might produce different results regarding regional flow data. Organ flow data captured during rewarming must be considered with caution.

8 Summary of results

8.1 Study 1

Maintaining intravenous volume mitigates hypothermia-induced myocardial dysfunction and accumulation of intracellular Ca^{2+}.

8.1.1 Main results

Cooling significantly reduced all hemodynamic parameters except for SV (increased) and TPR (unchanged). During rewarming, CO was significantly higher and $[Ca^{2+}]i$ was significantly lower in the treated groups compared to the control group.

8.1.2 Cardiovascular function

As shown in Figure 14, HR and MAP were both significantly reduced during cooling. TPR remained relatively unchanged and could not be measured during the 4 hours of hypothermia due to technical limitations. There were no significant differences between the three groups with regards to HR or MAP. TPR in the two treatment groups was significantly lower than in the control group during the first phase of rewarming, at 24 and 30°C, corresponding to the infusion period in the treatment groups.

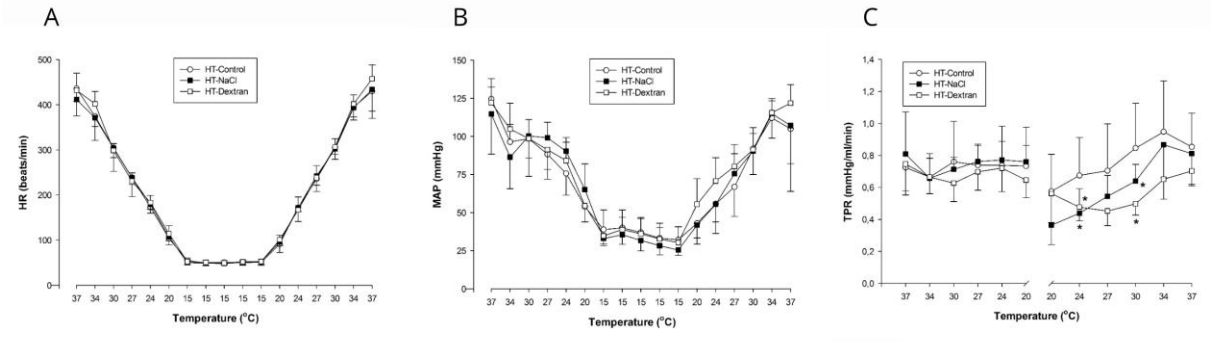

Figure 14 *- Hemodynamic variables in hypothermic rats.* ***A)*** *HR,* ***B)*** *MAP, and* ***C)*** *TPR. Control group (HT-control, n=7), crystalloid treated group (HT-NaCl, n=7), and dextran treated group (HT-Dextran, n=7). Values are mean ± SD. * - p < 0,05 vs control group.*

Figure 15 shows CO and SV. Neither CO nor SV were measured during the 4 hours of hypothermia due to technical limitations. During cooling, CO was reduced with no changes between groups (Fig 15 A). CO in the crystalloid group was significantly higher than in the control group during rewarming up to 30°C, corresponding with the infusion period. CO in the dextran group was significantly higher than in the control group during all stages of rewarming and significantly higher than in the crystalloid treated group from 24 to 37°C rewarming. CO in the treatment groups returned to pre-hypothermic levels after rewarming. SV showed an increase in all groups during cooling and was increased in both treatment groups compared to controls during rewarming to 30°C, corresponding to the infusion period. The dextran-treated animals displayed a sustained effect on SV, as it was significantly higher than controls at the end of rewarming.

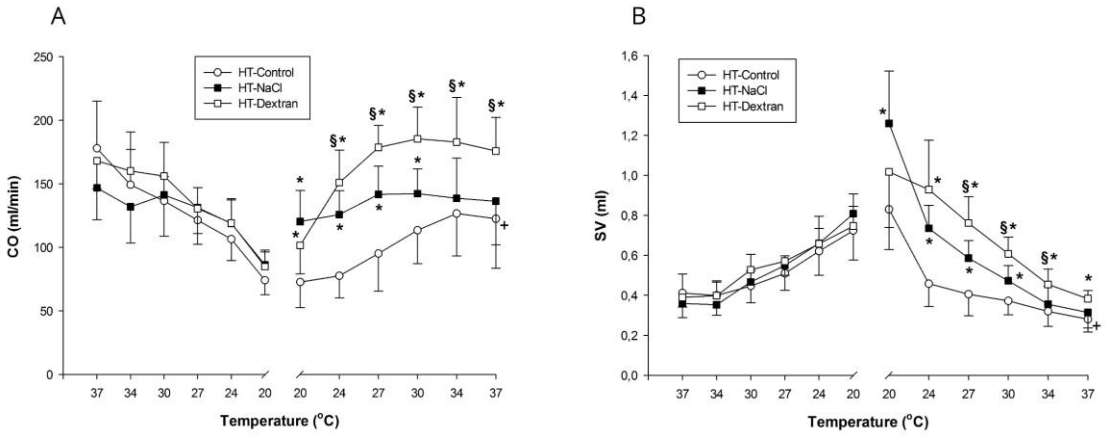

***Figure 15** – Hemodynamic variables in hypothermic rats. **A)** Cardiac output (CO) and **B)** Stroke volume (SV). Control group (HT-control, n=7), crystalloid treated group (HT-NaCl, n=7) and dextran treated group (HT-Dextran, n=7). Values are mean ± SD. * - p < 0,05 vs. control group. § - p < 0,05 vs. crystalloid treated group. + - p < 0,05 vs. baseline levels.*

The maximum and minimum rate of pressure change in the left ventricle (Left ventricular dP/dt_{max} and dP/dt_{min}) are shown in Figure 16. dP/dt_{max} decreased significantly during cooling in all groups and stayed low during the hypothermia period. It returned to pre-hypothermic baseline levels during rewarming in both treatment groups but not in the control group, where it was significantly reduced compared to baseline values. In the dextran group, dP/dt_{max} was significantly higher than the control group at 30°C rewarming. LV dP/dt_{min} increased during cooling in all groups and returned to baseline values after rewarming. It was significantly lower in the dextran group than in the control group from 24-30°C rewarming and significantly lower than the crystalloid group at 24°C rewarming.

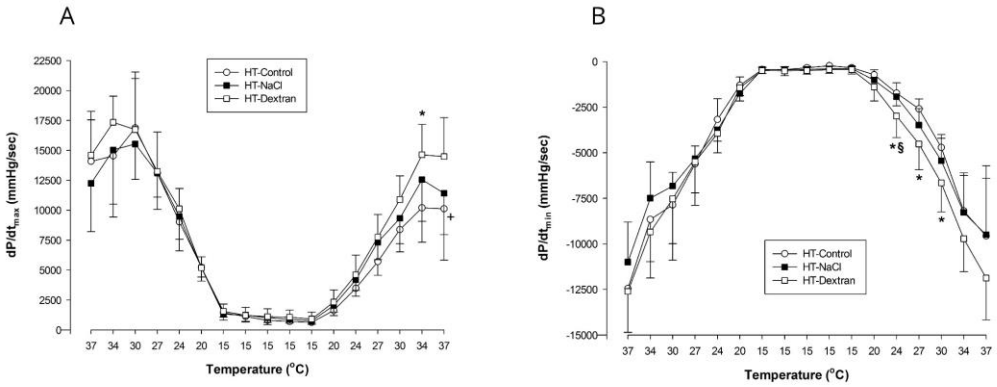

***Figure 16 – A)** Maximum level of LV pressure rise (LV dP/dt_{max}) and **B)** Maximum level of LV pressure decline (LV dP/dt_{min}) in hypothermic rats. Control group (HT-control, n=7), crystalloid treated group (HT-NaCl, n=7) and dextran treated group (HT-Dextran, n=7). Values are mean ± SD. * - p < 0,05 vs. control group. § - p < 0,05 vs. crystalloid treated group. + - p < 0,05 vs. baseline levels.*

Both treatment groups showed significantly reduced levels of myocardial intracellular Ca^{2+} content ($[Ca^{2+}]_i$) compared to the control group (Figure 17). A reduction of 47% in the saline and 49% in the dextran group compared to controls.

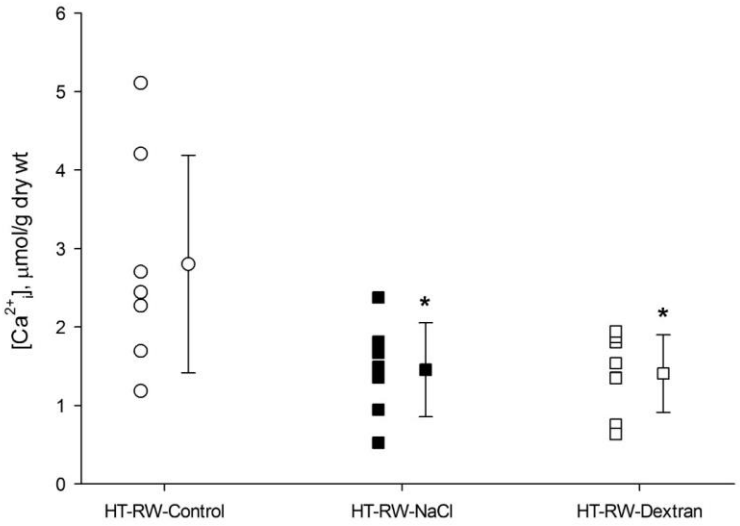

Figure 17 *- Intracellular calcium concentration ([ca^{2+}]$_i$)in hypothermic rats after rewarming. Control group (HT-RW-control, n=7), crystalloid treated group (HT-RW-NaCl, n=7) and dextran treated group (HT-RW-Dextran, n=7). Values are mean ± SD. * - p < 0.05 vs.control group.*

Table 1 shows arterial blood-gas values. There were no significant differences between groups, except for lower values of BE in both treatment groups after cooling to 15°C. After rewarming, all animals demonstrated significantly increased serum lactate in concert with reduced BE and pH.

	Group	**37°C**	**15°C**	**37°C rew**
pH	Control	7.36 (0.05)	7.24 (0.07) #	7.20 (0.06) #
	Crystalloid	7.35 (0.02)	7.18 (0.07) #	7.21 (0.06) #
	Dextran	7.33 (0.03)	7.21 (0.08) #	7.26 (0.03) #
$PaCO_2$ (kPa)	Control	4.0 (0.66)	5.0 (0.88)	2.8 (0.36)
	Crystalloid	4.4 (0.42)	6.2 (1.67) #	2.9 (0.33) #
	Dextran	4.3 (0.18)	5.4 (1.27)	3.3 (0.65)
PaO_2 (kPa)	Control	11.1 (4.1)	22.3 (6.3) #	12.9 (3.6)
	Crystalloid	10.6 (1.3)	26.9 (5.0) #	11.7 (2.1)
	Dextran	10.2 (1.7)	23.4 (5.9) #	10.8 (3.1)
Hb (g/dL)	Control	11.7 (1.2)	11.2 (2.0)	11.3 (1.8)
	Crystalloid	12.8 (1.4)	11.1 (1.5)	11.5 (1.6)
	Dextran	11.3 (1.0)	11.3 (1.1)	9.9 (1.5)
Hct (%)	Control	36.2 (3.7)	36.1 (3.7)	34.8 (5.5)
	Crystalloid	39.2 (4.1)	34.1 (4.4)	35.4 (4.8)
	Dextran	34.8 (3.0)	35.0 (3.1)	31.3 (4.5)
	Control	1.3 (0.6)	0.9 (0.4)	3.5 (1.3) #

Lactate (mmol/L)	Crystalloid	2.0 (1.3)	1.7 (0.8)	4.0 (1.2) #
	Dextran	1.3 (0.9)	1.6 (0.7)	3.7 (2.0) #
BE (mmol/L)	Control	-7.2 (1.7)	-7.0 (7.0)	-18.8 (2.3) #
	Crystalloid	-7.1 (1.5)	-10.6 (2.0) *	-18.3 (2.2) #
	Dextran	-7.9 (1.7)	-11.3 (2.0) #*	-15.1 (2.2) #
cTn-I (ng/ml)	Control	-	-	10 (5.6)
	Crystalloid			14.1 (7.8)
	Dextran			9.8 (5.8)
CBV (ml)	Control (n=5)	-	-	18.7 (5.3)
	Crystalloid (n=7)			20.1 (6.4)
	Dextran			-

Table 1 *- Arterial blood gases (n=7). Values are means and (SD). Haemoglobin (Hb), Haematocrit (Hct), Base Excess (BE), Cardiac troponin I (cTnI), Circulating blood volume (CBV). * - p < 0.05 vs control group. # - p < 0.05 vs. pre-hypothermic baseline.*

8.2 Study 2

Study of the effects of 3 h of continuous cardiopulmonary resuscitation at 27°C on global oxygen transport and organ blood flow.

In this study, normothermic to hypothermic CPR was compared over the first hour, 45 and 60 minutes, respectively, with an emphasis on hemodynamics, regional blood flow and oxygen transport. Further, the effects of 3 hours CPR at 27°C were investigated with regards to hemodynamics, oxygen transport and organ blood flow.

8.2.1 Main results

We hypothesised that the level of CO reproduced by CPR would be unaffected by temperature, allowing for a more significant portion of spontaneous CO at a reduced temperature to be replicated by CPR. Figure 18 A shows our hypothesis, and 17 B shows that CPR could not reproduce spontaneous CO completely at 27°C. We found CPR at 27°C to yield about 50% of spontaneous CO, whereas normothermic CPR produced about 25% of spontaneous CO.

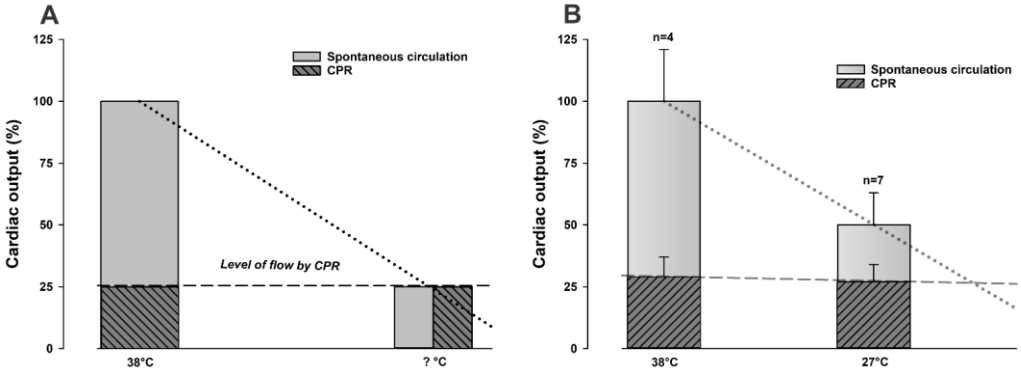

Figure 18 *- CO during CPR in normothermic (n=4) and hypothermic (n=7) animals.* ***A)*** *hypothesis* ***B)*** *Findings*

8.2.2 Comparing the effects of CPR: normothermic (38°C) vs hypothermic (27°C) groups

We found no statistically significant differences between groups in any of the variables recorded at the start of the experiments. Animals in both experimental groups had spontaneous circulation before induction of ventricular fibrillation (VF).

Hemodynamics

In the normothermic group, CO and MAP were significantly reduced from 3,0 l/min and 85 mmHg at baseline to 0,9 l/min (-71%) and 38 mmHg (-55%) after 15 minutes CPR (Figure 19 A, B). At 45 minutes, CO in the normothermic group decreased to 0,6 and MAP to 18 mmHg (-80% and -78%, respectively). In the hypothermic animals, 15 minutes of CPR led to significantly reduced CO and MAP, from 3,2 l/min and 89 mmHg to 0,8 l/min (-74%) and 33 mmHg (-33%). After 60 minutes of hypothermic CPR, CO and MAP were further reduced to 0,8 l/min and 31 mmHg (-74% and -65%, respectively.). No significant differences between groups were found after 15 minutes, but after 45 minutes of normothermic CPR, MAP was significantly lower than in the hypothermic group at 60 minutes.

Oxygen transport

In the normothermic group, DO_2 decreased significantly from 13,6 ml/kg/min at baseline to 4,8 ml/kg/min (-64%) at 15 minutes of CPR. At the same time oxygen consumption decreased significantly by 30%, from 5,8 to 4,1 ml/kg/min (Figure 19 C,D). Accordingly, O_2ER (Figure 20 A) was significantly increased at 15 minutes of CPR and exceeded the reported critical extraction ratio (ER_{crit} = 0,6 – 0,7) needed to support aerobic metabolism (151). At 45 minutes of CPR, the O_2ER remained significantly elevated at 0,84. Simultaneously, a reduction in mixed venous oxygen saturation (S_VO_2) was seen (Figure 21 B), from 56% during spontaneous circulation to 15% and 18% after 15 and 45 minutes of CPR.

In the hypothermic group, DO_2 decreased significantly from 13,7 to 3,6 ml/kg/min at 15 minutes CPR (-73%), and VO_2 from 5,7 to 2,0 ml/kg/min. (Figure 19 C,D). This caused a significant increase in the O_2ER from 0,42 to 0,55, but this value is lower than the reported critical extraction value (Figure 20 A). Simultaneously, S_VO_2 (Figure 21 B) fell from 58% at baseline to 46% at 15 minutes CPR and 27%

at 60 minutes CPR. Lactate (Figure 21 A) also increased; at 15 minutes CPR, it was 1,17, rising to 2,81 mmol/l at 60 minutes CPR.

Due to low core temperature, VO_2 was significantly lower in the hypothermic group than in the normothermic group, but no differences in DO_2 was found between groups. Also, the reduction in S_VO_2 was less severe in the hypothermic than in the normothermic group. The increase in lactate was significantly lower in the hypothermic than in the normothermic group. The higher S_VO_2, moderately increased lactate, and less severely increased O_2ER in the hypothermic group indicate better oxygen transport during CPR at 27°C.

Regional blood flow

15 minutes of CPR in the normothermic group led to significantly reduced blood flow to the heart, brain, kidneys, liver, stomach and small intestine (Figure 20 B, C, D). After 45 minutes of CPR, blood flow to these organs was negligible. A similar reduction in blood flow was seen in the hypothermic group at 15 minutes of CPR, but in contrast to the normothermic group, the reduced flow did not deteriorate further at 60 minutes of CPR.

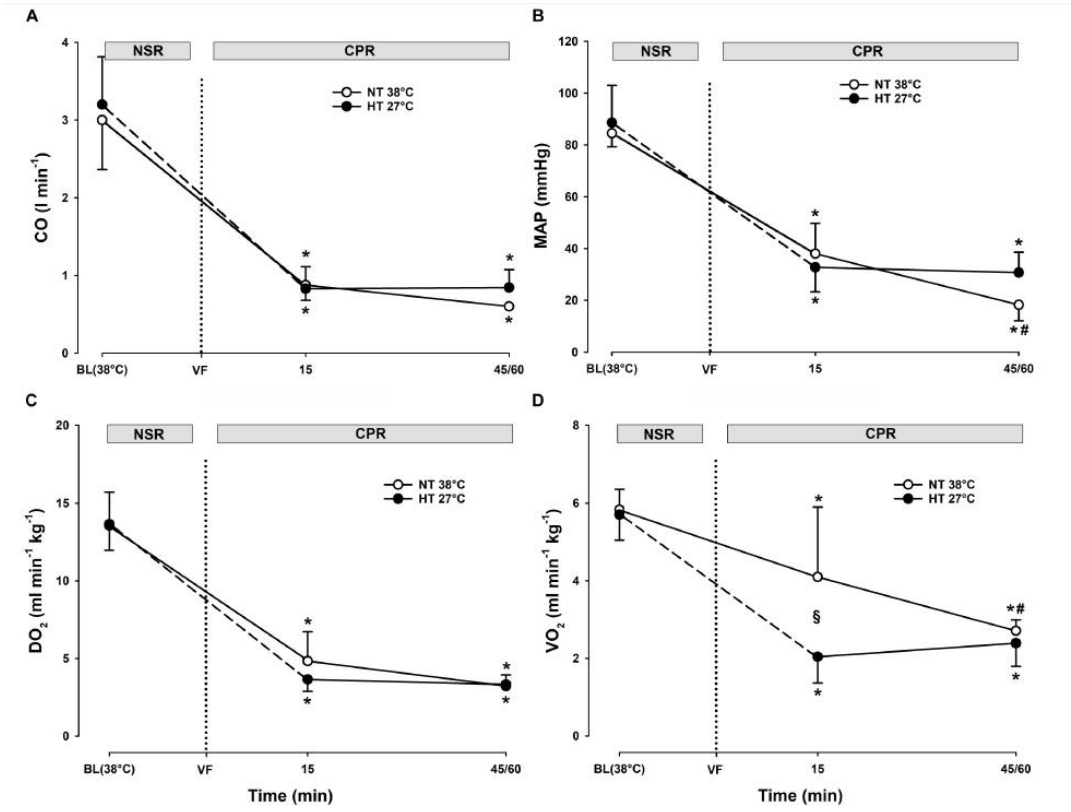

Figure 19 *- Hemodynamic variables, global O_2 transport and O_2 uptake during CPR at normothermia and hypothermia.* ***A)*** *Cardiac output (CO),* ***B)*** *mean arterial pressure (MAP),* ***C)*** *global oxygen delivery (DO_2), and* ***D)*** *global oxygen consumption (VO_2). CPR at normothermia (NT 38°C, n = 4), CPR at hypothermia (HT 27°C, n = 7). 38°C baseline (BL(38°C)), normal sinus rhythm (NSR), cardiopulmonary resuscitation (CPR), ventricular fibrillation (VF). Values are mean ± SD. * - p < 0.05 vs. intragroup 38°C baseline; # - p < 0.05 vs. intragroup 15 min of CPR; § - p < 0.05 vs. corresponding value between groups.*

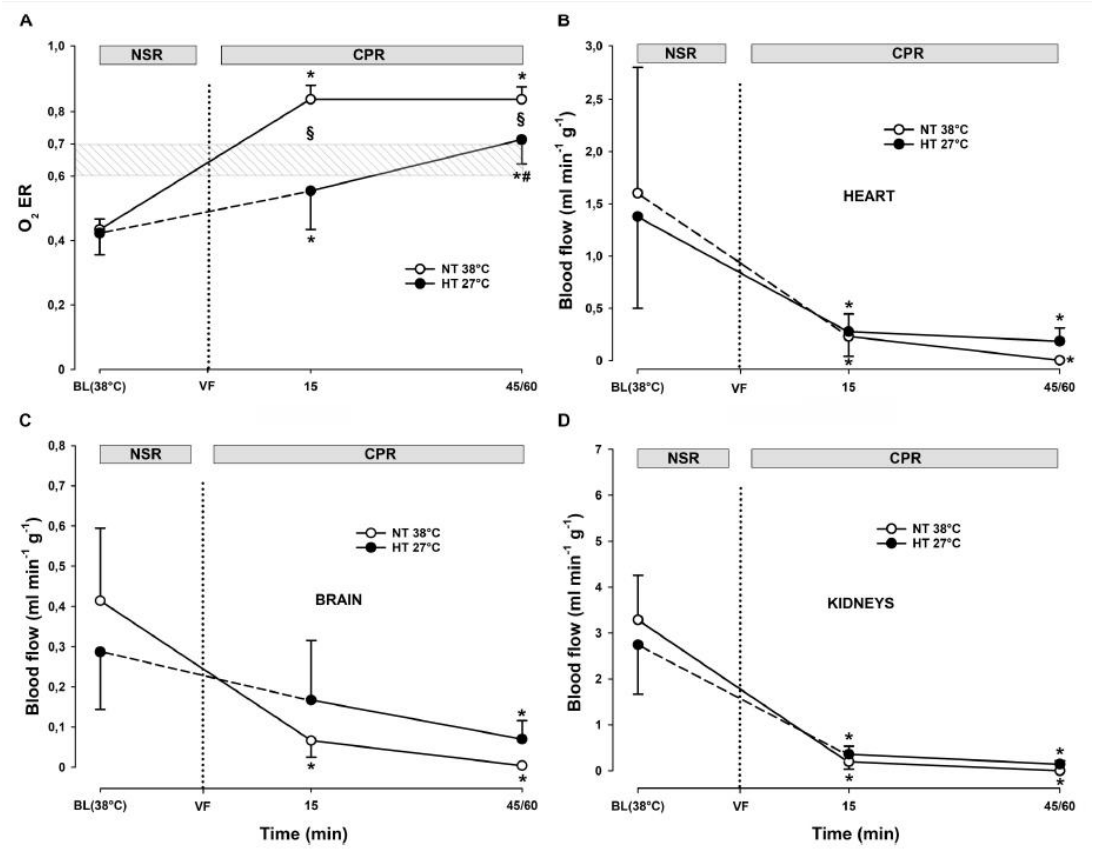

Figure 20 *- Oxygen extraction ratios and regional blood flow during CPR at normothermia and hypothermia.* ***A)*** *Oxygen extraction ratios (O_2 ER),* ***B-D)*** *regional blood flow in the heart, brain, and kidneys. CPR at normothermia (NT 38°C, n = 4), CPR at hypothermia (HT 27°C, n = 7). 38°C baseline (BL(38°C)), normal sinus rhythm (NSR), cardiopulmonary resuscitation (CPR), ventricular fibrillation (VF). Values are mean ± SD. * - p < 0.05 vs. intragroup 38°C baseline; # - p < 0.05 vs. intragroup 15 min of CPR; § - p < 0.05 vs. corresponding value between groups*

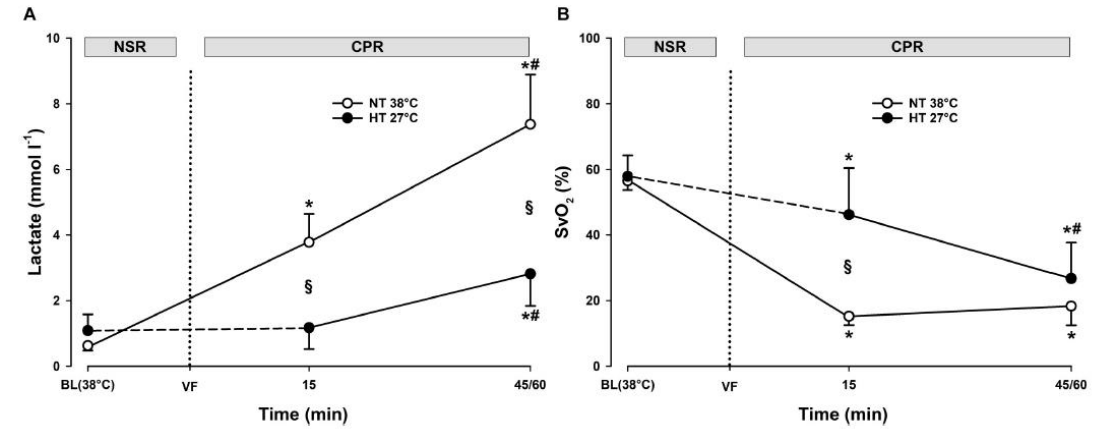

Figure 21 *- Lactate and mixed venous oxygen saturation during CPR at normothermia and hypothermia.* ***A)*** *Lactate and* ***B)*** *mixed venous oxygen saturation (SvO2). CPR at normothermia (NT 38°C, n = 4), CPR at hypothermia (HT 27°C, n = 7). 38°C baseline (BL(38°C)), normal sinus rhythm (NSR), cardiopulmonary resuscitation (CPR), ventricular fibrillation (VF). Values are mean ± SD. * - p < 0.05 vs. intragroup 38°C baseline; # - p < 0.05 vs. intragroup 15 min of CPR; § - p < 0.05 vs. corresponding value between groups.*

8.2.3 Cooling to 27°C under spontaneous circulation

Hemodynamics

Cooling to 27°C led to an almost linear decrease in CO and MAP (Figure 22 A, B). CO was reduced by 50% at 27°C, from 3,2 to 1,6 l/min, whereas MAP was decreased by 33%, from 89 to 60 mmHg.

Oxygen transport

DO_2 and VO_2 (Figure 22 C) were significantly reduced during cooling, DO_2 from 13,7 to 7,1 ml/kg/min (-48%) and VO_2 from 5,7 to 1,8 ml/kg/min (-68%). This resulted in an O_2ER of 0,26 (Figure 22 D), well below the critical threshold of 0,6-0,7(151).

Regional blood flow

Cooling to 27°C led to a reduction in blood flow to all organs measured; these changes were not statistically significant (Figure 23).

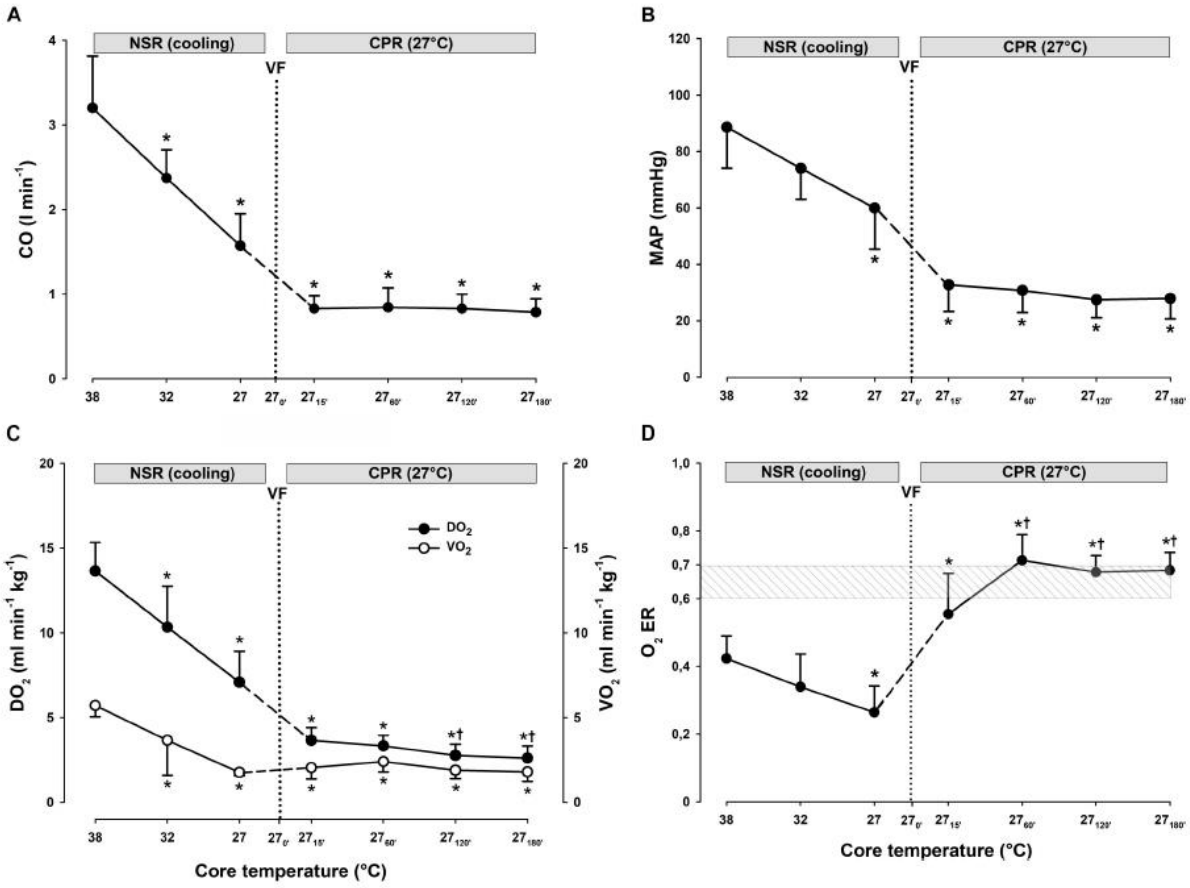

Figure 22 *- Hemodynamic function, global O_2 transport, global O_2 uptake, and O_2 extraction ratio during cooling and 3 h of CPR at 27°C (n = 7).* ***A)*** *Cardiac output (CO),* ***B)*** *mean arterial pressure (MAP),* ***C)*** *global oxygen delivery (DO_2), and global oxygen consumption (VO_2), and* ***D)*** *oxygen extraction ratio (O_2 ER). Normal sinus rhythm (NSR), cardiopulmonary resuscitation (CPR), ventricular fibrillation (VF). Values are mean ± SD. * - p < 0.05 vs. intragroup 38°C baseline; † - p < 0.05 vs. intragroup 15 min of CPR at 27°C.*

8.2.4 Effects of 3 hours CPR at 27°C

Hemodynamics

Compared to spontaneous circulation at 38°C, 15 minutes of CPR at 27°C led to significant reductions in CO and MAP (Figure 22 A, B). CO was reduced from 3,2 to 0,8 l/min (-74%), and MAP from 89 to 33 mmHg (-63%). These variables remained unchanged at the reduced levels throughout the 3 hour CPR period.

Oxygen transport

For the duration of the CPR period, the differences between DO_2 and VO_2 (Figure 22 C) were diminished. Compared to their corresponding values at 15 minutes of CPR, these values were significantly reduced after 2 hours. After 1 hour, the O_2ER was 0,71, thus reaching the critical value, ER_{crit}, of 0,6-0,7 (Figure 22 D). The O_2ER remained at or near the critical threshold for the remainder of the CPR period. S_VO_2 was reduced from 75% to 46% at 15 minutes CPR, further to 27% at 1 hour, and remained unchanged for the remainder of the CPR period (Figure 24). After 1 hour of CPR, lactate was significantly raised from 0,8 to 2,81 mmol/l. Lactate had a linear increase during the CPR period; at 3 hours, it was 5.56 mmol/l.

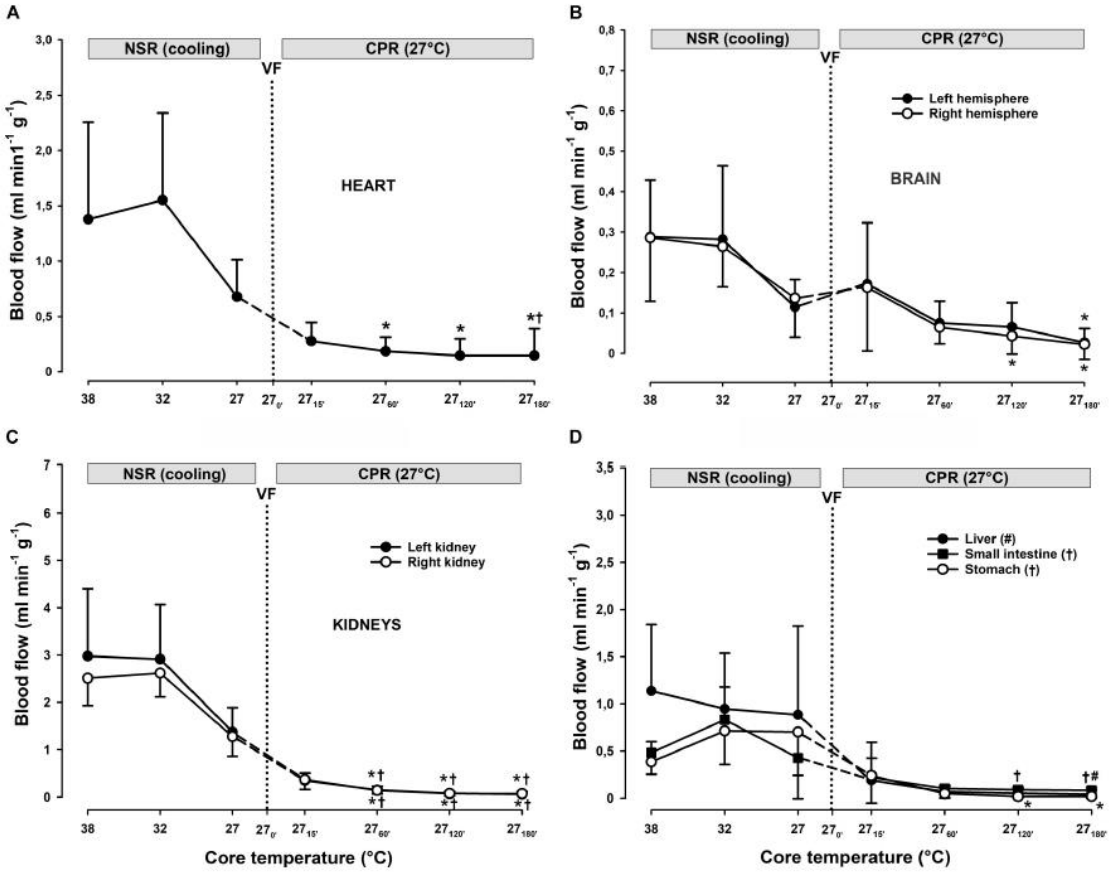

Figure 23 *- Regional blood flow during cooling and 3 hours CPR at 27°C (n = 7).* ***A)*** *Coronary blood flow,* ***B)*** *Cerebral blood flow,* ***C)*** *Renal blood flow, and* ***D)*** *Splanchnic blood flow. Normal sinus rhythm (NSR), cardiopulmonary resuscitation (CPR), ventricular fibrillation (VF). Values are mean ± SD. * - p < 0.05 vs. intragroup 38°C baseline; #, † - p < 0.05 vs intragroup 15 min of CPR at 27°C.*

Regional blood flow

Compared to values at 15 minutes CPR, coronary blood flow (Figure 23 A) remained unchanged in the first 2 hours of CPR but was significantly reduced at 3 hours, with blood flow deteriorating from 0,3 ml/g/min to 0,1 ml/g/min. Blood flow to both brain hemispheres (Figure 23 B) remained unchanged at 1 hour of CPR compared to 15 minutes. At the 2 hour mark, blood flow to the right hemisphere was significantly reduced, and at 3 hours, a significant reduction in blood flow to both hemispheres was seen. Renal blood flow was significantly reduced after 1 hour of CPR (Figure 23 C). Blood flow to the small intestine was significantly reduced after 2 hours CPR, to the liver and stomach after 3 hours CPR (Figure 23 D).

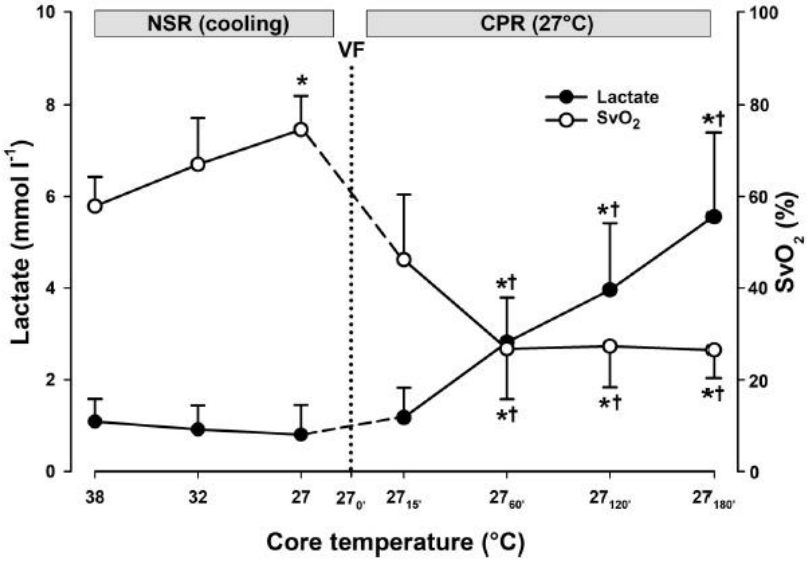

Figure 24 *- Lactate and mixed venous oxygen saturation during cooling and 3 hours CPR at 27°C (n = 7). Mixed venous oxygen saturation (SvO_2), normal sinus rhythm (NSR), cardiopulmonary resuscitation (CPR), ventricular fibrillation (VF). Values are mean ± SD. * - p < 0.05 vs. intragroup 38°C baseline; † - p < 0.05 vs. intragroup 15 min of CPR at 27°C.*

8.3 Study 3

Effects of rewarming with extracorporeal membrane oxygenation to restore oxygen transport and organ blood flow after hypothermic cardiac arrest in a porcine model.

With a basis in the results from study 2, indicating partially sustained aerobic metabolism, a group of pigs were rewarmed on ECMO following immersion cooling and 3 hours of CPR at 27°C. Hemodynamics, oxygen transport and regional blood flow during 3 hours of hypothermic CPR and the effects of ECMO rewarming on these variables were examined.

8.3.1 Main results

Rewarming on ECMO restored hemodynamics (CO and MAP), as well as oxygen transport. ECMO restored blood flow to the heart and parts of the brain. Some abdominal organs displayed significantly reduced blood flow during rewarming.

8.3.2 Immersion cooling and 3 hours CPR at 27°C

No statistically significant differences between animals in variables recorded at the start of the experiments were found. All animals had spontaneous circulation during cooling to 27°C. None of the pigs died during the experiment.

Hemodynamics

CO and MAP were significantly reduced during cooling and CPR (Figure 25). CO was reduced from 3,6 to 1,2 l/min (-56%) during cooling, fell to 0,9 l/min at 15 minutes CPR and persisted at this level during 3 hours of CPR. MAP was reduced from 88 to 76 mmHg (-24%) during cooling, fell to 38 mmHg (-58%) at 15 minutes CPR and remained at this level during the CPR period.

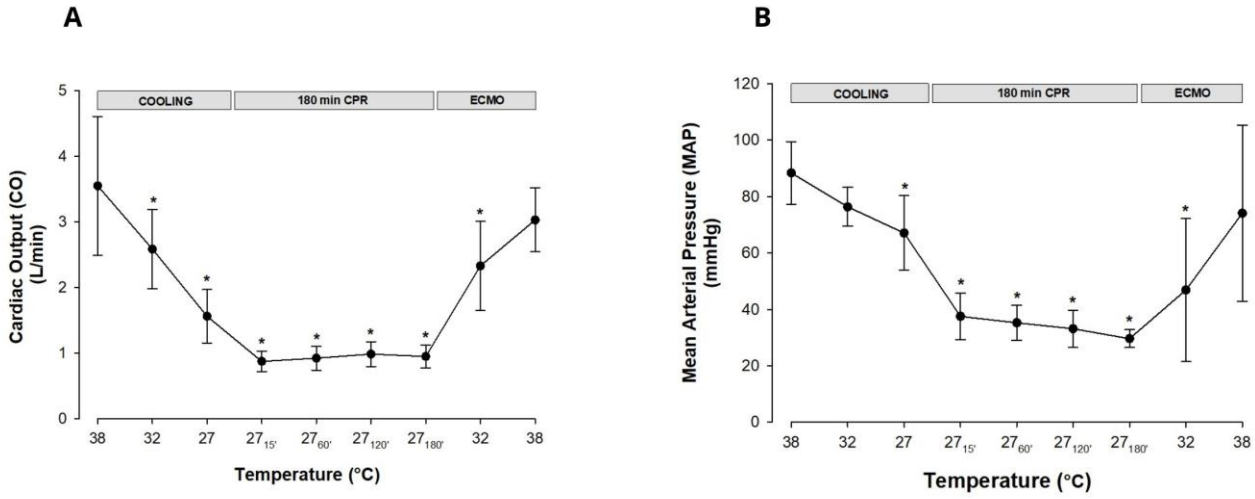

Figure 25 *– Hemodynamic variables during cooling, 3 hours CPR at 27°C and ECMO rewarming.* ***A)*** *Cardiac output (CO) and* ***B)*** *Mean arterial pressure (MAP). Cardiopulmonary resuscitation (CPR); Extracorporeal membrane oxygenation (ECMO). Values are mean ± SD. * - p < 0.05 vs. 38°C baseline.*

Oxygen transport

During cooling, DO_2 and VO_2 levels decreased significantly globally (Figure 26 A) and in the brain (Figure 26 B). Global DO_2 was decreased from 15,2 to 6,8 ml/min/100g (-55%) during cooling to 27°C, whereas VO_2 was reduced from 6,8 to 1,7 ml/min/100g (-75%). At 15 minutes of CPR, DO_2 was reduced to 4,3 ml/min/100g (-72%) and VO_2 to 2,2 ml/min/100g (-68%); both variables remained at this low level during the CPR period. The global O_2ER (Figure 26 C) was significantly decreased during cooling to 27°C, rose to 0,68 at 1 hour CPR and thereafter reached the critical extraction value of 0,7 needed to support aerobic metabolism (151). The cerebral O_2ER (Figure 26 C) was not significantly raised during CPR and stayed within the critical limit of 0,7 for the duration of the experiment. pH (Figure 26 D) showed an almost linear decline from 7,55 to 7,2 at 3 hours of CPR, lactate levels increased from 0,98 to 5,18 mmol/l, and S_VO_2 fell from 57 to 21%. Blood gas values, intracranial pressure (ICP) and central venous pressures are summarized in table 2.

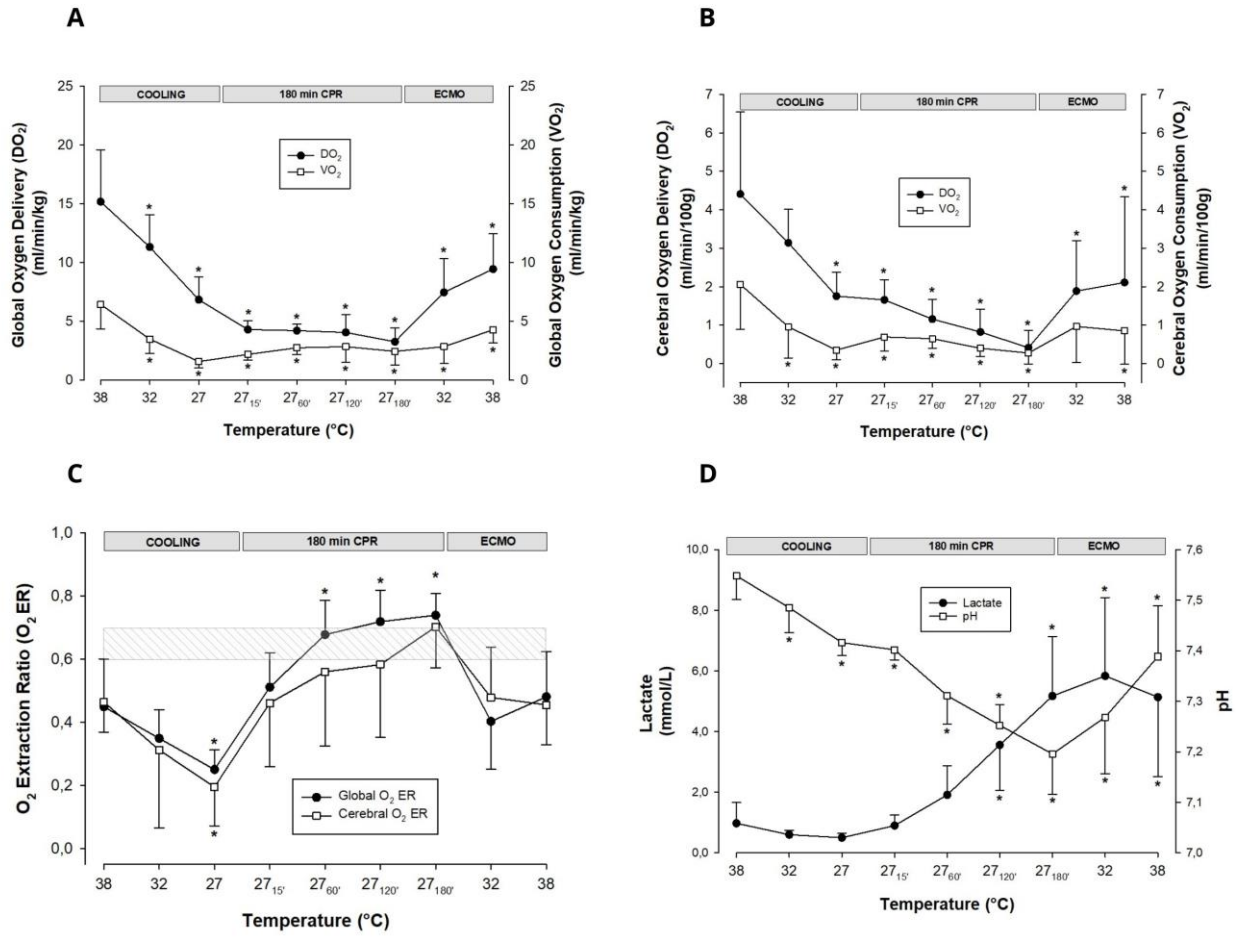

Figure 26 *– **A)** Global oxygen delivery (DO_2) and consumption (VO_2), **B)** Cerebral oxygen delivery ($DO_{2cerebral}$) and consumption ($VO_{2cerebral}$), **C)** Oxygen extraction ratios and **D)** pH and lactate levels during cooling, 3 hours CPR at 27°C and ECMO rewarming. Values are mean ± SD. * - p < 0.05 vs. intragroup 38°C baseline.*

	38°C	27°C	27°C$_{15\ min}$	27°C$_{3\text{-}h}$	RW 32°C	RW 38°C
pH	7.55 ± 0.05	7.42 ± 0.03*	7.4 ± 0.02*	7.20 ± 0.08*	7.27 ± 0.11*	7.39 ± 0.1*
Hb (g/dL)	8.2 ± 1.1	8.7 ± 1.2	9.2 ± 1.3	7.9 ± 0.9	5.5 ± 1.3*	5.3 ± 1.6*
Hct (%)	27 ± 2	27 ± 4	29 ± 4	25 ± 4	16 ± 4*	17 ± 5*
Lactate (mmol/l)	1.0 ± 0.7	0.5 ± 0.1	0.9 ± 0.3	5.2 ± 2.0	5.8 ± 2.6*	5.1 ± 2.6
BE (mmol/l)	5.6 ± 2.7	3.3 ± 3.5	2.2 ± 1.6*	-6.3 ± 2.4*	-8.9 ± 4.0*	-8.2 ± 3.3*
HCO_3- (mmol/l)	30 ± 3	27 ± 3	26 ± 1*	18 ± 2*	20 ± 2*	20 ± 3*
K^+ (mmol/l)	3.3 ± 0.4	2.6 ± 0.4	3.1 ± 0.3	5.3 ± 1.5*	4.5 ± 0.4	4.5 ± 0.7
PaO_2 (kPa)	13 ± 5	16 ± 4	46 ± 28*	15 ± 13	73 ± 3*	66 ± 2*
$PaCO_2$ (kPa)	4.4 ± 0.3	5.8 ± 0.8	5.8 ± 0.6	7.5 ± 1.5*	5.2 ± 1.9	3.7 ± 0.9
SaO_2 (%)	99 ± 2	99 ± 1	100 ± 0	82 ± 26	100 ± 0	100 ± 0
SvO_2 (%)	60 ± 11	78 ± 4	52 ± 15	21 ± 8*	68 ± 17	61 ± 17
SvO_2 jug.bulb (%)	58 ± 12	85 ± 13*	66 ± 21	26 ± 12*	59 ± 18	66 ± 12
CVP (mmHg)	6 ± 1	5 ± 2	19 ± 17*	14 ± 7	13 ± 6	15 ± 4
ICP (mmHg)	14 ± 3	13 ± 6	21 ± 5*	17 ± 4	17 ± 4	22 ± 7*
CPP (mmHg)	82 ± 11	62 ± 12	17 ± 9*	13 ± 5*	32 ± 21*	52 ± 32

Table 2 *- Blood gas variables, intracranial pressure (ICP) and central venous pressure (CVP) values (n=8). Values are mean ± SD. * - p < 0,05 vs. 38°C baseline.*

Regional blood flow

Coronary blood flow (Figure 27 A) was significantly reduced during cooling to 27°C, from 153 to 71 ml/min/100g (-54%). After 15 minutes of CPR, the coronary flow was further reduced to 24 ml/min/100g and remained at this level during 3 hours of CPR. Blood flow to the temporal lobes (Figure 27 B) declined significantly during cooling, from 35 to 12 ml/min/100g (-66%) in the right temporal lobe and from 32 to 16 ml/min/100g (-50%) in the left. After 15 minutes of CPR, flow to the temporal lobes was further reduced to 10 ml/min/100g (-68%) in the right and 13 ml/min/100g (-64%) in the left lobe. After 3 hours of CPR, blood flow to the temporal lobes was reduced by 87% in the right and 86% in the left lobe. Cerebellar blood flow (Figure 27 C) shows the same pattern as the temporal lobes. Cooling to 27°C led to a reduction in flow, from 42 to 14 ml/min/100g (-67%) in the left and from 41 to 16 ml/min/100g in the right cerebellum. After 3 hours of CPR, flood flow was reduced by 86% in the left and 87% in the right cerebellum, similar to the temporal lobes. Regional blood flow values are summarized in table 3.

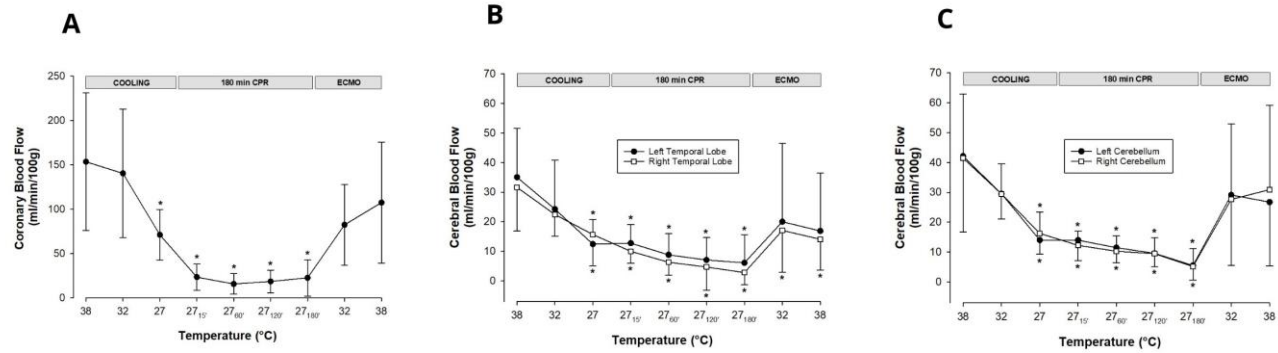

Figure 27 *- Regional blood flow values during cooling and 3 hours CPR at 27°C.* ***A)*** *coronary blood flow.* ***B)*** *blood flow to the temporal lobes and* ***C)*** *blood flow to the cerebellum. Values are mean ± SD. * - p < 0.05 vs. intragroup 38°C baseline.*

The abdominal organs showed a varying response to cooling and CPR. Renal blood flow (Figure 28 A) was significantly reduced during cooling and CPR. The flow was reduced by 44% in the right and 43% in the left kidney. After 3 hours of CPR, renal blood flow was reduced by 97% in the right and 96% in the left kidney. Blood flow to the spleen (Figure 28 B) declined significantly during cooling (-66%) and CPR (-99%). Hepatic blood flow was unaltered by cooling to 27°C but remained significantly reduced during CPR (-97%). Blood flow to the stomach and small intestine (Figure 28 C) initially increased by 38% and 23% at the start of cooling but was significantly reduced after 1 hour of CPR. After 3 hours of CPR, blood flow to the stomach was reduced by 90% and the small intestine by 79%.

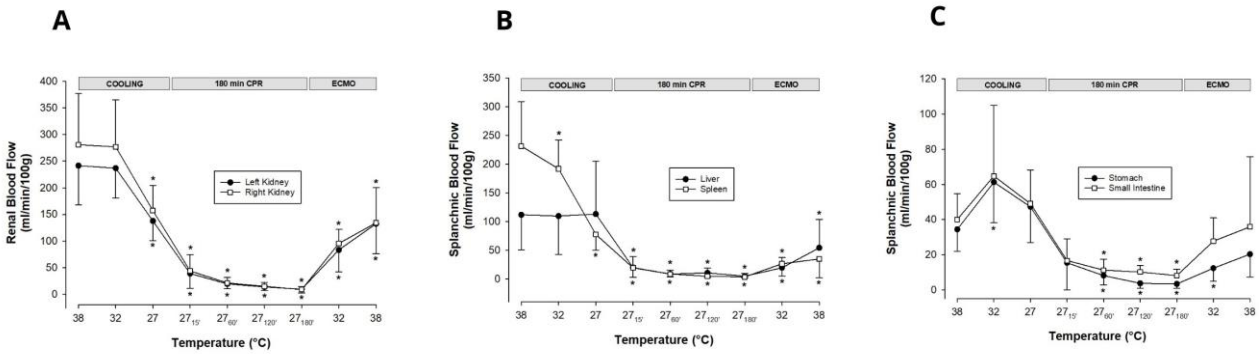

Figure 28 *- Regional blood flow to abdominal organs during cooling and 3 hours CPR at 27°C.* ***A)*** *kidneys,* ***B)*** *liver and spleen, and* ***C)*** *stomach and small intestine. Values are mean ± SD. * - p < 0.05 vs. intragroup 38°C baseline.*

	38°C	27°C	27°C$_{15\ min}$	27°C$_{3\text{-}h}$	RW 32°C	RW 38°C
Heart	153 ± 78	71 ± 28*	24 ± 15*	23 ± 20*	82 ± 46	107 ± 68
Left temp. lobe	35 ± 17	12 ± 7*	12 ± 6*	6 ± 9*	20 ± 26	17 ± 20
Right temp. lobe	32 ± 15	16 ± 5*	10 ± 4	3 ± 4*	17 ± 14*	14 ± 10*
Left cerebellum	42 ± 21	14 ± 5*	14 ± 3*	6 ± 6*	29 ± 24	27 ± 21
Right cerebellum	41 ± 24	16 ± 7*	12 ± 5*	5 ± 4*	27 ± 22	31 ± 28
Left kidney	242 ± 74	138 ± 37*	39 ± 28*	9 ± 7*	83 ± 41*	132 ± 56*
Right kidney	281 ± 96	157 ± 47*	44 ± 31*	9 ± 5*	95 ± 26*	134 ± 66*
Liver	112 ± 61	113 ± 92	20 ± 19*	5 ± 5*	19 ± 14*	54 ± 49*
Stomach	34 ± 13	47 ± 20	15 ± 15	3 ± 3*	12 ± 7*	20 ± 13
Small intestine	40 ± 15	49 ± 19	17 ± 12	8 ± 4*	28 ± 13	36 ± 40
Spleen	231 ± 77	78 ± 28*	19 ± 16*	4 ± 1*	26 ± 11*	35 ± 32*

***Table 3** – Organ bloow flow (ml/min/100 g). (n=8). Values are mean and SD. * - p < 0,05 vs. 38°C baseline.*

8.3.3 ECMO rewarming

All pigs were rewarmed to 38°C. Mean rewarming times were 17 minutes to a temperature of 32°C, and 45 minutes to a temperature of 38°C.

Hemodynamics

Between 2000 and 6000 ml (mean 3750ml) Ringer's solution was added to the ECMO circuit to maintain venous access pressure above -100 mmHg. All 8 pigs achieved sinus rhythm on ECMO: one spontaneously at 29°C, two after cardioversion at 32°C, two after cardioversion at 38°C and three after sternotomy, pericardiotomy and internal cardioversion at 38°C.

ECMO flow was adjusted to mimic the hemodynamic characteristics of the individual animal during cooling. At 27°C, it was started at 0,8-1 l/min and gradually increased alongside core temperature to 2-2,5 l/min at 32°C and 3-3,5 l/min at 38°C. CO and MAP were restored in all animals (Figure 25).

Oxygen transport

Global DO_2 (Figure 26 A) increased during ECMO rewarming but was at 38°C still significantly decreased from baseline, 15,2 vs 9,4 ml/min/100g (-38%). Global VO_2 (Figure 26 A) showed similar characteristics, significantly reduced at 38°C vs baseline, 6,8 vs 4,3 ml/min/100g (-34%). Cerebral DO_2 and VO_2 (Figure 26 B) increased during rewarming, but were still significantly reduced at 38°C compared to baseline: $DO_{2cerebral}$ 4,41 vs 2,11 ml/min/100g (-50%), $VO_{2cerebral}$ 2,08 vs. 0,85 ml/min/100g (-56%). Global and cerebral O_2ER (Figure 26 C) fell below 0,7 during rewarming and were at 38°C unchanged from baseline values.

After rewarming to 38°C, arterial pH (Figure 26 D) was restored: 7,55 at baseline vs 7,39 after rewarming. Serum lactate peaked during rewarming at 32°C, at 5,8 mmol/l, displayed a declining tendency, but was at 38°C still significantly elevated at 5,1 compared to the baseline value of 0,98 mmol/l. S_VO_2 was restored to 60% after rewarming.

Regional blood flow

Rewarming led to increased blood flow in all organs measured, but statistical analysis showed restoration of blood flow in only a few organs. Flow values are summarised in table 3. Coronary blood flow (Figure 27 A) was restored. Blood flow to the right temporal lobe (Figure 27 B) was significantly reduced after rewarming, whereas flow to the left temporal lobe was restored. Flow to both cerebellar hemispheres (Figure 27 C) was restored. In most abdominal organs, rewarming led to partial restoration of blood flow. Blood flow to the stomach and small intestine was restored (Figure 28 C). After rewarming, renal blood flow (Figure 28 A) was significantly reduced compared to baseline, in the left kidney 242 vs 132 ml/min/100g (-45%), and in the right 281 vs 134 ml/min/100g (-53%). Blood flow to the liver (Figure 28 B) and spleen was significantly reduced after rewarming, the liver 112 vs 54 ml/min/100g (-52%), and the spleen 231 vs 35 ml/min/100g (-82%).

Plasma biomarkers

Three hours of CPR led to a significant increase in Aspartate aminotransferase (ASAT), which remained after rewarming. The level of glial fibrillary acidic protein (GFAP) was increased after rewarming. The plasma biomarkers are summarized in table 4.

	38°C	**27°C**	**27°C$_{3\text{-h}}$**	**RW 38°C**
Brain				
S100β (pg/ml)	64 ± 44	40 ± 28	85 ± 56	89 ± 53
UCHL1 (pg/ml)	91 ± 12	108 ± 48	92 ± 9	95 ± 15
GFAP (pg/ml)	8.8 ± 5.6	8.4 ± 5.1	9.8 ± 4.9	14.7 ± 8.0*
NSE (ng/ml)	-	-	-	-
Heart				
CK-MB (ng/ml)	1.7 ± 1.9	2.0 ± 2.1	1.0 ± 0.8	1.2 ± 1.0
Troponin T (pg/ml)	65.8 ± 51.2	54.1 ± 47.2	47.4 ± 39*	49.2 ± 46.5*
Kidney				
Carbamide (mmol/l)	1.6 ± 0.3	2.0 ± 0.5	2.4 ± 0.6*	2.2 ± 0.7*
Creatinine (μmol/l)	57.1 ± 7.8	49.3 ±11.3*	61.6 ± 12.5	47.4 ± 9.9*
Activin-A (pg/ml)	-	-	-	-
Liver/pancreas				
ASAT (U/l)	40.6 ± 5.3	51.4 ± 9.6	264.0 ± 81.6*	374.1 ± 226.5*
ALAT (U/l)	72.6 ± 16.8	67.2 ± 15.1*	70.4 ± 12.6	55.7 ± 12.1*
γ-GT (U/l)	31.1 ± 9.4	27.1 ± 6.8	22.8 ± 4.8*	15.4 ± 5.2*
Amylase (U/l)	1744 ± 562	1591 ± 478	1359 ± 466*	879 ± 352*
Lipase (U/l)	-	-	-	-
Bilirubin (μmol/l)	-	-	-	-
ALP (U/l)	-	-	-	-

***Table 4** – serum biomarkers for organ function and organ injury. UCHL1, Ubiquitin Carboxyl-terminal Esterase-L1; GFAP, Glial Fibrillary Acidic Protein; NSE, Neuron-Specific Enolase; CK-MB, Creatine Kinase-Muscle/Brain isozyme; ASAT, Aspartate Aminotransferase; ALAT, Alanine; Aminotransferase; γ-GT, γ-Glutamyl Transpeptidase; ALP, Alkaline Phosphatase. (n = 8). Values are mean and SD. *p < 0.05 vs. 38°C baseline. - indicate serum level below level of detection.*

9 Discussion

9.1 Effects of cooling

Hypothermia comes with pathophysiologic mechanisms that cause vasoconstriction and reduced blood pressure and CO (37). In all three studies, the hemodynamic variables show a declining trend during cooling. In study 1, a reduction in HR, CO and MAP is seen, similar to other studies in rats by our group (31,56). In studies 2 and 3, MAP is reduced by 33 and 24%, and CO is reduced by 50 and 56%, respectively. This corresponds well with previous studies done by our group (32). The hemodynamics and regional flow values are intercomparable between the two studies.

All organs measured in studies 2 and 3 displayed flow reductions in response to cooling, except for the liver, stomach and small intestine, in which flow was unchanged or increased. These organ blood flow values are comparable to those in our previous study, where spontaneous circulation was maintained for 3 hours at 27°C (32).

Other animal studies show similar trends in organ blood flow compared to studies 2 and 3. Su et al. studied regional blood flow in monkeys subjected to progressive cooling to 20°C (152). They showed diminished flow to the heart, brain and kidneys, whereas splanchnic flow increased at the start of cooling. Cerebral blood flow was reduced by 28% at 30°C and by 30% at 25°C. Rosomoff and Holaday studied anaesthetised dogs, where progressive cooling showed an almost linear reduction in cerebral blood flow with a decrease of about 60% at 26°C (153). Two studies on neonatal pigs have shown reduced cerebral blood flow as a response to systemic cooling. Okubo et al. studied cerebral blood flow in newborn piglets and found that brain blood flow at 32°C was only 41% of blood flow at 39°C (154). Busija et al. did a similar study in newborn piglets showing a 40-50% reduced cerebral blood flow at 34-35°C (155). These studies show that newborn animals seem to have more severe reductions in cerebral blood flow as a response to cooling. We used juvenile pigs in studies 2 and 3, yet our data showed cerebral blood flow to be unchanged at 32°C, with a decrease of about 50% at 27°C. Our data seem to correlate well with findings from studies in mature animals.

9.2 CPR during hypothermia

The European Resuscitation Council 2015 guidelines state that accidental hypothermia victims in cardiac arrest should receive immediate CPR, which should be continued during evacuation and transport (24). The guidelines advocate automated CPR devices in special situations, for instance, in rescue helicopters. During transport, a limited number of people are available to perform CPR in a cramped environment, and rescuer fatigue (117) would quickly be an issue. However, there is still an ongoing debate whether mechanical CPR devices are superior to manual CPR (119,147,156). If continuous CPR is not possible, the 2015 guidelines advocate the use of intermittent CPR, 5 minutes of CPR with hands-off time < 5minutes in patients with core temperatures > 20°C (157). Our data show that prolonged CPR is feasible during hypothermia, but when looking at the O_2ER during hypothermic CPR, the margin against anaerobic metabolism is narrow. Data from our study support continuous CPR and should encourage mechanical CPR devices in adverse environments.

Different CPR techniques have been explored in experimental pig models to optimize pressure generation and organ blood flow during normothermia (103,104,158). The techniques explored are

active compression-decompression CPR with or without an impedance threshold valve compared to standard CPR. These studies consistently report better pressure and blood flow generation during active compression-decompression, attributed to the negative intrathoracic pressure between compressions, which facilitates blood flow to the heart. Mechanical chest compression devices often offer active decompression-compression, as did the one we used in our experiments. However, due to the shape of the pig's chest, the degree of active decompression was minimal, as this depends on a suction cup. The LUCAS device is designed for human use, and one might speculate that its intended use generates better CO and organ flow, providing more available oxygen for tissues.

Several studies have used pigs to study normothermic CPR, where most of the recorded data were captured during the first 10 minutes of CPR (159,160). In studies 2 and 3, the first data were collected after 15 minutes. Our data are comparable with those of Steen et al. (146), reporting a 45 % decrease in MAP and a 73 % decrease in CO after 5 minutes of CPR. Forty-five minutes of CPR during normothermic conditions is well past the point of termination in most clinical situations (109), and few other researchers have followed CPR this long. Carretero et al. reported a similar reduction (74 %) of CO after 15 min of CPR, but the reduction in CO after 45 min was less (83 % vs our 93 %)(161). Wik et al. did a CPR study on pigs where they cycled through different CPR methods with varying compression depths and modes with active decompression (103). They found pre-arrest blood flow rates in the heart and brain comparable to our data. They did not present their data along a time axis, but the total CPR time was 24±1 minutes. In their study, the reduction in blood flow to the brain and heart is comparable to our data at 15 minutes CPR. Sunde et al. did a similar CPR study on pigs using different active decompression and cycling through different compression rates, 60 – 90 – 120 compressions/minute (104). Their total CPR time was 16±1 minutes. They show pre-arrest organ blood flow rates that are comparable to our data. At 90 compressions/minute, the heart and blood flow rates reported are higher than our data at 15 minutes. The reason for this is probably a combination of using active decompression and a shorter duration of CPR at the time of measurement, as the study by Wik et al. showed myocardial and brain blood flow to increase by 53 and 37%, respectively, when using active decompressions (103). However, our blood flow data from the brain and heart after 15 minutes of CPR are comparable to studies by Gervais et al. (110) and Moore et al. (162), which measure blood flow in organs at 15 minutes CPR.

A study on pigs cooled to 28°C and then to cardiac arrest at around 22°C followed by resuscitation with a chest compression device showed reductions in hemodynamic variables and blood flow rates comparable to our data from studies 2 and 3 (163). MAP is reduced by 75% with values after 20 minutes of CPR at 26,1 mmHg, similar to our findings. In the referred study, basal organ flows are higher than our data. Still, the blood flow reductions after 20 minutes of CPR correlates well with our data, specifically 13% of basal cerebral flow, 7% of basal myocardial flow, 5% of renal and hepatic blood flow, and 14% of basal flow to the small intestine. The reduced organ blood flow seen in studies 2 and 3 are not as severe, but the trends are comparable. One must consider that the referred study allowed the pigs to breathe spontaneously during cooling from 28°C until cardiac arrest at around 22°C, and this can thoroughly explain the more pronounced reductions in organ blood flow seen. In pilot studies, the pigs we used went into spontaneous cardiac arrest between 26 and 27°C; this is most likely due to physiological differences between different breeds of pigs. These physiological differences may also explain the differences in organ blood flow.

9.3 Oxygen transport and metabolism during cooling, 3 hours CPR at 27°C and rewarming

During normal resting conditions, DO_2 exceeds VO_2 to a great extent, thereby making VO_2 largely independent of delivery to secure aerobic organ metabolism. When oxygen delivery is limited, VO_2 will eventually become dependent on DO_2. The O_2ER is a valuable tool to visualise the oxygen deficit, forcing the body to switch to anaerobic metabolism. With declining DO_2, this ratio approaches a critical value (O_2ER_{crit}), where the oxygen supply rules tissue oxygen consumption (39). In normothermia, the O_2ER_{crit} is reported to be 0,6-0,7 (151). During hypothermia, the critical value is reported to be reduced (≈ 0.65), perhaps due to increased vascular tone, which may interfere with local feedback, leading to changes in blood flow distribution (43).

In study 2, we found a simultaneous reduction in DO_2 and VO_2 during cooling, similar to what we have reported in other studies (33,71). The O_2ER balances on the critical threshold during the 3 hours of hypothermic CPR; it barely exceeds this threshold at 1 hour of CPR but is within limits at 2 and 3 hours. In a recent study in the pig model, we documented hemodynamics, oxygen transport and regional blood flow during cooling to 27°C, 3 hours hypothermia with spontaneous circulation and subsequent rewarming (32). DO_2 and VO_2 between these two studies are similar and show the same trends during cooling. During hypothermia with spontaneous circulation, the global O_2ER approached critical values, indicating a marginal oxygen supply during stable hypothermia. This implies that the physiologic increase in oxygen extraction in situations with deficiencies in delivery works in hypothermic conditions; this has also been documented in other animal species (31). Based on our findings, we suggest that oxygen consumption in the hypothermic CPR group is use-dependent. In support of our suggestion, we documented a moderate elevation of lactate levels to 5,56 mmol/l at 3 hours of CPR and a reduction of SvO_2 to 27%. This supports the notion that 3 hours of CPR at 27°C provides marginal but sufficient oxygen transport for vital organs. In contrast, the O_2ER in the normothermic CPR group exceeds the critical threshold after just 15 minutes of CPR, lactate levels approached 8 mmol/l at 45 minutes of CPR, indicating the existence of supply-dependent oxygen consumption and inability to sustain aerobic metabolism.

In study 3, we demonstrate similar findings as in study 2: during CPR, there is a rise in the O_2ER towards and slightly above the critical value of 0,7. We demonstrated DO_2 means during hypothermic CPR between 3,2 and 4,3 ml/kg/min. For comparison, a study in dogs during exsanguination found the critical DO_2 at 34°C to be 5,6 ml/kg/min (43). A study on hypothermic and normothermic sheep on cardiopulmonary bypass found critical oxygen delivery rates at 3,17 ml/kg/min at 33°C (164). Extrapolating the results from the studies mentioned, we speculate that we are balancing on the edge of critical oxygen delivery rates during the hypothermic CPR period in our experiments. A study on progressively hypoxic pigs at 29°C found that critical oxygen delivery rates correlated well with an acute rise in lactate levels (41). The referred study found normoxic lactate levels at 29°C to be 3,16 mmol/l. Critical DO_2 during hypothermia was found to be 5,2 ml/kg/min and critical S_vO_2 to be 29,7%. Our data from study 3 show lactate levels of 5,8 mmol/l and S_vO_2 of 20,6% at 3 hours CPR. A moderately elevated lactate but a more severely impaired S_vO_2 strengthen our speculations that aerobic metabolism is at least partially supported.

When discussing outcomes of patients with cardiac arrest, our main interest is not only survival, but more importantly, neurological function and health-related quality of life (165). In this regard, the main question in any resuscitation scenario is if the hypoxic period has been short enough for the brain to survive without significant sequelae. Nozari et al. did a study on dogs divided into four groups and subjected to 20+20 minutes of CPR, with two groups being cooled to 34 or 27°C via veno-venous extracorporeal shunt during the last 20 minutes of CPR (94). They found that in the two normothermic groups, dogs remained unconscious, whereas the animals subjected to hypothermia regained consciousness and had normal behavioural scores. The experiments in studies 2 and 3 involve surgical procedures so severe that the animals had to be euthanised after the conclusion of the experimental protocol. We, therefore, have no behavioural scale to assess brain function in animals. Data from study 2 show significantly reduced flow to the right cerebral hemisphere after 2 hours of CPR and left hemisphere after 3 hours. Data from study 3 show the same trends during CPR as study 2, but also that upon rewarming, blood flow is restored to the cerebellum and the left temporal lobe. Cerebral O_2ER is elevated during CPR but lower than global O_2ER, just barely exceeding the critical value of 0,7 after 3 hours of CPR and normalising during rewarming. ECMO rewarming led to the restoration of both global and cerebral O_2ER, with values well below ER_{crit}. Rewarming increased S_VO_2 to 61%, and lactate showed a declining trend. We interpret this as indicative of a sufficient DO_2 to meet the metabolic demands of organs during and after rewarming. Biomarkers of brain injury showed no pathological changes as GFAP and UCHL1, which are highly selective of central nervous system (CNS) injury(166), were within normal control levels in pigs(50). However, the question of neurologic function remains unanswered throughout our experiments.

9.4 Volume substitution during rewarming from hypothermia

In several studies, our group has shown that rewarming rats from hypothermia is complicated by hypothermia-induced cardiac dysfunction, with decreased SV and CO (77,132,169). In study 1, the low SV and CO were counteracted by intravenous fluids, which raised SV and CO in both groups. A sustained effect was only seen in the dextran group, whereas the crystalloid-treated group showed increased CO and SV only during the infusion period.

Crystalloid solutions, like saline, are rapidly redistributed to the extracellular compartment, with only 25% of infused saline present in the vascular space after one hour (168) and around 10% of Ringer's solution present in plasma after 3 hours (169). During hypothermia, capillary integrity is impaired(77), aggravating fluid redistribution(89). On the other hand, colloids tend to stay longer in the vascular space, with most of the infused volume present after one hour (168). In clinical practice, patients presenting with hypovolemic shock are often treated with intravenous fluids. The advanced trauma life-support (ATLS) protocol used to have a liberal approach to intravenous fluids but have in recent updates adjusted recommendations to 1000 ml crystalloids in adults and 20 ml/kg in patients < 40 kg (170). The potential side effects of colloids, such as allergic reactions, coagulopathies and the risk of kidney injury, has led to restricted use in critically ill patients(171). Recent studies, including trauma victims and critically ill patients, have shown that the ratio of crystalloids to colloids necessary to achieve the same physiologic targets is about 1,5:1 (172,173). Based on this, we chose 25 ml/kg of saline 0,9% and 12 ml/kg of dextran, giving a 2:1 ratio of crystalloids to colloids. In study 1, there was no change in haematocrit between groups to indicate the presence of hemodilution. Due to technical limitations, circulating plasma volume was measured in control- and crystalloid-treated groups only,

but no inter-group differences were found after rewarming. This may be due to increased extravasation of crystalloids at low core temperatures (174,175), limiting the volume effect to the period of ongoing infusion, also indicated by the temporary mitigating effect of crystalloid treatment on hemodynamic function.

The animals loaded with dextran displayed a sustained increase in SV and CO, which can be explained by the sustained effect in the circulation by colloids. Lauri et al. (176) infused 40 ml/kg 6% dextran in hypothermic Beagle dogs, showing similar results with elevated SV and reduced TPR without effects on HR or MAP. However, the lack of increased contractility (dP/dT_{max}) after dextran infusion pointed towards possible heart failure due to hypervolemia. It led him to caution the use of intravenous fluids during hypothermia and rewarming. However, due to the hypothermia induced cardiac dysfunction, a reduced dP/dT_{max} is observed both during hypothermia and after rewarming(72,167). Study 1 showed increased dP/dT_{max} at 34°C by use of dextran compared to the non-treatment group. After rewarming to 37°C, the dP/dT_{max} in the dextran group was unchanged compared to baseline values, but the non-treated animals displayed significantly reduced dP/dT_{max}. As a surrogate marker of systolic LV function, dP/dT_{max} is influenced by end-diastolic volume (EDV)(177,178) and is more accurate for assessing contractility in euvolemia(179).

Rewarming from hypothermia and reperfusion after ischemia during normothermia share the same goal: to restore macro-vascular perfusion to optimize micro-vascular perfusion. The factors determining micro-vascular blood flow are plasma viscosity, haematocrit, RBC deformability and RBC aggregation (180). All of these are affected by hypothermia, with reduced CO and organ blood flow. Consequently, rewarming is associated with elevated TPR (11), microvascular aggregation of RBCs (51–53), and fluid extravasation, leading to reduced circulating blood volume (181,182). The positive effects of dextran to combat RBC aggregates during induced hypothermia is well documented (51,52,183), and the reason for choosing it in one of the intervention groups.

Synthetic colloids have gained notoriety through studies showing increased incidence of acute kidney injury and bleeding in intensive care patients (178). Their use is more or less discontinued in clinical practice in Scandinavia (184). These recommendations were based on two controlled randomized trials (185,186) that have been controversial for using clinically unlikely regimens, the sole use of colloid solutions and very high volumes of colloid solutions. The debate is ongoing, and colloid solutions are still on the market. Data from study 1 point towards using colloids in general and dextran with its rheological properties in particular as adjuncts during rewarming from hypothermia.

9.5 Myocardial calcium control in hypothermia

Impaired calcium control in cardiac cells is a key factor in the pathophysiology of heart failure (187). In response to cooling in rodents, a decrease in myofilament calcium sensitivity and an increase in SV occurs. These seemingly contradictory changes are evident already at a temperature of 30°C (188), and the increase in contractility is associated with increased intracellular calcium (189). The increase in cytoplasmic calcium enhances cardiac contractility by increasing the number of cross-bridges recruited. Still, it seems that the elevated calcium levels over time are dysfunctional, leading to calcium overload and mechanical dysfunction (187). Our group has previously shown that increased calcium levels are present in the formation of post-hypothermic cardiac failure (55,56). After 30

minutes at 15°C, intracellular calcium levels remain unaltered (56), whereas a 4-hour exposure to 15°C led to a more than six-fold increase in calcium levels. Rewarming after 4 hours led to a slight reduction (15%) in calcium levels. Elevated levels of intracellular calcium are labelled “a characteristic” of the hypothermic beating heart, aggravated by exposure and relatively unresponsive to rewarming (55). Study 1 shows post-hypothermic calcium levels in the control group comparable to previous studies (55,56). We measured reduced levels of intracellular calcium in both treatment groups compared to the control group, but all groups present supra-normal values of intracellular calcium.

The causal connection between intravenous volume substitution and reduced myocardial calcium is not clear. In the volume loading groups, we found increased SV and diminished calcium levels compared to controls. Two main factors increase CO as a response to an increased circulating volume: nervous stimulation and intrinsic factors. Nervous stimulation increases CO by increasing HR and increasing the strength of the cardiac muscle(79). We have previously reported diminished β-adrenoceptor mediated cardiac responses during hypothermia (136). The lack of effect on HR in the treatment groups also points away from nervous stimulation playing a pivotal role. As far as the intrinsic factors are concerned, an increased preload in the two treatment groups would activate the Frank-Starling mechanism: strengthening the force of contraction due to increased preload (79). The Frank-Starling mechanism is calcium-independent but on the cellular level due to increased myofilament sensitivity to calcium (81). This increased calcium sensitivity at longer sarcomere lengths (190) opposes the hypothermia-induced PKA mediated phosphorylation of cardiac troponin I responsible for reduced calcium sensitivity (60,61,191,192). The animals in study 1 were subjected to 4 hours of hypothermia at 15°C, altering their myocardial calcium homeostasis. However, after rewarming, the treatment groups displayed significantly lower levels of intracellular calcium than controls. Our group did a similar study on hypothermic rats treated with levosimendan, a calcium sensitizer, during rewarming. It showed that levosimendan ameliorated the hypothermia induced cardiac dysfunction through an increase in SV and CO (72). Both levosimendan and stretching of the myofilaments (Frank-Starling effect) produce cellular effects of increased calcium sensitivity, which can explain the similarity in results. The similar effects of dextran and levosimendan are quite striking. It should encourage clinicians to strive for euvolemia during the rewarming of hypothermic patients and consider synthetic colloids for intravenous volume replacement therapy.

9.6 ECMO rewarming

An overwhelming number of case histories of hypothermic cardiac arrest patients successfully rewarmed by ECLS (CPB or ECMO) has led to its status as the gold standard (67). Rewarming patients with hypothermic cardiac arrest on ECLS relieves both pulmonary and cardiac function. The goal is to re-establish circulation and oxygen delivery to limit end-organ dysfunction and increase core temperature. In study 3, rewarming restored CO and MAP to baseline levels and provided adequate DO_2 to support aerobic metabolism. A study in a porcine model of normothermic cardiac arrest, CPR with the Lucas device and circulatory support with veno-arterial ECMO shows similar restoration of MAP as in our study (193).

During ECMO rewarming cardiac output is controlled by the ECMO circuit in terms of a target flow. Venous drainage, in turn, limits the flow. Centralised circulation due to hypothermia limits venous

drainage (77), which prompted a veno-veno-arterial circuit (194). Ringer's solution was added to the circuit when venous access pressure exceeded -100 mm Hg, indicating impaired venous drainage (204). The mean volume of fluid added during rewarming was 2,5 ml/kg/min. Reduced core temperature increases fluid requirements during extracorporeal circulation (182), reported being 0,5-0,9 ml/kg/min during hypothermic CPB if only crystalloids are used for priming and fluid additions (182,195). These values are considerably lower than what we found in study 3. We interpret these differences due to the cooling methods used: immersion cooling vs cooling by extracorporeal circulation. Preclinical studies have documented a more significant loss of plasma proteins in surface cooled animals (48,50,182), implying a more severely damaged vascular barrier resulting from surface cooling. Animals in study 3 were also subjected to a longer duration of hypothermia, including 3 hours CPR at 27°C, which may have caused additional capillary damage.

After ECMO rewarming, animals in study 3 displayed the markers that predict a favourable outcome in survivors of normothermic cardiac arrest: the return of pH to physiologic levels, low lactate and a shockable cardiac rhythm (196). With a 3-hour duration of CPR-generated flow, it seems evident that the element of hypothermic protection must be significant, but details of the protective effects are not well documented. However, we have reported hypothermia-induced cardiac dysfunction and alterations in renal tissue morphology after rewarming (56,197). The severity of organ dysfunction is linked to the level and duration of hypothermia. The effects of normothermic ischemia as seen in cardiac arrest are similar, where the length of the low flow period impacts the likelihood of survival and neurologic restitution. All animals in study 3 had shockable rhythms after ECMO-rewarming, and all were converted to sinus rhythm spontaneously or after defibrillation. These results, although encouraging, only speak to the viability of the heart. We did not assess whether the animals could be taken off ECMO support. Patients with hypothermic cardiac arrest usually require prolonged cardiopulmonary support before weaning is attempted(92).

Organ blood flow data from study 3 showed similar results as study 2 during cooling and 3 hours of CRP at 27°C. Rewarming restored blood flow to the heart, parts of the brain, the small intestine and the stomach. We have limited knowledge about how individual organs respond to reperfusion, but they are all exposed to complex pathophysiologic processes that alter organ function, labelled the post-cardiac arrest syndrome (198). A recent study applied a global metabolome analysis to an animal model of 20 minutes of cardiac arrest followed by 30 minutes of CPB reperfusion to assess the impact of cardiac arrest (199). Reperfusion alleviated the metabolic dysregulation in the heart and liver but exacerbated it in the brain and kidneys. These findings have some similarities to ours, as rewarming led to a fall in serum lactate, indicating some clearance by the liver and the absence of diuresis despite partial restoration of renal blood flow.

ECMO rewarming restored CO and MAP. Global DO_2 and VO_2 remained significantly reduced, but the O_2ER returned well below the critical threshold (ER_{crit} = 0,6 – 0,7), and the S_VO_2 normalized. This reduced VO_2 might be a mirror image of reduced organ function after rewarming. After cooling, 3 hours CPR and 62 minutes rewarming, most organs have suffered marginal blood flow and oxygen delivery to maintain organ function. The expected consequence of raised core temperature is therefore not an immediate restoration of organ flow and function. This heterogeneity in restitution can be seen

in study 3, where blood flow was only restored in the heart, stomach, small intestine, and parts of the brain.

Our research group studied blood flow distribution in rats during hypothermia and rewarming (77). This study found that rewarming favoured blood flow to the abdominal skin and skeletal muscle and not the brain, heart, kidneys, liver or small bowel, indicating the absence of autoregulation or irreversible shock. The data obtained from study 3 show that restoration of regional blood flow on ECMO support seems to favour flow to critical organs, especially the heart, brain and gastrointestinal tract. This leads us to conclude that the autoregulation of flow must be somewhat intact during ECMO rewarming.

A study on 1-2 week old piglets subjected to 32°C hypothermia for 1 hour and then allowed to rewarm passively showed regional flow trends similar to our research (200). Of note here is diminished flow to heart, CNS, kidney, stomach and bowel with the restoration of regional flow after rewarming only in the heart, CNS and kidneys. These neonatal piglets were surface cooled and kept at 32°C for 60 minutes before passive rewarming at room temperature. The hypothermia was moderate in level and duration compared to our experiment. The data from study 3 differ from the referred study in that we saw the additional restoration of flow to the stomach and small intestine, whereas renal blood flow was still significantly reduced. The reduced renal blood flow is probably a result of the duration of the hypothermic insult, coupled with reduced blood pressure during 3 hours of CPR. We have previously documented alterations in kidney morphology similar to acute tubular necrosis in rewarmed rats following 4 hours of profound hypothermia (197). A study in a rat model of 7 minutes cardiac arrest followed by resuscitation and ROSC showed acute kidney injury in 65% of surviving animals (201). The mechanism for reduced renal blood flow and GFR after rewarming from prolonged hypothermia is unknown. Sustained vasoconstriction and elevated hormone levels from the renin-angiotensin-aldosterone system might play a role in this (202). It is more difficult to explain why a prolonged hypothermic insult in mature piglets led to the restoration of blood flow to the intestine, whilst moderate hypothermia in neonatal piglets displayed depressed blood flow after rewarming(200). Schneider et al. found small intestinal injury in 94% of neonatal piglets subjected to 4,5 hours of moderate hypothermia, linking it to the necrotizing enterocolitis seen in human neonates(203). The more mature piglets in study 3 probably have more developed bowel systems, accounting for these differences. One might also argue that the bowel is a critical organ; one cannot survive without a minimum of the small intestine intact. Acute mesenteric ischemia is a life-threatening vascular emergency with a 60-80% mortality, where early restoration of bowel blood flow is vital to prevent necrosis and patient death (204).

ECMO rewarming restored coronary flow. Since the heart does not do any external work, one might be led to conclude that this flow is superfluous. However, increased coronary perfusion pressure is associated with higher rates of ROSC in a study using an iCPR device (impella) to increase coronary perfusion (205). A study using ECMO in resuscitating pigs in cardiac arrest also reported higher coronary perfusion pressure in animals that achieved ROSC (193).

The brain is the most sensitive to ischemic injury and is the limiting factor for survival after cardiac arrest (206). In our study, flow to the CNS was restored in the cerebellum and only partially restored

to the parietal lobe after rewarming. A study on hypothermic piglets (207) showed higher flow in the cerebellum than in the neocortex on cardiopulmonary bypass at 32°C, and these findings are similar to our results. During rewarming, we observed a significant reduction in cerebral DO_2 and VO_2, despite restoration of flow in some parts of the brain and cerebral O_2ER well below ER_{crit}. Compared to human data after cardiac arrest, decreased cerebral blood flow coupled with reduced oxygen consumption might indicate patency of cerebral autoregulation (208). Mezrow studied the safety of 1 hour hypothermic cardiac arrest at 8 and 13°C vs low flow CPB at 18°C in dog puppies (209). 27 of 32 animals survived, and all but two of them were reported to have restitution of neurologic function. Further, better cerebral protection was reported when using low flow CPB at 18°C than in non-perfused controls; this supports the use of CPR during hypothermic cardiac arrest. Also, reduced cerebral blood flow was reported due to increased vascular resistance in all groups, lasting up to 8 hours after rewarming. Our findings of reduced cerebral blood flow after rewarming indicate the same elevated vascular resistance.

9.7 Potential translational value

9.7.1 Study 1

This experiment demonstrates the effects of volume substitution during rewarming to enhance cardiac mechanical function and contractility comparable to pharmacologic interventions (62,69,72,210). This study used a crystalloid solution (saline) or a synthetic colloid (dextran) as volume expanders in the two treatment groups. Dextran produced a more profound and protracted effect than saline. We measured significantly lower levels of myocardial intracellular calcium in response to volume substitution, but calcium levels were supra-normal in all groups after rewarming. With this background, we advocate using intravenous fluids to maintain euvolemia during rewarming from long-lasting profound hypothermia. The rheological properties of dextran coupled with the limited volume infused compared to crystalloids may prove valuable tools in expanding circulating volume and limiting oedema during rewarming and should be investigated further.

9.7.2 Study 2 and 3

Study 2 and 3 strives to emulate a clinical scenario in our university hospital, where patients have survived prolonged periods of hypothermic CPR with little or no neurologic sequelae after rewarming (2). Northern Norway is well equipped with ambulance aeroplanes and helicopters to retrieve patients from remote areas. Still, these evacuations take time, and 3 hours of CPR is, in reality, a conservative timeframe.

In a study that included 222 patients, the researchers did autopsies on patients not successfully resuscitated (149). They found that the use of mechanical devices is associated with an increased risk of CPR-related injuries, but no injuries were deemed fatal. Reviews have found no differences between manual and automated CPR with regards to survival in out-of-hospital cardiac arrest (211,212). They do, however, advocate the use of compression devices in particular circumstances, for instance, during ambulance transport and as a bridge to ECMO. This appears to be sound advice as it is well recognized that the quality of chest compressions deteriorate quickly after the first minute of CPR (213). The effects of prolonged use of mechanical CPR devices have not been thoroughly studied. We know little about the consequences of 3 hours of continuous mechanical chest

compressions on the thoracic skeleton and the heart muscle. From a clinical perspective, the only way of treating hypothermic patients with cardiac arrest through evacuation and transport is with an automated CPR device. Therefore, the University hospital of North Norway aims to equip all its ambulances with mechanical CPR devices. The beneficial physiologic effects of prolonged CPR at reduced core temperatures emerge to build a new basis with unknown potential for patient survival after in-hospital rewarming.

Study 3 shows that ECMO rewarming, following 3 hours CPR at 27°C, generates adequate global blood flow, pressure and oxygen delivery to support aerobic metabolism. Our results indicate the presence of intact autoregulation after ECMO rewarming following 3 hours of CPR at 27°C; flow is preferred to critical organs. Data from studies 2 and 3, showing moderately elevated lactate, acceptable pH levels and normokalemia, as well as restoration of cardial electro-mechanical activity after rewarming, support our conclusion that aerobic metabolism must be partially sustained during hypothermic CPR and rewarming. This should encourage the aggressive and sustained resuscitation of hypothermic cardiac arrest victims, and reinforces the need for rapid evacuation to hospitals with ECLS capabilities.

10 Conclusion

From the experiments done in studies 1 – 3, we draw the following conclusions:

- Maintaining euvolemia during rewarming from hypothermia has a positive effect on the cardiac failure often seen during rewarming. Unlike crystalloid solutions, colloid solutions provide a sustained effect to support cardiac function after the infusion period.
- The CO generated by CPR is unaffected by temperature, providing favourable effects on organ survival when instituted at 27°C compared to 38°C. Hypothermic CPR favours flow to critical tissues, brain and heart, which infers the existence of intact autoregulation. Global oxygen delivery borders on delivery dependence during hypothermic CPR, but metabolic variables indicate that aerobic metabolism is at least in part sustained.
- ECMO rewarming from hypothermic CPR restores global hemodynamics and provides organ blood flow fully or partially to critical organs in order of importance. Further, restoration of cardiac electro-mechanical activity in concert with only moderate alterations of metabolic variables supports our conclusion that aerobic metabolism is sustained during prolonged hypothermic CPR and ECMO rewarming.

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Paper 1

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RESEARCH PAPER

Maintaining intravenous volume mitigates hypothermia-induced myocardial dysfunction and accumulation of intracellular Ca^{2+}

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Abstract

Previous research exploring pathophysiological mechanisms underlying circulatory collapse after rewarming victims of severe accidental hypothermia has documented post-hypothermic cardiac dysfunction and hypothermia-induced elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in myocardial cells. The aim of the present study was to examine if maintaining euvolaemia during rewarming mitigates cardiac dysfunction and/or normalizes elevated myocardial $[Ca^{2+}]_i$. A total of 21 male Wistar rats (300 g) were surface cooled to 15°C, then maintained at 15°C for 4 h, and subsequently rewarmed to 37°C. The rats were randomly assigned to one of three groups: (1) non-intervention control (n = 7), (2) dextran treated (i.v. 12 ml/kg dextran 70; n = 7), or (3) crystalloid treated (24 ml/kg 0.9% i.v. saline; n = 7). Infusions occurred during the first 30 min of rewarming. Arterial blood pressure, stroke volume (SV), cardiac output (CO), contractility (dP/dt_{max}) and blood gas changes were measured. Post-hypothermic changes in $[Ca^{2+}]_i$ were measured using the method of radiolabelled Ca^{2+} ($^{45}Ca^{2+}$). Untreated controls displayed post-hypothermic cardiac dysfunction with significantly reduced CO, SV and dP/dt_{max}. In contrast, rats receiving crystalloid or dextran treatment showed a return to pre-hypothermic control levels of CO and SV after rewarming, with the dextran group displaying significantly better amelioration of post-hypothermic cardiac dysfunction than the crystalloid group. Compared to the post-hypothermic increase in myocardial $[Ca^{2+}]_i$ in non-treated controls, $[Ca^{2+}]_i$ values with crystalloid and dextran did not increase to the same extent after rewarming. Volume replacement with crystalloid or dextran during rewarming abolishes post-hypothermic cardiac dysfunction, and partially mitigates the hypothermia-induced elevation of $[Ca^{2+}]_i$.

KEYWORDS
microcirculation, rewarming shock, volume replacement

1 | INTRODUCTION

Successful rewarming of patients after accidental hypothermia is often complicated by hypothermia-induced myocardial dysfunction, clinically ranging from a minor depression of cardiac output (CO) to a fulminant circulatory collapse ('rewarming shock') (Maclean & Emslie-Smith, 1977; Tveita, 2000). Hypothermia-induced myocardial dysfunction presents as a left ventricular systolic dysfunction during and after rewarming (Filseth et al., 2010). The pathophysiological mechanisms are not completely understood, but preclinical experiments have revealed that at least part of the dysfunction is caused by impairment of the contractile apparatus within cardiomyocytes. In addition, significant elevation of intracellular [Ca^{2+}] ([$Ca^{2+}]_i$) takes place during hypothermia (Wold et al., 2013), and it remains elevated after rewarming (Kondratiev et al., 2008; Wold et al., 2013).

Depending on the depth and severity of hypothermic exposure, cooling and rewarming can disrupt a number of physiological processes. For example, in response to cooling, there are changes in circulatory parameters, which include a progressive reduction of heart rate (HR), mean arterial pressure (MAP) and CO (Filseth et al., 2010; Tveita et al., 1996). Furthermore, there is a profound increase in blood viscosity during hypothermia, which gives rise to a marked elevation of total peripheral resistance (TPR) that is aggravated by a simultaneous increase in vascular tone (Brown et al., 2012). The resulting low-flow state induced by cooling disrupts shear forces and can lead to intravascular aggregation of red blood cells, which has been demonstrated in hypothermic microcirculation (Grossman & Lewis, 1964; Lofstrom, 1959). These red blood cell aggregates can become lodged at the entrance to capillaries and block flow through individual micro-vessels, impairing effective circulation as red blood cells are sequestered in peripheral tissues (Lipowsky, 2005). Along with a hypothermia-induced impairment of the vascular barrier and a subsequent increase in fluid extravasation from the intravascular to the interstitial space (Hammersborg et al., 2005), there may be a significant loss of plasma volume and circulating blood volume in the hypothermic patient.

It remains unclear whether hypovolaemia is an essential factor in rewarming shock (Tveita, 2000). Based on the observation that hypothermia-induced loss of plasma volume and circulating blood volume may reverse upon rewarming, some have advocated caution against administering large volumes of fluid to accidental hypothermia patients (Lloyd, 1996). However, there is preclinical evidence that fluid loss does not necessarily resolve, especially after prolonged hypothermic exposure (Kondratiev et al., 2006; Tveita et al., 1996). Thus, to avoid intravascular hypovolaemia during rewarming, fluid loss must be compensated by fluid administration, and often in considerable amounts (Brown et al., 2012; Farstad & Husby, 2014; Paal et al., 2016; Truhlar et al., 2015). Still, there is a lack of consensus concerning the type of fluid to be given, with some recommending liberal use of warm crystalloid solutions (Brown et al., 2012), while others routinely administer colloid solutions only during rewarming from severe hypothermia (Farstad et al., 2006; Suominen et al., 2010). Compared to crystalloid solutions, administering colloids during rewarming from hypothermia is associated with improved post-hypothermic haemodynamic function, and reported to limit oedema formation and total fluid requirements (Farstad & Husby, 2014). Dextrans, specifically, are demonstrated to counteract the formation of red blood cell aggregates in the hypothermic microcirculation (Lofstrom, 1959).

In a rat model of hypothermia–rewarming shock, we previously observed post-hypothermic reductions in CO and stroke volume (SV), as well as a 15–20% loss of circulating blood volume after rewarming (Kondratiev et al., 2006). In the present study, we hypothesized that post-hypothermic myocardial dysfunction and elevation of $[Ca^{2+}]_i$ is mitigated by maintaining euvolaemia during rewarming, and that the use of colloids could have beneficial haemodynamic effects surpassing crystalloid solutions.

New Findings

- **What is the central question of this study?**
  Detailed guidelines for volume replacement to counteract hypothermia-induced intravascular fluid loss are lacking. Evidence suggests colloids might have beneficial effects compared to crystalloids. Are central haemodynamic function and level of hypothermia-induced calcium overload, as a marker of cardiac injury, restored by fluid substitution during rewarming, and are colloids favourable to crystalloids?
- **What is the main finding and its importance?**
  Infusion with crystalloid or dextran during rewarming abolished post-hypothermic cardiac dysfunction, and partially mitigated myocardial calcium overload. The effects of volume replacement to support haemodynamic function are comparable to those using potent cardio-active drugs. These findings underline the importance of applying intravascular volume replacement to maintain euvolaemia during rewarming.

2 | METHODS

2.1 | Ethical approval

Adult male Wistar rats (250–350 g; Harlan UK Ltd, UK) were used in the present study. The experimental protocol was approved by the Norwegian Animal Research Authority (ref. no.: 08/62182-1) in accordance with the recommendations of the Federation of European Laboratory Animal Science Associations. On arrival, the animals were

quarantined for 1 week, provided *ad libitum* access to food and water, and housed in accordance with guidelines for accommodation and care of animals (article 5 of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986).

2.2 | Anaesthesia

Anaesthesia was induced by an i.p. injection of 50 mg/kg pentobarbital sodium, followed by a continuous infusion of 7.5 mg/kg/h through an intravenous line in the right jugular vein, extended to the right auricle. Due to hypothermia-induced anaesthesia and reduced drug metabolism, infusion was terminated at temperatures <30°C during cooling, and reintroduced at 30°C during rewarming. Animals were continuously monitored by toe-pinch for any sign of discomfort, and additional anaesthesia was provided if necessary. No neuromuscular blockers were used at any time during the experiment. After rewarming to 37°C and subsequent data sampling, animals were euthanised by an i.v. injection of 1 ml pentobarbital sodium (50 mg/ml).

2.3 | Respiratory support

The rats were placed on an operating table in the supine position. The trachea was incised, and a 14 G tracheal tube inserted. All animals had spontaneous and sufficient ventilation (monitored by P_{aCO_2}) at core temperatures >20°C. At core temperatures <20°C, normo-ventilation (P_{aCO_2}, 5.18–6.39 kPa) was achieved by a volume-controlled small-animal respirator (New England rodent ventilator, model 141, New England Instruments, Medway, MA, USA) using room air.

2.4 | Core cooling and rewarming

Animals were cooled and rewarmed by circulation of cold or warm water (recirculating water bath heater, RTE-110, Neslab Instruments, Newington, NH, USA) through U-shaped polyethylene tubes placed in the oesophagus and the lower bowels. Also, water from the same water bath circulated through the double layered operating table made of hollow aluminium. Core temperature was continuously monitored using a thermocouple wire positioned in the aortic arch via the right femoral artery, connected to a thermocouple controller (Thermoalert TH-5, Columbus Instruments, Columbus, OH, USA).

2.5 | Haemodynamic measurements

Previously, we used a pressure–volume conductance catheter to monitor left ventricular cardiac function. However, in the present experiment, as a consequence of infusing relatively large intravenous volumes, significant changes in the electrical conductance of blood precluded reliable volume measurements using this conductance cather. Therefore, CO was measured using the thermodilution technique, first described by Fegler (1954), by injecting 0.1–0.15 ml of 0.9% saline precooled in ice water through an intravenous line positioned in the right auricle. The change in temperature was recorded from the thermocouple positioned in the aortic arch. Thermodilution signals were recorded on a Linearcorder (Mark II, WR3101, Watanabe Instruments, Tokyo, Japan), digitalized (at 1 kHz sampling rate) using a Calcomp digitizing table (model 23180, Calcomp Digitzer Products Division, Anaheim, CA, USA) and analysed without further signal processing. CO was calculated according to the method described by Hanwell & Linzell (1972), with a program designed with the LabView package (LabVIEW 6.0, National Instruments, Austin, TX, USA) and calculated as the mean of three consecutive measurements.

A 22 G, fluid-filled catheter was placed in the left femoral artery for continuous recording of arterial pressure. The signals from the blood pressure transducer were amplified and digitized (12-bit analog-to-digital converter; BNC 2090, National Instruments) at a 1 kHz sampling rate. Signal processing and data analysis were performed with the help of a unique computer program developed at our department using a LabView package.

2.6 | Blood gases and acid–base parameters

Blood gases, O_2 saturation, pH and base excess were measured in 0.15 ml arterial blood samples taken from the femoral artery at the start of the experiment, at 15°C, and after rewarming to 37°C. Samples were analysed by a RapidLab 800 blood gas analyser (Chiron Diagnostics, Emeryville, CA, USA).

2.7 | Measurement of $[Ca^{2+}]_i$

Total myocardial $[Ca^{2+}]_i$ was measured using a method previously described in detail (Kondratiev et al., 2008), which was based on the incorporation of radiolabelled Ca^{2+} ($^{45}Ca^{2+}$) and adapted to an *in vivo* experiment. In brief, 20 μCi of $^{45}Ca^{2+}$ (ARX-102 Calcium-45, American Raidolabeled Chemicals Inc., St Louis, MO, USA) was injected at the start of the experiment. Pilot experiments revealed a rapid reduction of $^{45}Ca^{2+}$ activity in the plasma, reaching a steady state level by 120 min after injection. In order to wash out extracellular $^{45}Ca^{2+}$ in the myocardium, the hearts were excised and perfused in a Langendorff system with Krebs–Hensleit bicarbonate buffer containing 11.1 mM glucose and 2.4 mM Ca^{2+} at room temperature. We found that extracellular $^{45}Ca^{2+}$ was washed out after 1 min, and a washout period of 3 min was chosen, after which the hearts were freeze clamped, vacuum dry frosted (Christ Alpha 1–4; Medizinischer Apparatebau, Osterode, Harz, Germany) and subsequently pulverized by a micro-dismembrator (Braun Messungen AG, Melsungen, Germany). In the homogenate, 80–90 mg was extracted in perchloric acid, centrifuged at 7000g (Kubota 1700 centrifuge; Cubota Corp., Tokyo, Japan), and the $^{45}Ca^{2+}$ activity in the supernatant was determined. To determine the specific activity of the isotope, an arterial blood sample, drawn immediately before terminating the experiment, was centrifuged at 9000g, and the $^{45}Ca^{2+}$ activity and Ca^{2+} concentration in plasma were determined

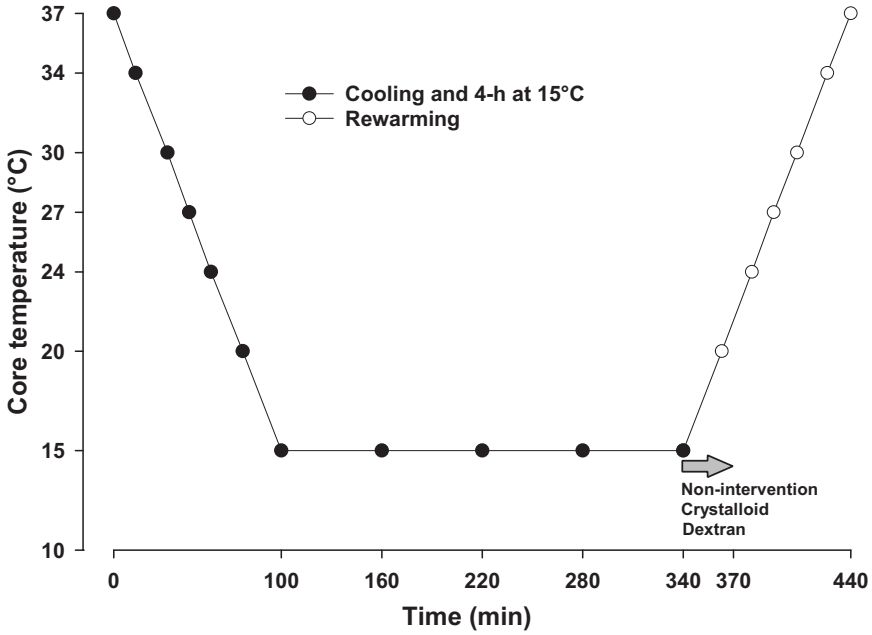

FIGURE 1 Experimental protocol

using a liquid scintillation spectrometer (Model 1900 TR, Packard Instrument Co., Downers Grove, IL, USA) and RapidLab 800 blood gas analyser (Chiron Diagnostics Corp.) respectively. Ultimately, $[Ca^{2+}]_i$ was calculated from the tissue radioactivity, the specific activity of the plasma and the dry weight of the heart tissue. As this method cannot distinguish between intracellular Ca^{2+} compartments, that is, cytosolic, sarcoplasmic reticulum and mitochondrial, only total myocardial $[Ca^{2+}]_i$ was measured.

2.8 | Blood volume determination

Blood volume was determined at the end of the experiment using the method described by Tschaikowsky et al. (1997), in which hydroxyl ethyl starch (HES) is used as a dilution marker. Blood (0.4 ml) was drawn from the arterial line just before injection of 0.5 ml of HES into the venous line and again 5 min after the injection. Haematocrit was determined 5 min after injection of HES that had been centrifuged at 11400g (Centri A 13, Jouan, Saint Nazaire, France) for 5 min at 12,000 rpm. From the same blood samples, plasma glucose levels were determined. In accordance to the method of Tschaikowsky et al. (1997), concentrated HCl was added to plasma samples to hydrolyse glucose from HES. Plasma glucose was determined by the Cobas Fara II Chemistry System using a glucose kit (Roche Diagnostics, Basel, Switzerland). Total blood volume (BV) was determined as follows: $BV = 3082 \times Vol_{HES}/\Delta glucose/(1 - Hct)$, where Δglucose is the difference in plasma glucose levels before and after HES (mg%), 3082 (mg%) is a standard factor given by Tschaikowsky et al. (1997), and Vol_{HES} is the volume of HES injected (ml).

After surgical instrumentation, animals were allowed to rest for 45 min before starting the experiment and obtaining baseline measurements. After cooling and the 4-h period at 15°C, animals were randomized into one of three experimental groups (Figure 1):

Group 1 ($n = 7$), non-intervention control. The animals were cooled from 37°C to 15°C during a 100-min period, maintained at 15°C for 4 h, and then rewarmed over a 100-min period before being euthanised. No intravenous fluids were given except the fluids accompanying anaesthesia.

Group 2 ($n = 7$), dextran treated. The animals were cooled from 37°C to 15°C during a 100-min period, maintained at 15°C for 4 h, and then rewarmed over a 100-min period before being euthanised. During the rewarming period, these animals were given an i.v. infusion of 12 ml/kg dextran 70 (60 mg/ml dextran in 0.9% saline) in addition to the fluids accompanying anaesthesia.

Group 3 ($n = 7$), crystalloid treated. The animals were cooled from 37°C to 15°C during a 100-min period, maintained at 15°C for 4 h, and then rewarmed over a 100-min period before being euthanised. During the rewarming period, these animals were given an i.v. infusion of 25 ml/kg 0.9% saline, in addition to the fluids accompanying anaesthesia.

2.9 | Calculations

Stroke volume (SV) was calculated as: CO/HR. TPR was calculated as: MAP/CO.

2.10 | Statistics

Results are presented as means and SD. Hemodynamic variables in Figure 4 and 5, and myocardial $[Ca^{2+}]_i$ values in Figure 6 are presented as median with interquartile range, 10th and 90th

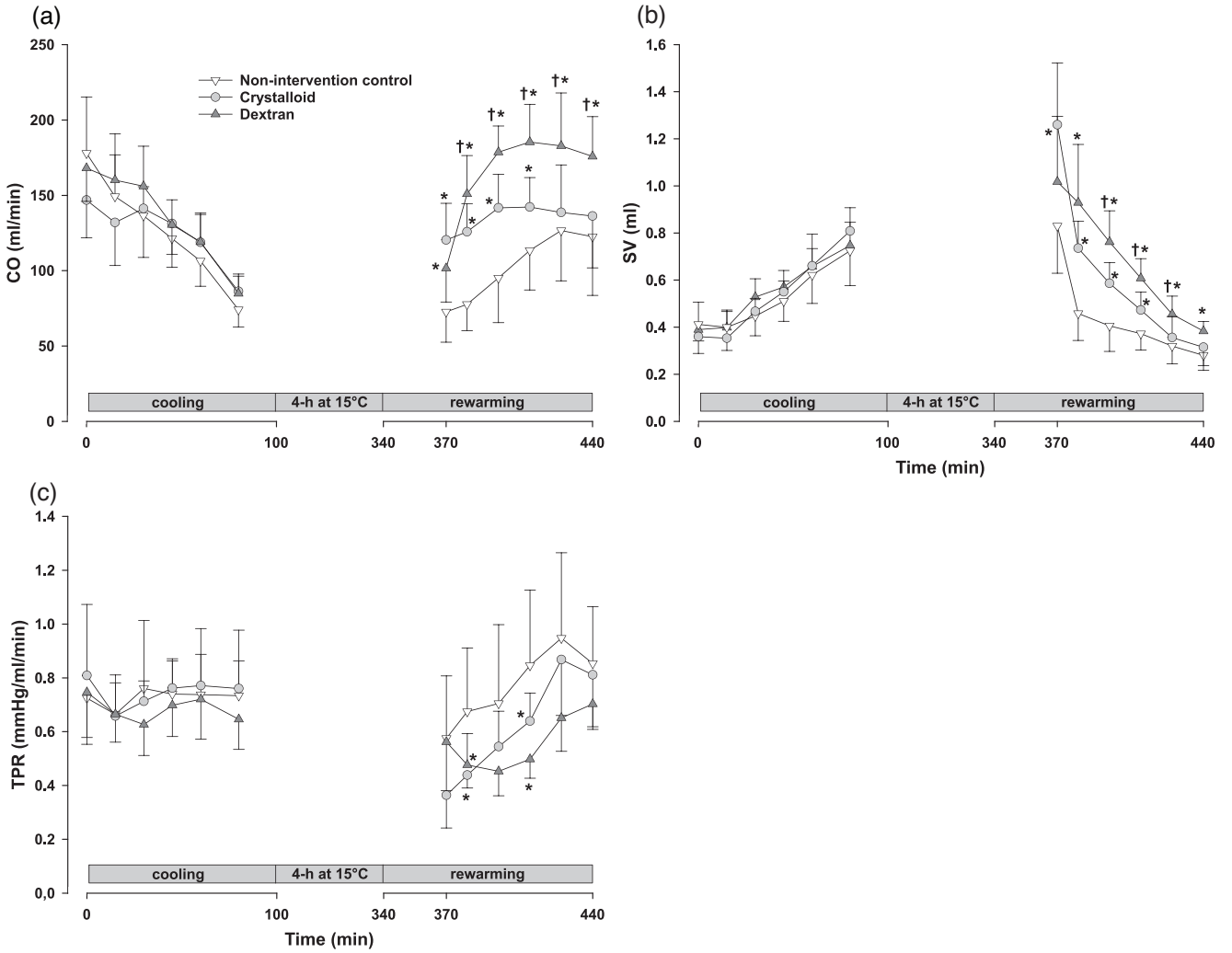

FIGURE 2 Haemodynamic function during hypothermia and rewarming. (a) Cardiac output (CO); (b) stroke volume (SV); (c) total peripheral resistance (TPR). Values are means ± SD. Each group $n = 7$; * $P < 0.05$ vs. corresponding value in the non-intervention control group; † $P < 0.05$ vs. corresponding value in the crystalloid group

percentile. For between-group comparisons of haemodynamic variables and myocardial $[Ca^{2+}]_i$, a one-way ANOVA was used. When significant differences were found, *P*-values were obtained by using Scheffe's test in hypothermic groups. For within-group comparisons of normothermic baseline against post-hypothermic end point, a paired Student's *t*-test was used. To compare variables measured in plasma samples, a two-way RM ANOVA with Dunnett's *post hoc* test was used. Differences were considered significant at $P < 0.05$.

3 | RESULTS

3.1 | Haemodynamic function (Figures 2 and 3)

As in previous studies using this animal model (Haheim et al., 2017; Wold et al., 2013), we found that haemodynamic function was stable during normothermic conditions. There were no differences in pre-hypothermic baseline haemodynamic values among the three groups.

3.1.1 | Cooling and 4 h at 15°C

Compared to pre-hypothermic baseline, cooling to 15°C caused a reduction in most haemodynamic variables. Due to technical limitations related to both the conductance catheter and thermodilution techniques, CO could not be measured below 20°C, and consequently, calculations of TPR and SV could not be made below this temperature. At 20°C a substantial reduction in CO was measured (~50%; Figure 2a), whereas TPR remained unchanged (Figure 2c), and SV was increased (~200%; Figure 2b). At 15°C the following haemodynamic variables were substantially reduced: HR (~87%; Figure 3a), MAP (~57%; Figure 3b), dP/dt_{max} (~88%; Figure 3c) and dP/dt_{min} (~93%; Figure 3d). No further changes were measured in

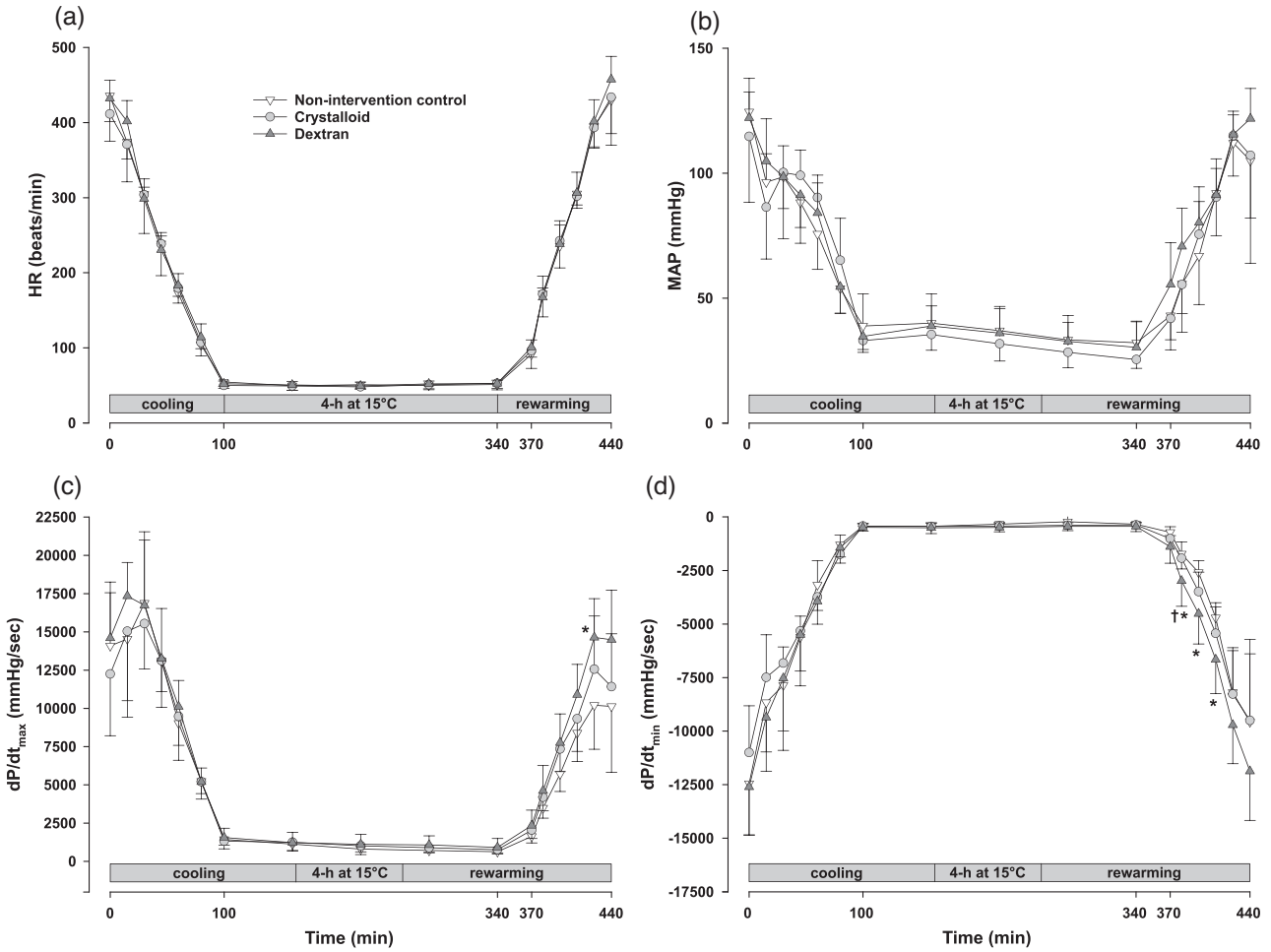

FIGURE 3 Haemodynamic function during hypothermia and rewarming (cont.). (a) Heart rate (HR); (b) mean arterial pressure (MAP); (c) maximum rate of LV pressure rise (dP/dt_{max}); (d) maximum rate of LV pressure decline (dP/dt_{min}). Values are means ± SD. Each group, $n = 7$; * $P < 0.05$ compared to corresponding value in the non-intervention control group; † $P < 0.05$ compared to corresponding value in the crystalloid group

any of the haemodynamic variables during 4-h maintenance of core temperature at 15°C.

3.1.2 | Rewarming to 37°C

Comparisons among groups

In the dextran-treated group, CO was significantly increased compared to both the crystalloid-treated and the non-intervention groups, and remained elevated throughout rewarming to 37°C (Figure 2a). In response to cooling and rewarming, HR underwent substantial changes (Figure 3a), but there was no differences among groups in HR, and therefore, the increase in CO in response to dextran was due to the significant increase in SV (Figure 2b), over that of the two other groups, during rewarming. In contrast, in the crystalloid-treated group, there was a significant increases in CO and SV compared to the non-intervention group, but these effects lasted only half way through the 100 min rewarming period (Figure 2a, b).

3.1.3 | Pre-hypothermic versus post-hypothermic differences (Figures 4 and 5)

In contrast to the non-intervention group, where there were significant reductions in CO (Figure 4a), SV (Figure 4b), and the index of left ventricular contractility, dP/dt_{max} (Figure 5c) after rewarming, all of these haemodynamic variables returned to pre-hypothermic baseline values after rewarming in both treatment groups.

3.2 | Post-hypothermic myocardial $[Ca^{2+}]_i$ (Figure 6)

Compared to the non-intervention control group, post-hypothermic myocardial $[Ca^{2+}]_i$ was significantly lower in both the crystalloid-treated (−47%) and the dextran-treated (−49%) groups.

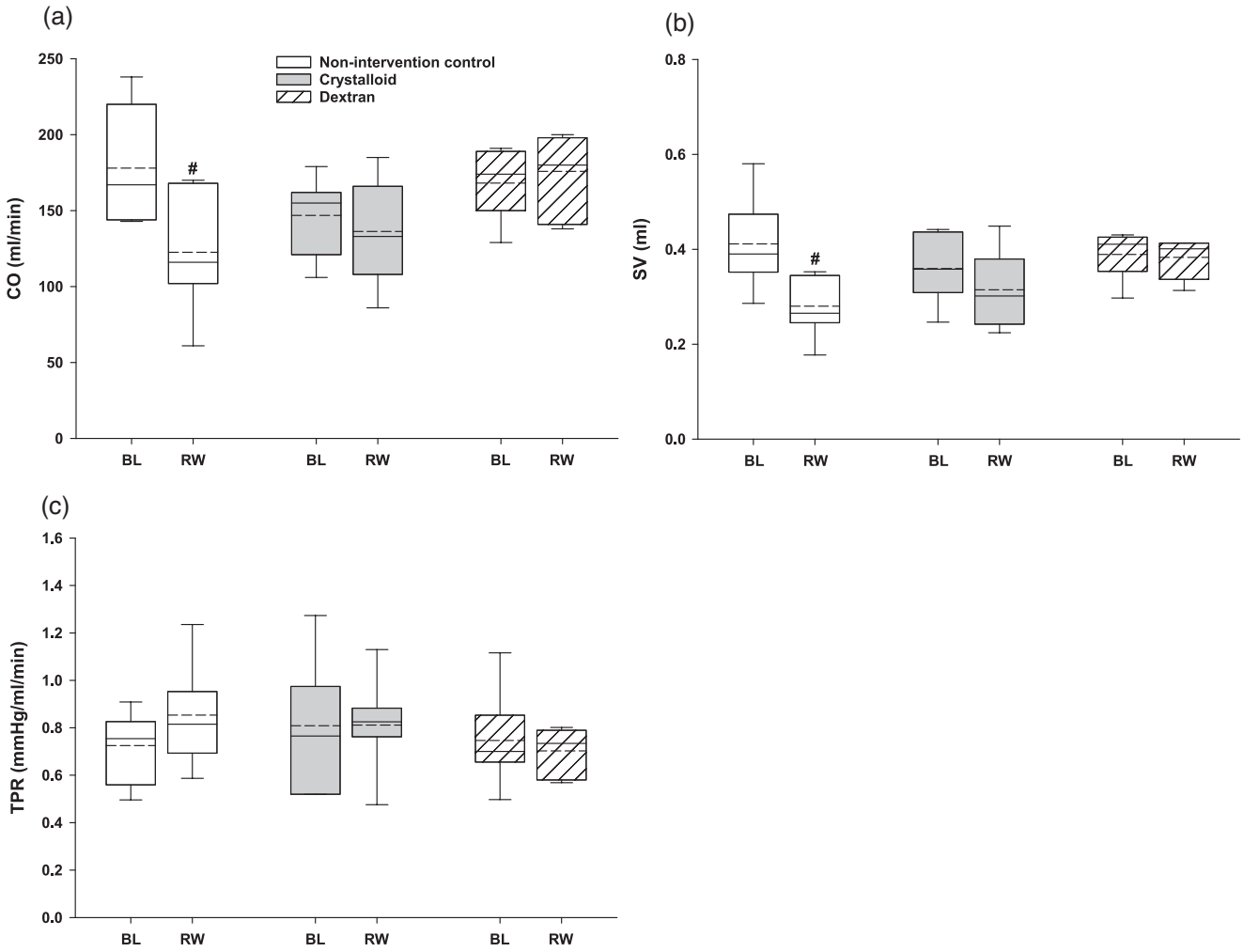

FIGURE 4 Pre-hypothermic vs. post-hypothermic haemodynamic function. (a) Cardiac output (CO); (b) stroke volume (SV); (c) total peripheral resistance (TPR). BL, pre-hypothermic baseline; RW, after rewarming to 37°C. Each group, $n = 7$. Values are presented as vertical boxes with median (solid line), mean (dashed line), interquartile range with 10th and 90th percentile error bars. #$P < 0.05$ vs. intragroup pre-hypothermic baseline

3.3 | Post-hypothermic arterial gas levels (Table 1)

Compared to their corresponding pre-hypothermic values, cooling to 15°C was associated with a significant reduction in pH in all groups. In the crystalloid-treated group, there was an elevation of P_{aCO_2}, but within physiological levels. Base excess (BE) was lower in the crystalloid and dextran-treated groups compared to the non-intervention control group.

After rewarming, when compared to their corresponding pre-hypothermic control values, animals in all groups demonstrated a significant increase in serum lactate levels in concert with reduced BE and pH, and a compensatory hyperventilation. Blood volume was measured in the non-intervention control group and the crystalloid-treated group only, but no differences between the two groups were found after rewarming. There were no differences among groups in post-hypothermic levels of serum cardiac troponin I. However, these levels were elevated (7–10 times) when compared to levels previously reported for normothermic time-matched control animals (Dietrichs et al., 2014). This suggests that hypothermia/rewarming induces cardiac tissue damage in this model.

4 | DISCUSSION

This study demonstrated that intravenous volume replacement, using crystalloid or dextran treatment during rewarming from hypothermia, significantly improved post-hypothermic haemodynamic function and mitigated the hypothermia-induced elevation of myocardial $[Ca^{2+}]_i$. This is in contrast to non-intervention control animals in which hypothermia/rewarming induced reductions in SV, CO, dP/dt_{max}, and significantly higher $[Ca^{2+}]_i$. In the crystalloid-treated group, the effects of volume replacement to support haemodynamic function was limited to the period of fluid administration, whereas in the dextran-treated

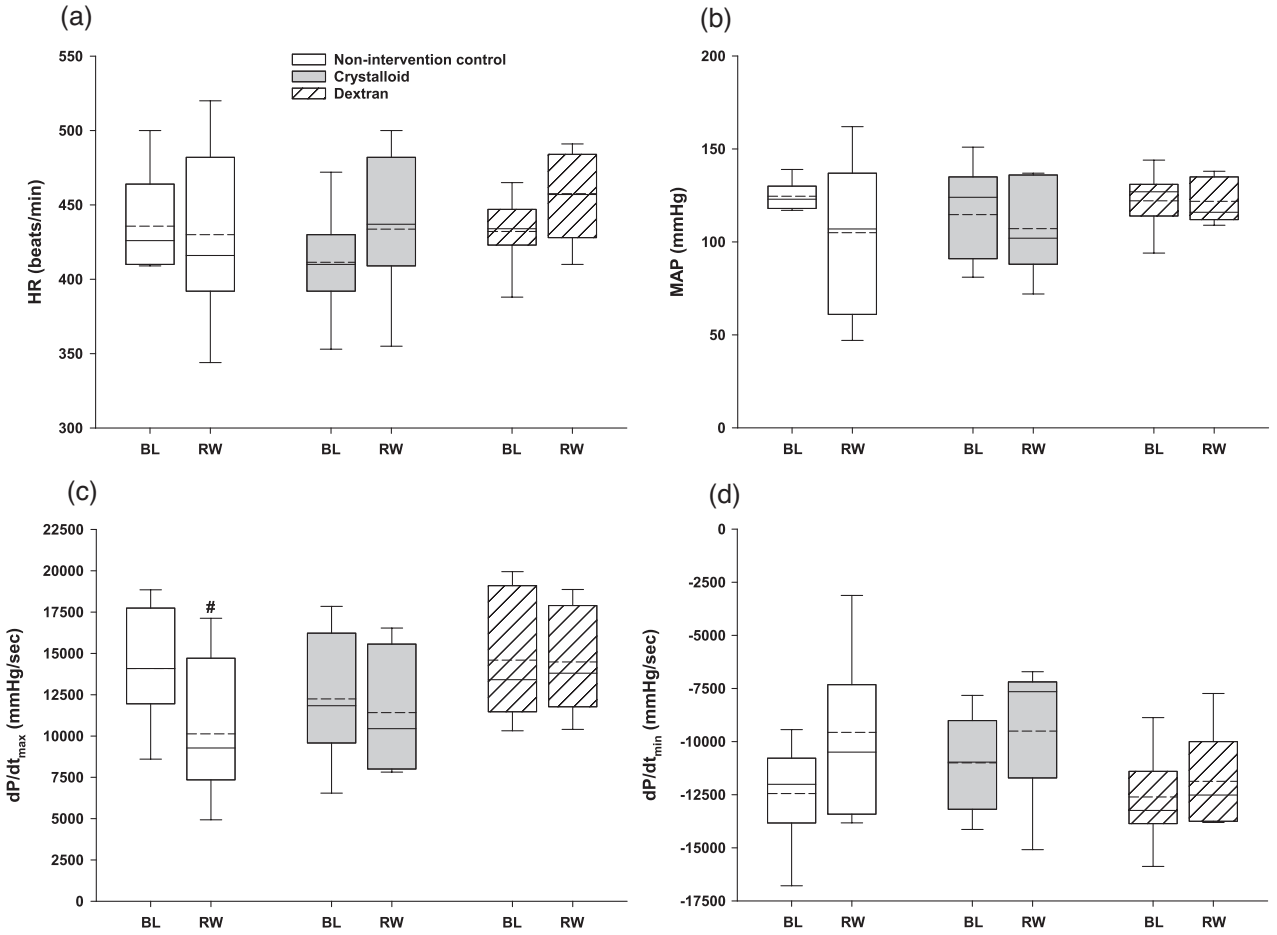

FIGURE 5 Pre-hypothermic vs. post-hypothermic haemodynamic function (cont.). (a) Heart rate (HR); (b) mean arterial pressure (MAP); (c) maximum rate of LV pressure rise (dP/dt_{max}); (d) maximum rate of LV pressure decline (dP/dt_{min}). Each group, $n = 7$. Values are are presented as vertical boxes with median (solid line), mean (dashed line), interquartile range with 10th and 90th percentile error bars. #$P < 0.05$ vs. intragroup pre-hypothermic baseline

group the improved haemodynamic function remained throughout rewarming.

The effects of volume replacement during rewarming to elevate cardiac mechanical function and contractility are comparable to those of previous experiments documented in response to pharmacological interventions (Dietrichs et al., 2014; Kondratiev et al., 2006; Tveita & Sieck, 2012). However, in this study the actual intervention protocol prevented us from using the conductance catheter, which is otherwise routinely used in this experimental model. Therefore, continuous detailed information about left ventricular pressure/volume changes in response to volume infusions could not be monitored and this challenged our detailed interpretation of causal effects of this treatment.

Rewarming from hypothermia and reperfusion after hypo-perfusion or ischaemia during normothermia share the same treatment strategy: restoration of macro-vascular perfusion in an attempt to optimize micro-vascular blood flow. Essential determinants of micro-vascular blood flow are plasma viscosity, haematocrit, red blood cell deformability and red blood cell aggregation (Surgenor, 2013). All of these determinants are seriously affected during low-flow hypothermia. As a consequence, rewarming is often challenged by a marked elevation of SVR (Brown et al., 2012), microvascular aggregation of red blood cells (Grossman & Lewis, 1964; Lipowsky, 2005; Lofstrom, 1959) and fluid extravasation (Hammersborg et al., 2005), causing plasma volume loss and subsequent reduction of circulating blood volume (Chen & Chien, 1978; Farstad et al., 2003). The presence of red blood cell aggregates creates a situation of heterogeneous micro-vascular blood flow where perfused capillaries appear in close proximity to non-perfused capillaries (Lofstrom, 1959; Svanes, 1966), causing organ hypoxia despite normalized global O_2 transport and CO during rewarming.

In the present study, a plausible explanation for the effects of intravenous volume replacement during rewarming in mitigating reduced haemodynamic function appears straightforward. However, a causal relationship between intravenous volume replacement and the mitigation of hypothermia/rewarming-induced myocardial $[Ca^{2+}]_i$ overload is not as obvious. In animals receiving crystalloid or dextran treatment, the increased circulating blood volume will increase venous return thereby increasing preload, which will subsequently elevate SV and improve contractility via the Frank–Starling mechanism

TABLE 1 Variables measured in plasma samples

Parameter	Group	Pre-hypothermic	15°C	Post-hypothermic
pH	Control	7.36 (0.05)	7.24 (0.07)#	7.20 (0.06)#
	Crystalloid	7.35 (0.02)	7.18 (0.07)#	7.21 (0.06)#
	Dextran	7.33 (0.03)	7.21 (0.08)#	7.26 (0.03)#
P_{aCO_2} (kPa)	Control	4.0 (0.66)	5.0 (0.88)	2.8 (0.36)
	Crystalloid	4.4 (0.42)	6.2 (1.67)#	2.9 (0.33)#
	Dextran	4.3 (0.18)	5.4 (1.27)	3.3 (0.65)
P_{aO_2} (kPa)	Control	11.1 (4.1)	22.3 (6.3)#	12.9 (3.6)
	Crystalloid	10.6 (1.3)	26.9 (5.0)#	11.7 (2.1)
	Dextran	10.2 (1.7)	23.4 (5.9)#	10.8 (3.1)
Hb (g/dl)	Control	11.7 (1.2)	11.2 (2.0)	11.3 (1.8)
	Crystalloid	12.8 (1.4)	11.1 (1.5)	11.5 (1.6)
	Dextran	11.3 (1.0)	11.3 (1.1)	9.9 (1.5)
Hct (%)	Control	36.2 (3.7)	36.1 (3.7)	34.8 (5.5)
	Crystalloid	39.2 (4.1)	34.1 (4.4)	35.4 (4.8)
	Dextran	34.8 (3.0)	35.0 (3.1)	31.3 (4.5)
Lactate (mmol/l)	Control	1.3 (0.6)	0.9 (0.4)	3.5 (1.3)#
	Crystalloid	2.0 (1.3)	1.7 (0.8)	4.0 (1.2)#
	Dextran	1.3 (0.9)	1.6 (0.7)	3.7 (2.0)#
BE (mmol/l)	Control	−7.2 (1.7)	−7.0 (7.0)	−18.8 (2.3)#
	Crystalloid	−7.1 (1.5)	−10.6 (2.0)*	−18.3 (2.2)#
	Dextran	−7.9 (1.7)	−11.3 (2.0)#*	−15.1 (2.2)#
cTn-I (ng/ml)	Control	—	—	10 (5.6)
	Crystalloid			14.1 (7.8)
	Dextran			9.8 (5.8)
CBV (ml)	Control ($n = 5$)	—	—	18.7 (5.3)
	Crystalloid ($n = 7$)			20.1 (6.4)
	Dextran			—

Values are means (SD), $n = 7$; *$P < 0.05$ compared to non-intervention control group; #$P < 0.05$ compared to pre-hypothermic baseline. BE, base excess; CBV, circulating blood volume; cTn-I, cardiac troponin I; Hb, haemoglobin; HCT, haematocrit.

(Guyton, 1977). This fundamental property of the heart, by some researchers suggested to be the consequence of increased myofilament Ca^{2+} sensitivity at longer sarcomere lengths (de Tombe et al., 2010), would oppose the reduced Ca^{2+} sensitivity induced by hypothermia–rewarming (Han et al., 2010, 2018; Schaible et al., 2016; Tveita et al., 2019).

During volume infusion, there was a significant increase in SV, CO and heart work, which would provide an increase in coronary blood flow. The absence of an increase in serum lactate levels during rewarming, over that in non-treated control, indicates the presence of a patent coronary autoregulation to provide an adequate myocardial O_2 supply–consumption balance to meet the increased heart work during volume replacement. In this case, volume replacement might have increased myocardial microcirculation, which, in the dextran-treated group, remained throughout the rewarming phase. To speculate, an increase in myocardial micro-vascular blood flow in response to volume infusion also suggests increased clearance of the hypothermia-induced $[Ca^{2+}]_i$ overload. In support of this suggestion is the well-documented (Fukusumi & Adolph, 1970; Grossman & Lewis, 1964; Lofstrom, 1959) positive effect of dextran treatment in preventing hypothermia-induced red blood cell aggregates, which is the background for using dextran in the present experiment.

Intravenous volume replacement during rewarming should preferentially correct intravascular hypovolaemia, restore microcirculatory function, while limiting oedema formation and fluid overload, factors which in clinical medicine are related to increased patient mortality (Chappell et al., 2008).

By adding crystalloid solutions during normothermia, the intravascular volume effect is only about 20%, as crystalloids are evenly distributed throughout the extracellular fluid compartment (Chappell et al., 2008), and this effect may be further reduced by hypothermia (Schanche et al., 2019; Roberts et al., 1985). In contrast, infusing colloid solutions reduces fluid extravasation and oedema formation during hypothermia, and these solutions are routinely administered during

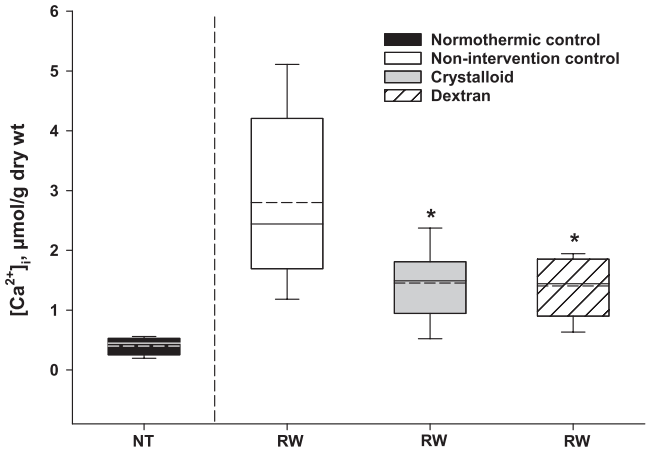

FIGURE 6 Concentration of $[Ca^{2+}]_i$ in cardiac tissue in normothermic controls and after rewarming. Each group, n = 7. Values are presented as vertical boxes with median (solid line), mean (dashed line), interquartile range with 10th and 90th percentile error bars. *$P < 0.05$ vs. non-intervention control. Normothermic control values included are from previous studies to illustrate normothermic baseline levels of $[Ca^{2+}]_i$ concentration (Kondratiev et al., 2008; Wold et al., 2013)

rewarming from severe hypothermia in some institutions (Farstad et al., 2006; Suominen et al., 2010). Still, the fear of potential side effects such as allergic reactions, coagulopathies and risk of kidney injury, has led to restricted use of synthetic colloids in critically ill patients (Reinhart et al., 2012). Several recent studies, including information on normothermic trauma victims and critically ill patients, has shown that a ratio of crystalloids to colloids necessary to achieve the same physiological targets is about 1.5:1 (Annane et al., 2013; Orbegozo et al., 2015; Spahn et al., 2019). Based on this, we chose to use a 2:1 crystalloid to colloid ratio in the present study.

The maintenance of euvolaemia during rewarming in the treatment groups was indicated by the fact that haematocrit levels did not change, that is, there was no evidence of haemodilution. Due to technical limitations, circulating plasma volumes were measured only in the non-intervention control and crystalloid-treated groups, but there was no difference in circulating blood volume between these two groups after rewarming. This may be the consequence of increased extravasation of crystalloids at low core temperatures (Farstad et al., 2005, 2006), limiting the volume effect only to the period of ongoing infusion, also indicated by the temporary mitigating effect of crystalloid treatment on haemodynamic function. The moderate but significant reduction in pH and elevated plasma lactate levels in all groups indicate the absence of massive organ hypoxia during hypothermia/rewarming. In support, we found normal values of global O_2 partial pressure in arterial blood.

In previous studies using animal models of hypothermia/rewarming, we observed time-dependent elevation of myocardial $[Ca^{2+}]_i$ (Kondratiev et al., 2008; Wold et al., 2013). After 30 min at 15°C $[Ca^{2+}]_i$ remained unaltered (Wold et al., 2013), whereas after 4 h at 15°C, there was a more than six-fold increase in $[Ca^{2+}]_i$ compared to pre-hypothermic levels (Wold et al., 2013). After rewarming, myocardial $[Ca^{2+}]_i$ only partially recovered (−15%), but remained substantially increased (Wold et al., 2013). The post-hypothermic elevation of myocardial $[Ca^{2+}]_i$ levels observed in non-intervention control animals in the present study were comparable to those previously reported (Kondratiev et al., 2008; Wold et al., 2013). Importantly, with volume replacement in the treatment groups, myocardial $[Ca^{2+}]_i$ levels were significantly lower after rewarming when compared to the non-intervention control group. Impaired homeostasis of myocardial $[Ca^{2+}]_i$ is a key factor in the pathophysiology of normothermic heart failure (Vassalle & Lin, 2004). In response to hypothermia, there is a decrease in myofilamental Ca^{2+}-sensitivity (Han et al., 2010, 2018; Harrison & Bers, 1989; Schaible et al., 2016; Tveita et al., 2019). These two, seemingly contradictory functional changes, are already present at 30°C (Kusuoka et al., 1991), and the increase in force is associated with an elevation of $[Ca^{2+}]_i$ (Puglisi et al., 1996) in response to cooling. The increase in cytoplasmic $[Ca^{2+}]$ enhances cardiac contractility by increasing the number of cross-bridges recruited for force development, but seemingly, due to a dysfunctional elevation of this ion over time, Ca^{2+} overload occurs (Tani & Neely, 1989; Vassalle & Lin, 2004), which results in mechanical dysfunction that may entail cardiac failure (Aasum & Larsen, 1997; Aasum et al., 1997; Bers et al., 1989; Gambassi et al., 1994; Puglisi et al., 1996; Schiffmann et al., 2001; Shattock & Bers, 1987; Shutt & Howlett, 2008; Steigen et al., 1994; Stowe et al., 1995, 1999, 2000; Groban et al., 2002). Studies using papillary muscle (Han et al., 2010) or isolated cardiomyocytes (Schaible et al., 2016) to investigate excitation–contraction coupling at low temperatures (15°C) have reported that the mechanism for the hypothermia-induced calcium overload over time is related to the prolongation of evoked Ca^{2+} transient in response to stimulation, leaving insufficient time for the evoked transient to return to baseline before the next stimulus. Further, with relevance to outcome after continuous haemodynamic interventions during and after rewarming, we have reported spontaneous recovery of contractile dysfunction and return of calcium overload during a 2-h follow-up period after rewarming in these isolated, perfused and stimulated cells (Schaible et al., 2016).

4.1 | Summary and conclusion

The positive haemodynamic effects were both more pronounced and more protracted with dextran than with crystalloid solution. In addition, we measured significantly lower $[Ca^{2+}]_i$ in cardiac tissue in response to volume replacement, but post-hypothermic levels are still substantially elevated. On this background, we advocate using volume replacement aimed at maintaining euvolaemia during rewarming from long-lasting accidental hypothermia.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Conception or design of the work: J.H.N., T.K., O.H. and T.T. Acquisition, analysis or interpretation of data for the work: J.H.N., T.S., T.K., O.H., G.C.S. and T.T. Drafting of the work or revising it critically for important intellectual content: J.H.N., T.S., T.K., O.H., G.C.S. 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 or 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.

DATA AVAILABILITY STATEMENT

All data are available upon reasonable request to the authors.

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Paper 2

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Study of the Effects of 3 h of Continuous Cardiopulmonary Resuscitation at 27°C on Global Oxygen Transport and Organ Blood Flow

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Aims: Complete restitution of neurologic function after 6 h of pre-hospital resuscitation and in-hospital rewarming has been reported in accidental hypothermia patients with cardiac arrest (CA). However, the level of restitution of circulatory function during long-lasting hypothermic cardiopulmonary resuscitation (CPR) remains largely unknown. We compared the effects of CPR in replacing spontaneous circulation during 3 h at 27°C vs. 45 min at normothermia by determining hemodynamics, global oxygen transport (DO_2), oxygen uptake (VO_2), and organ blood flow.

Methods: Anesthetized pigs (n = 7) were immersion cooled to CA at 27°C. Predetermined variables were compared: (1) Before cooling, during cooling to 27°C with spontaneous circulation, after CA and subsequent continuous CPR (n = 7), vs. (2) before CA and during 45 min CPR in normothermic pigs (n = 4).

Results: When compared to corresponding values during spontaneous circulation at 38°C: (1) After 15 min of CPR at 27°C, cardiac output (CO) was reduced by 74%, mean arterial pressure (MAP) by 63%, DO_2 by 47%, but organ blood flow was unaltered. Continuous CPR for 3 h maintained these variables largely unaltered except for significant reduction in blood flow to the heart and brain after 3 h, to the kidneys after 1 h, to the liver after 2 h, and to the stomach and small intestine after 3 h. (2) After normothermic CPR for 15 min, CO was reduced by 71%, MAP by 54%, and DO_2 by 63%. After 45 min, hemodynamic function had deteriorated significantly, organ blood flow was undetectable, serum lactate increased by a factor of 12, and mixed venous O_2 content was reduced to 18%.

Conclusion: The level to which CPR can replace CO and MAP during spontaneous circulation at normothermia was not affected by reduction in core temperature in our

setting. Compared to spontaneous circulation at normothermia, 3 h of continuous resuscitation at 27°C provided limited but sufficient O_2 delivery to maintain aerobic metabolism. This fundamental new knowledge is important in that it encourages early and continuous CPR in accidental hypothermia victims during evacuation and transport.

Keywords: accidental hypothermia, cardiac arrest, hemodynamics, organ blood flow, cardiopulmonary resuscitation (CPR)

INTRODUCTION

Over the past years, patient case reports of accidental hypothermia with cardiac arrest (CA) indicate favorable neurologic outcome after in-hospital rewarming (Walpoth et al., 1990, 1997; Gilbert et al., 2000; Mark et al., 2012; Wanscher et al., 2012; Hilmo et al., 2014). These favorable outcomes appear to be linked to the good quality of pre-hospital emergency medical treatment provided including early start and continued cardiopulmonary resuscitation (CPR) (Monsieurs et al., 2015; Truhlar et al., 2015) in accordance to the latest international guidelines (Truhlar et al., 2015).

Normothermic CPR can provide ∼30% of pre-arrest cardiac output (CO) (Wik et al., 1996; Sunde et al., 1998; Pytte et al., 2006) but is usually considered discontinued after 20–30 min if the return of spontaneous circulation is not achieved, as these patients show poor clinical outcome (Torke et al., 2015; Perkins et al., 2015a,b). Preclinical studies (Wik et al., 1996; Gervais et al., 1997; Schwarz et al., 2002; Tomte et al., 2010; Welbourn and Efstathiou, 2018) also support this clinical notion, but the maximum duration of CPR in normothermic patients has so far not been determined (Welbourn and Efstathiou, 2018).

It is well documented that cooling with spontaneous circulation slows metabolic rate and, therefore, reduces CO in parallel with reductions in global O_2 transport (DO_2) and O_2 consumption (VO_2) (Black et al., 1976; Kondratiev et al., 2006; Valkov et al., 2019). In addition, low core temperature *per se* prolongs end-organ survival during hypothermic CPR (Gilbert et al., 2000; Mark et al., 2012; Wanscher et al., 2012). In an intact pig model, we validated these findings and also documented that O_2 transport (Filseth et al., 2010) and tissue blood flow (Valkov et al., 2019) are maintained during 1–3 h of stable hypothermia (25°–27°C) as well as during rewarming. However, to date, the effects of prolonged (hours) CPR on global DO_2 and organ blood perfusion have not been assessed in an intact hypothermic animal model. A report from 1986 documented that CPR at 28°C replaced normothermic CO during spontaneous circulation by only 7% after 20 min (Maningas et al., 1986). Subsequent pharmacologic studies on pigs (Krismer et al., 2000; Kornberger et al., 2001) reported the maintenance of coronary perfusion pressure at 28°C during short-lasting CPR, but neither of these studied applied the latest standard CPR algorithm (Truhlar et al., 2015).

New devices make it possible to sustain CPR during evacuation and transport for a longer period of time with a limited number of rescuers (Perkins et al., 2015b). UNN (University Hospital of North Norway) is located in a scarcely populated, subarctic catchment area, making flying time for patients with ambulance airplanes typically around 90 min. This makes evacuation and transportation time 3–4 h for accidental hypothermia patients with CA in need of in-hospital rewarming. Under these circumstances, continuous prehospital CPR is often the only treatment option for these patients.

It is well documented that standard CPR can replace CO only to a certain low level. We, here, hypothesize that this level is largely unaffected by core temperature, and thus, cooling with spontaneous circulation will reduce CO to a level, which eventually can be replaced by CPR. Therefore, the aim of this study was to evaluate the value of CPR as part of prehospital interventions for hypothermic patients with CA in more detail using our intact porcine model of CPR instrumented to determine hemodynamics, global DO_2, VO_2, and organ blood flow during continuous CPR for 3 h at 27°C core temperature. To study the effects of low temperature only, ischemic episodes were avoided before CPR, and 27°C corresponds to the core temperature of surviving patients after hypothermic CA. To more closely mimic a clinical scenario, the porcine model was equipped with a commercially available automated chest compression device.

MATERIALS AND METHODS

Ethical Approval

The Norwegian Food Safety Authority approved the study (ref. number: 14/56323). Eleven castrated male pigs (wt. 20–29 kg, age 3 months) from NOROC stock were used. On arrival, the animals were acclimated for 2–5 days before the terminal experiment. Animals were fed twice daily, had free access to water at all times, and received humane care in accordance to the Norwegian Animal Welfare Act.

Anesthesia and Instrumentation

We previously reported the detailed methods for hemodynamic monitoring, immersion cooling, and blood flow measurements using the porcine animal model (Valkov et al., 2019). Briefly, after fasting the animals overnight, premedication was induced by an intramuscular bolus of ketamine hydrochloride (20 mg kg^{-1}), midazolam (30 mg), and atropine (1 mg), and anesthesia was induced by a bolus infusion of fentanyl (10 μg kg^{-1}) and pentobarbital-sodium (10 mg kg^{-1}) in an ear vein. After

Abbreviations: ACD-CPR, active compression–decompression; CA, cardiac arrest; CPR; ITV-CPR, impedance threshold valve CPR; CPR, cardiopulmonary resuscitation; CVP, central venous pressure; DO_2, global oxygen transport; ER O_2, extraction ratio; HR, heart rate; MAP, mean arterial pressure; VO_2, oxygen uptake; CO, cardiac output; VF, ventricular fibrillation.

tracheotomy and intubation, the animals were connected to a respirator (Siemens Servo 900D, Solna, Sweden), adjusted to maintain $PaO_2 > 10$ kPa and $PaCO_2$ at 4.5–6.0 kPa uncorrected for temperature (α-stat management). After instrumentation, animals were randomly assigned to two groups [hypothermic CA and CPR for 3 h ($n = 7$), or normothermic CA and CPR for 45 min ($n = 4$)]. During ventricular fibrillation (VF) and CPR, FiO_2 was set to 1.0. Infusion of fentanyl (20 μg kg^{-1} h^{-1}), midazolam (0.3 μg kg^{-1} h^{-1}), and pentobarbital-sodium (4 mg kg^{-1} h^{-1}) was continued via a femoral vein catheter. In the hypothermia group, anesthesia was discontinued at 27°C. Microspheres were injected to the left ventricle through a 6F fluid filled pigtail catheter (Cordis Corporation, Miami, FL, United States). Core temperature, CO, and venous and mixed venous blood gases were measured via a 7F Swan–Ganz thermodilution catheter (Edwards Lifesciences LLC, Irvine, CA, United States) positioned in the pulmonary trunk. Thermodilution is described as the gold standard for measuring CO during CPR (Carretero et al., 2010). The tip of another 7F Swan–Ganz thermodilution catheter was positioned in the aortic arch via the left femoral artery to monitor arterial blood gas and mean arterial pressure (MAP) and to collect reference blood samples for the microsphere technique. Urinary output was followed from a 14F urinary bladder catheter introduced via a lower abdominal incision. The animals were given 5,000 IE Heparin and allowed to stabilize for 45 min before the start of the experimental protocol.

Regional Blood Flow Measurements

To determine organ blood flow, stable isotope-labeled microspheres (BioPAL Inc., Worcester, MA, United States) were injected into the left ventricle (Reinhardt et al., 2001). Simultaneously, a reference blood sample was drawn from the tip of a catheter inserted in the left femoral artery and advanced to the aortic arch to calculate regional blood flow. Blood flow was determined in tissue samples from the brain (temporal lobes), kidneys, liver, heart, small intestine, and stomach based on a technique of neutron activation to analyze microsphere content as already described in detail (Valkov et al., 2019).

Experimental Protocol

Animals in the hypothermia group were immersion cooled in ice water to a blood temperature of 27°C, and VF was induced by stimulating the epicardial surface using an alternating current (5–20 mA, 6 Hz, and 30 V) conducted via a 15 cm-long needle electrode (**Figure 1**). The needle was inserted in the epigastric area and pointed toward the heart apex. Correct needle placement was confirmed when aspirating arterial blood from the left ventricle. CA was defined as asystoly or VF on ECG associated with the absence of fluctuation in arterial pressure. After 90 s of CA, an automated chest compression device (LUCAS chest compression system, Physio-Control Inc., Lund, Sweden) was started. The piston on this compression device was equipped with a suction cup to ensure active decompression with a continuous mode compression/decompression duty cycle of 50 ± 5% at a rate of 100 ± 5 compressions/min, and compression depth was 4–5 cm. In the normothermia group, CPR was continued for 45 min, whereas in the hypothermia group, CPR was continued for 3 h.

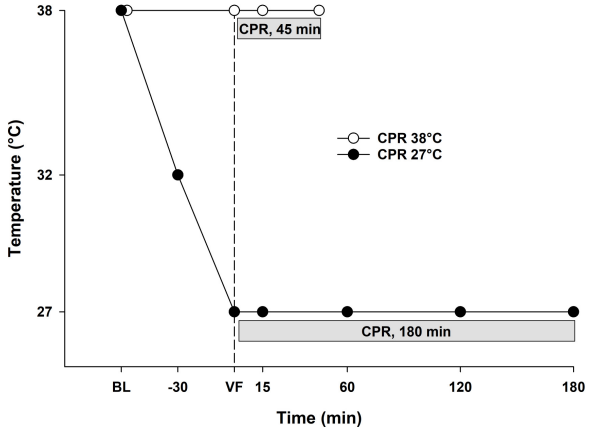

FIGURE 1 | Experimental protocol. 38°C Baseline (BL); Cardiopulmonary resuscitation (CPR); Ventricular fibrillation (VF).

Frequency of Measurements

In the normothermia group: during spontaneous circulation at 38°C before VF, and at 15 and 45 min during CPR. In the hypothermia group: during spontaneous circulation at 38°C, during cooling at 32° and 27°C before VF, and during CPR at 15 min and hourly thereafter. Simultaneously, we recorded electrocardiogram (standard leads), heart rate (HR), MAP, central venous pressure (CVP), and urine output, and microspheres were injected. Blood gases were analyzed by simultaneous sampling from arterial, central venous, and mixed venous blood.

Statistics

Statistical analyses were performed using SigmaPlot statistical software version 14 (Systat Software Inc., Richmond, CA, United States). Normal distribution was checked using the Shapiro–Wilk test. Comparisons between normothermia and hypothermia groups as well as intragroup comparisons at 38°C baseline, 15 min, and 45/60 min of CPR were performed by two-way repeated measures ANOVA. Where significant differences were found, all pairwise multiple comparison procedures were done using the Holm–Sidak test. To compare values within the hypothermia group, we used one-way repeated measures ANOVA for normal distributed variables, and Friedman repeated measures ANOVA on ranks for non-normal distributed variables. Where significant differences were found, Dunnett's test was used to compare all values within the hypothermia group vs. 38°C baseline, as well as values obtained during CPR at 60, 120, and 180 min vs. CPR at 15 min. The level of significance was set at $p < 0.05$. Data are presented as means and SD.

RESULTS

No statistically significant differences were found between groups in any of the variables recorded at the start of the experiments.

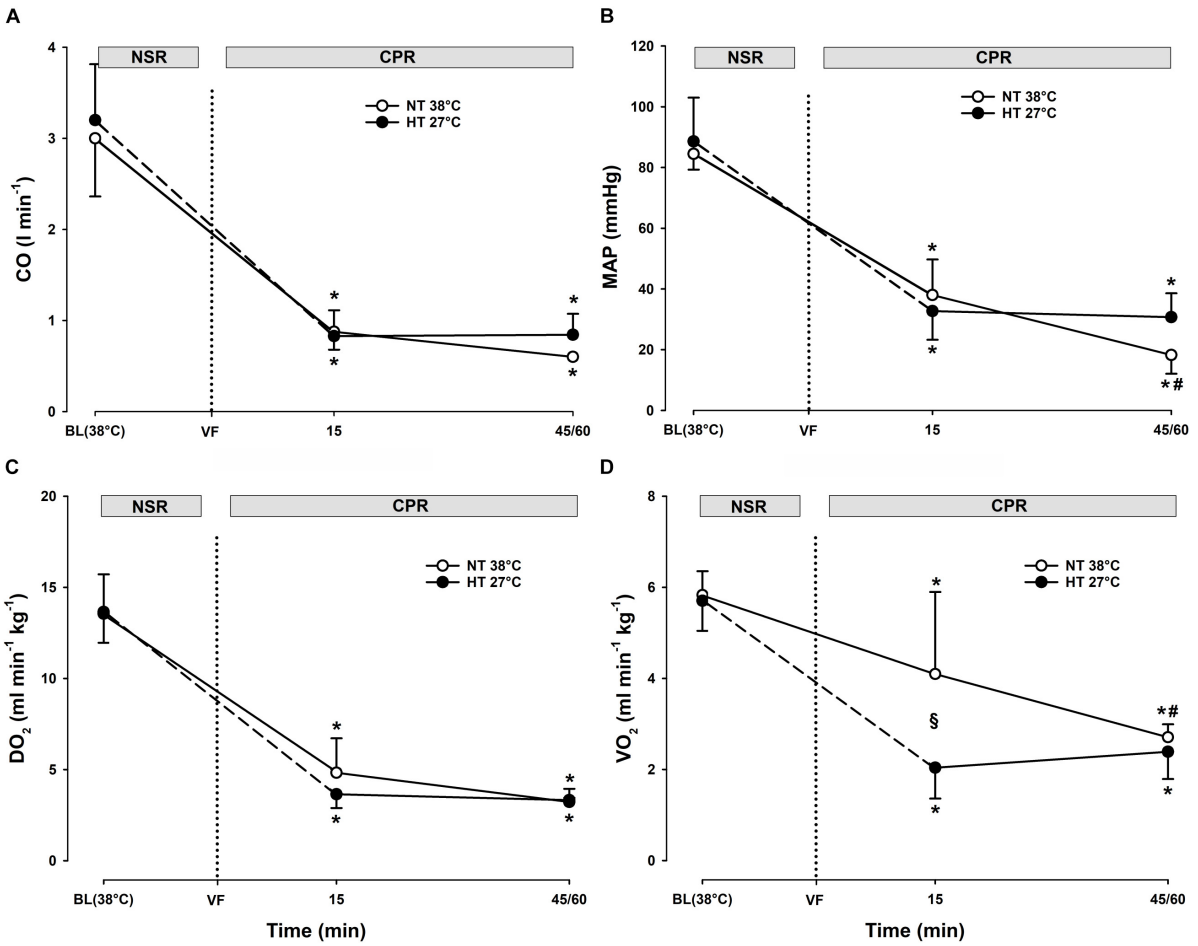

FIGURE 2 | Hemodynamic variables, and global O_2 transport and O_2 uptake during CPR at normothermia and hypothermia. **(A)** Cardiac output (CO), **(B)** mean arterial pressure (MAP), **(C)** global oxygen delivery (DO_2), and **(D)** global oxygen consumption (VO_2). CPR at normothermia (NT 38°C, $n = 4$), CPR at hypothermia (HT 27°C, $n = 7$). 38°C baseline (BL) (38°C); normal sinus rhythm (NSR); cardiopulmonary resuscitation (CPR); ventricular fibrillation (VF). Comparisons between groups and intragroup comparisons at 38°C baseline, 15 and 45/60 min CPR were done using two-way repeated measures ANOVA and pairwise multiple comparison using Holm–Sidak test. Values are mean ± SD. $^{*}p < 0.05$ vs. intragroup 38°C baseline; $^{\#}p < 0.05$ vs. intragroup 15 min of CPR; $^{\S}p < 0.05$ vs. corresponding value between groups.

Animals in both groups had spontaneous circulation before induction of VF. Stability of the actual pig model related to hemodynamic function and organ blood flow at normothermia has previously been documented in experiments lasting up to 7 h (Filseth et al., 2010; Valkov et al., 2019). Multiple costal fractures occurred in all animals after CPR, and after 3 h of CPR also sternal fractures were observed in six out of seven animals.

Comparing Effects of CPR for VF After 15 and 45/60 min, at the Two Different Core Temperatures, 38° and 27°C

Hemodynamic Variables

38°C.

Compared to spontaneous circulation at 38°C, after CPR for 15 min at this temperature, CO was significantly reduced from 3.0 ± 0.6 to 0.9 ± 0.2 L min^{-1} (-71%) and MAP was significantly reduced from 85 ± 5 to 38 ± 12 mmHg (-55%) (**Figures 2A,B**). After 45 min of CPR at 38°C, CO and MAP ended up being reduced by 80 and 78% (0.6 ± 0 L min^{-1} and 18 ± 6 mmHg), respectively, but the reduction in MAP was statistically significant.

27°C.

Compared to spontaneous circulation at 38°C, after CPR for 15 min at 27°C, CO was significantly reduced from 3.2 ± 0.6 to 0.8 ± 0.1 L min^{-1} (-74%) and MAP from 89 ± 14 to 33 ± 9 mmHg (-63%). After 60 min of CPR at 27°C, CO and MAP were reduced by 74 and 65% (0.8 ± 0.2 L min^{-1} and 31 ± 8 mmHg), respectively.

Comparisons.

No significant differences between groups in CO or MAP were found after 15 min of CPR. In the 38°C group, MAP after 45 min CPR was significantly lower than MAP after 60 min of CPR in the

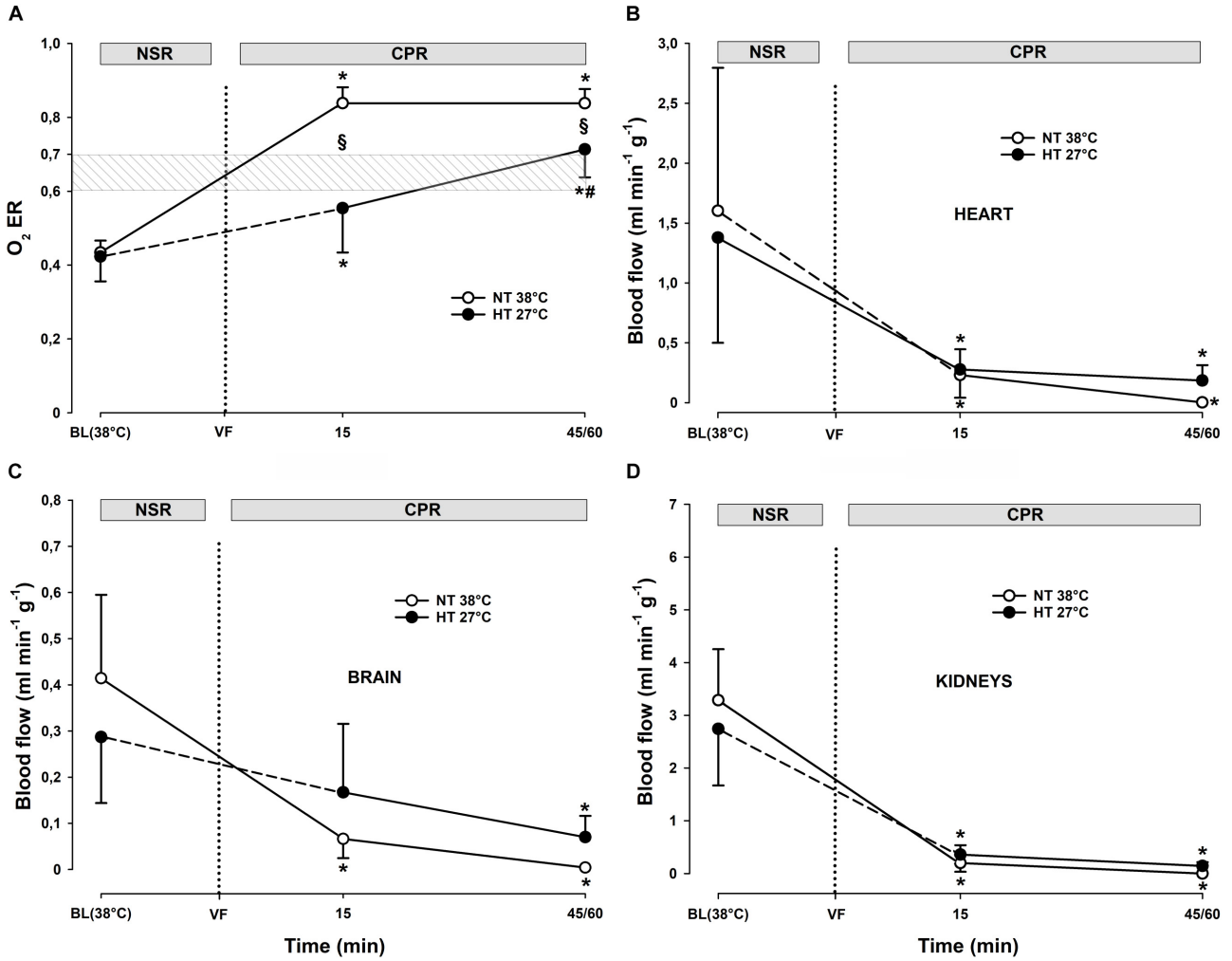

FIGURE 3 | Oxygen extraction ratio, and regional blood flow during CPR at normothermia and hypothermia. **(A)** Oxygen extraction ratio (O_2 ER), **(B–D)** regional blood flow in the heart, brain, and kidneys. CPR at normothermia (NT 38°C, $n = 4$), CPR at hypothermia (HT 27°C, $n = 7$). 38°C baseline (BL) (38°C); normal sinus rhythm (NSR); cardiopulmonary resuscitation (CPR); ventricular fibrillation (VF). Comparisons between groups and intragroup comparisons at 38°C baseline, 15 and 45/60 min of CPR were done using two-way repeated measures ANOVA and pairwise multiple comparison using the Holm–Sidak test. Values are mean ± SD. $^{*}p < 0.05$ vs. intragroup 38°C baseline; $^{\#}p < 0.05$ vs. intragroup 15 min of CPR; $^{\S}p < 0.05$ vs. corresponding value between groups.

27°C group. This is in essential contrast to the 27°C group where both MAP and CO remained unchanged during 60 min of CPR.

Oxygen Transport and Extraction

38°C.

Compared to during spontaneous circulation at 38°C, after 15 min of CPR, DO_2 decreased significantly from 13.6 ± 2.2 to 4.8 ± 1.9 ml min^{-1} kg^{-1} (-64%), and VO_2 from 5.8 ± 0.5 to 4.1 ± 1.8 ml min^{-1} kg^{-1} (-30%) (**Figures 2C,D**). Accordingly, the O_2 extraction ratio (ER; DO_2/VO_2) (**Figure 3A**) was significantly increased after 15 min of CPR (from 0.43 ± 0.03 to 0.84 ± 0.04), exceeding the reported critical extraction ratio (ER_{crit}) value (0.6–0.7) to provide aerobic metabolism (Leach and Treacher, 1992). After 45 min, ER remained significantly elevated (0.84 ± 0.04). Simultaneously, a reduction in mixed venous O_2 saturation (SvO_2) (**Figure 4B**) from 56 ± 3% during spontaneous circulation to 15 ± 3 and 18 ± 6% after 15 and 45 min of CPR, respectively, took place in parallel with a significant increase in the serum lactate level (**Figure 4A**), from 0.63 ± 0.15 during spontaneous circulation to 3.78 ± 0.87 after 15 min of CPR increasing to 7.38 ± 1.51 mmol L^{-1} after 45 min of CPR, underlining the existence of inadequate O_2 transport throughout the last 30 min of CPR at 38°C.

27°C.

Compared to during spontaneous circulation at 38°C, after 15 min of CPR at 27°C, DO_2 decreased significantly from 13.7 ± 1.7 to 3.6 ± 0.8 ml min^{-1} kg^{-1} (-73%), and VO_2 from 5.7 ± 0.7 to 2.0 ± 0.7 ml min^{-1} kg^{-1} (-64%) (**Figures 2A,B**). This caused a significant increase in ER from 0.42 ± 0.07 to 0.55 ± 0.12%, but this value is below ER_{crit} (0.6–0.7) (**Figure 3A**). Simultaneously, SvO_2 (**Figure 4B**) fell from 58 ± 6 at 38°C to

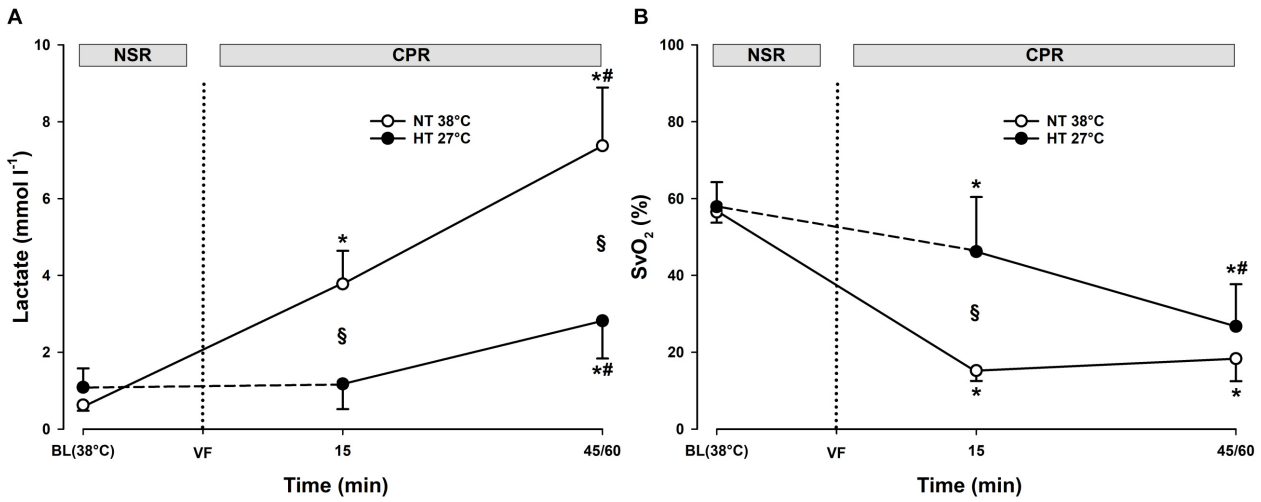

FIGURE 4 | Lactate and mixed venous oxygen saturation during CPR at normothermia and hypothermia. **(A)** Lactate and **(B)** mixed venous oxygen saturation (SvO_2). CPR at normothermia (NT 38°C, $n = 4$), CPR at hypothermia (HT 27°C, $n = 7$). 38°C baseline (BL) (38°C); normal sinus rhythm (NSR); cardiopulmonary resuscitation (CPR); ventricular fibrillation (VF). Comparisons between groups and intragroup comparisons at 38°C baseline, 15 and 45/60 min CPR were done using two way repeated measures ANOVA and pairwise multiple comparison using the Holm–Sidak test. Values are mean ± SD. $^{*}p < 0.05$ vs. intragroup 38°C baseline; $^{\#}p < 0.05$ vs. intragroup 15 min of CPR; $^{\S}p < 0.05$ vs. corresponding value between groups.

46 ± 14 after 15 min of CPR, and after 60 min of CPR, SvO_2 was reduced to 27 ± 11%, and lactate (**Figure 4A**) increased after 15 min and 60 min of CPR from 1.17 ± 0.65 to 2.81 ± 0.98 mmol L^{-1}, respectively.

Differences

Owing to the low core temperature, VO_2 in the 27°C group was significantly lower than VO_2 in the 38°C group, whereas no differences between groups in DO_2 were found. The reduction in SvO_2 was significantly lower in the 27°C group than in the 38°C group. Also, the increase in lactate in the 27°C group was significantly lower than in the 38°C. Taken together, the higher the SvO_2, the modest increase in lactate, and the less increase in ER during 60 min of CPR in the 27°C group indicates a better O_2 transport than in the 38°C group.

Organ Blood Flow

Already after 15 min of CPR in the 38°C group, there was a statistically significant reduction in blood flow to the brain, heart, kidneys, stomach, liver, and small intestine (**Figures 3B,D**). After 45 min of CPR, practically no organ blood flow was detectable. A similar reduction in organ blood flow was measured after 15 min of CPR in the 27°C group, but in essential contrast to the 38°C group, organ blood flow was maintained at this reduced level throughout 60 min of CPR.

Cooling to 27°C With Spontaneous Circulation

Hemodynamic Variables

Compared to corresponding values at 38°C, cooling with maintained spontaneous circulation gave an almost linear reduction in CO and MAP (**Figures 5A,B**). At 27°C, CO was significantly reduced from 3.2 ± 0.6 to 1.6 ± 0.4 L min^{-1} (-50%), and MAP significantly reduced from 89 ± 14 to 60 ± 14 mmHg (-33%).

Oxygen Transport and Extraction

Global DO_2 and VO_2 (**Figure 5C**) were significantly reduced from 13.7 ± 1.7 to 7.1 ± 1.8 ml min^{-1} kg^{-1} (-48%), and from 5.7 ± 0.7 to 1.8 ± 0.2 ml min^{-1} kg^{-1} (-68%), respectively, giving an ER (**Figure 5D**) of 0.26, which is far below the ER_{crit} of 0.6–0.7 (Leach and Treacher, 1992).

Organ Blood Flow

During cooling to 27°C, an apparent reduction in blood flow to all organs measured took place, but these changes did not reach statistical significance (**Figure 6**).

Effects of Continuous CPR for VF During 3 h at 27°C

Hemodynamic Variables

CPR for 15 min at 27°C gave a significant reduction in CO from 3.2 ± 0.6 to 0.8 ± 0.1 L min^{-1} (-74%), and a significant reduction in MAP from 89 ± 14 to 33 ± 9 mmHg (-63%) when compared to corresponding values during spontaneous circulation at 38°C (**Figures 5A,B**). Both CO and MAP remained unchanged at these reduced levels throughout the 3 h of CPR at 27°C.

Oxygen Transport and Extraction

During 3 h of CPR, the differences between DO_2 and VO_2 diminished (**Figure 5C**), but when compared to their corresponding values after 15 min of CPR at 27°C (7.1 ± 1.8 and 1.8 ± 0.2 ml min^{-1} kg^{-1}, respectively), DO_2 and VO_2 were further significantly reduced first after 2 h of CPR (2.8 ± 0.7 and 1.9 ± 0.5 ml min^{-1} kg^{-1}, respectively). By 1 h of CPR, ER_{crit} (**Figure 5D**) was approached (0.71 ± 0.08) and remained at or

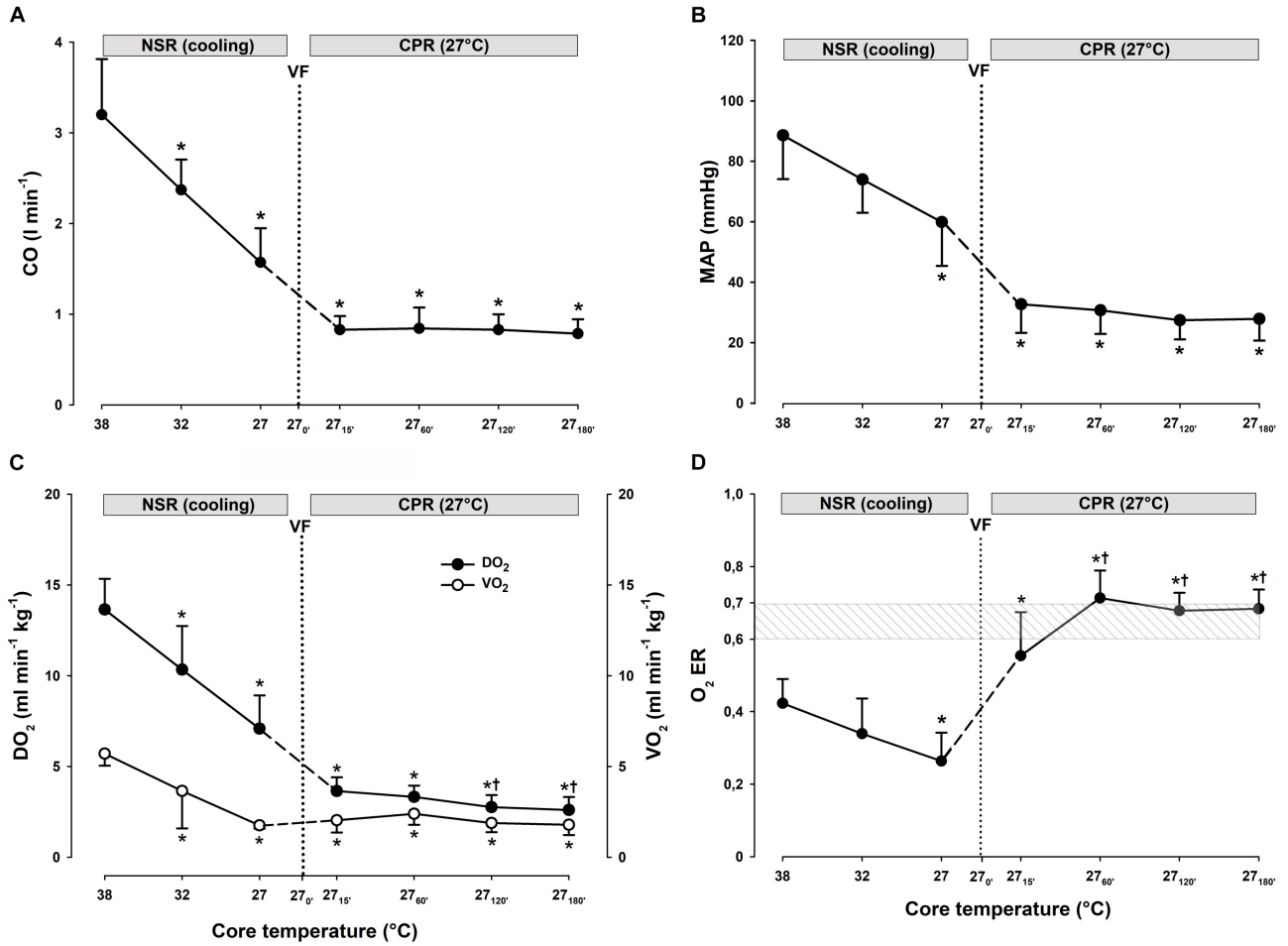

FIGURE 5 | Hemodynamic function, global O_2 transport, global O_2 uptake, and O_2 extraction ratio during cooling and 3 h of CPR at 27°C (n = 7). **(A)** Cardiac output (CO), **(B)** mean arterial pressure (MAP), **(C)** global oxygen delivery (DO_2), and global oxygen consumption (VO_2), and **(D)** oxygen extraction ratio (O_2 ER). Normal sinus rhythm (NSR); cardiopulmonary resuscitation (CPR); ventricular fibrillation (VF). To compare values within groups, we used one-way repeated measures ANOVA for normal distributed variables and Friedman repeated measures ANOVA on ranks for non-normal distributed variables. Dunnett's test was used to compare all values obtained vs. 38°C baseline and also for values obtained during CPR vs. corresponding values after CPR for 15 min. Values are mean ± SD. *$p < 0.05$ vs. intragroup 38°C baseline; †$p < 0.05$ vs. intragroup 15 min of CPR at 27°C.

near this critical value over the next 2 h of CPR (0.68 ± 0.05). After 15 min of CPR, SvO_2 (**Figure 7**) was reduced from 75 ± 7 to 46 ± 14% followed by a further gradual reduction in SvO_2 over the next 1 h to 27 ± 11%, but with no change during the next 2 h. After 1 h of CPR at 27°C, a modest but significant elevation of serum lactate from 0.8 ± 0.65 to 2.81 ± 0.98 mmol L^{-1} took place. Serum lactate level increased linearly during the rest of the CPR period reaching 5.56 ± 1.84 mmol L^{-1}after 3 h.

Organ Blood Flow

Compared to after 15 min of CPR at 27°C, blood flow to the heart remained unaltered during the first 2 h of CPR (**Figure 6A**). A significant reduction in blood flow to the heart from 0.3 ± 0.2 to 0.1 ± 0.2 ml min^{-1} g^{-1} was found first after 3 h of CPR. Blood flow to both brain hemispheres (**Figure 6B**) remained unaltered after 1 h of CPR when compared to flow after 15 min of CPR, but flow to both left hemispheres was significantly reduced after 2 h of CPR. First, after 3 h of CPR blood flow to both hemispheres was significantly reduced compared to 15 min of CPR: left hemisphere from 0.16 ± 0.16 to 0.02 ± 0.02 and right hemisphere from 0.17 ± 0.15 to 0.03 ± 0.03, all values in ml $min^{-1}g^{-1}$. Blood flow to kidneys (**Figure 6C**) was significantly reduced already after 1 h of CPR, liver blood flow was significantly reduced after 3 h, whereas flow to the small intestine and stomach was reduced after CPR for 2 and 3 h (**Figure 6D**).

DISCUSSION

This experiment, comparing hemodynamic function, O_2 transport, and organ blood flow during spontaneous circulation at 38°C, and during subsequent CPR for VF at 38°C or 27°C, demonstrated that after 15 min of CPR, these variables were

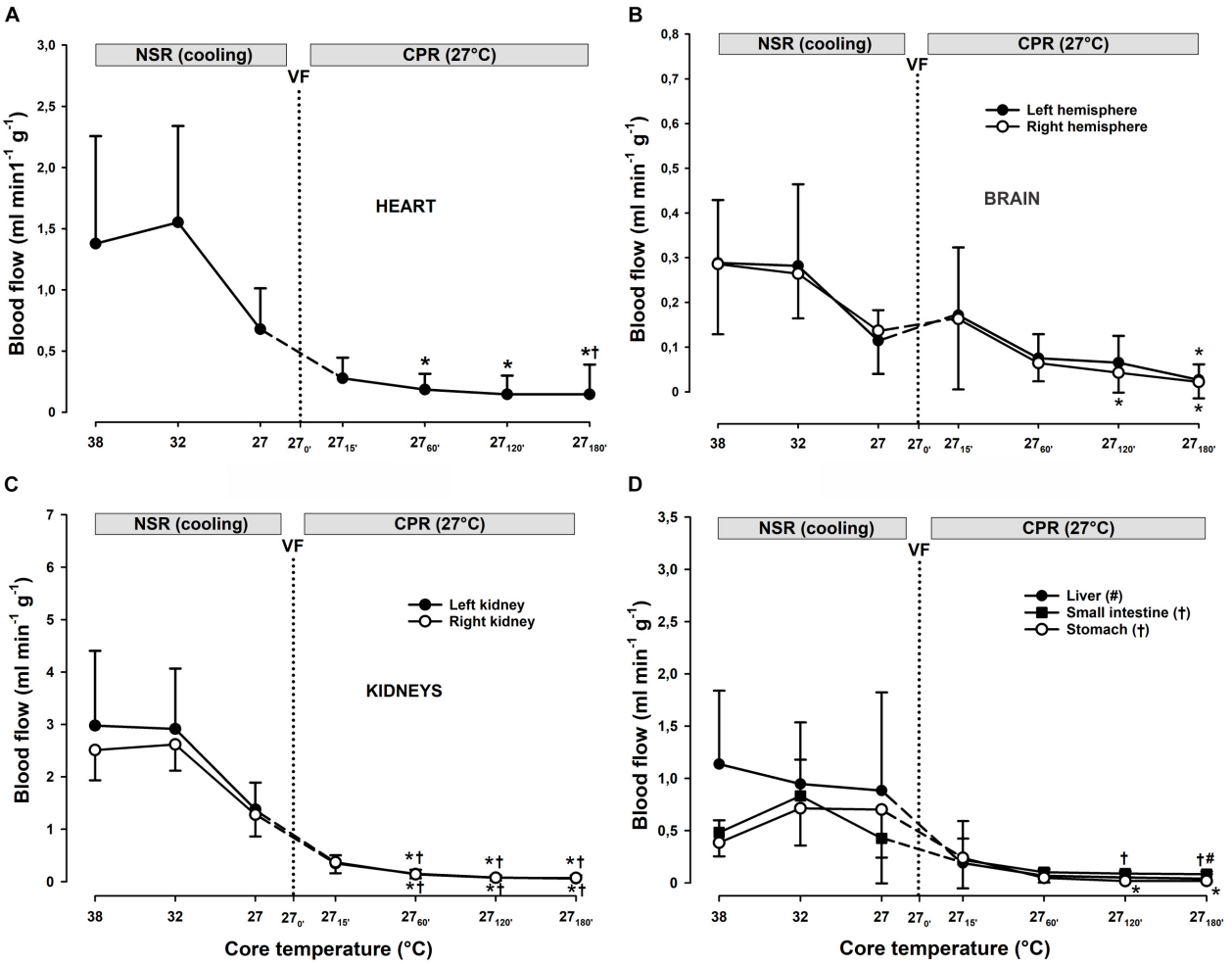

FIGURE 6 | Regional blood flow during cooling and 3 h CPR at 27°C (n = 7). **(A–D)** Regional blood flow. Normal sinus rhythm (NSR); cardiopulmonary resuscitation (CPR); ventricular fibrillation (VF). To compare values within groups, we used one-way repeated measures ANOVA for normal distributed variables, and Friedman repeated measures ANOVA on ranks for non-normal distributed variables. Dunnett's test was used to compare all values obtained vs. 38°C baseline and also for values obtained during CPR vs. corresponding values after CPR for 15 min. Values are mean ± SD. $^{*}p < 0.05$ vs. intragroup 38°C baseline; $^{\#}$, $^{\dagger}p < 0.05$ vs. intragroup 15 min of CPR at 27°C.

generated at the same reduced levels irrespective of the core temperature. This corresponds with our working hypothesis. Continued CPR for a total of 3 h at 27°C maintained MAP and CO, blood flow to the brain and heart at the same reduced level, and provided adequate DO_2 to support aerobic metabolism. The results also indicate the presence of a compensatory autoregulation of organ blood flow during hypothermic CPR with critically reduced circulation. By contrast, after 45 min of CPR at 38°C, MAP was significantly reduced, and blood flow to vital organs became undetectable in parallel with derangements in organ metabolism.

Cardiopulmonary Resuscitation

The European Resuscitation Council guidelines (2015) (Truhlar et al., 2015) recommend that in accidental hypothermia patients with CA, CPR should be started immediately and continued during evacuation and transport. These guidelines also advocate using mechanical chest compression devices in special scenarios, e.g., during rescue of hypothermic patients in a moving ambulance, even though it is still debated if compression devices are superior to manual compression for CA (Rubertsson and Karlsten, 2005; Aufderheide et al., 2011; Putzer et al., 2013).

Several studies using the pig as experimental model have explored the effects of different techniques for CPR on pressure generation and organ blood flow during normothermia, with and without the aid of compression devices (Wik et al., 1996; Sunde et al., 1997, 1998; Langhelle et al., 2002; Schwarz et al., 2002; Pytte et al., 2006; Tomte et al., 2010; Karlis et al., 2014). Alternative techniques most often tested are active compression–decompression CPR (ACD-CPR) with and without use of an impedance threshold valve CPR (ITV-CPR) in comparison to standard CPR (Langhelle et al., 2002; Schwarz et al., 2002). These studies consistently reported better pressure end flow generation during ACD-CPR, attributed the elevated negative intrathoracic

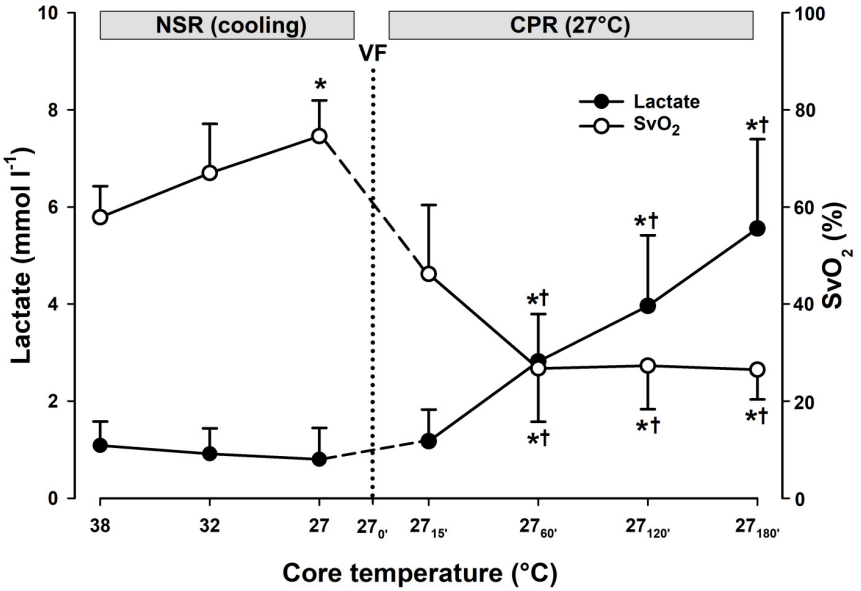

FIGURE 7 | Lactate and mixed venous oxygen saturation during cooling and 3 h of CPR at 27°C (n = 7). Mixed venous oxygen saturation (SvO_2); normal sinus rhythm (NSR); cardiopulmonary resuscitation (CPR); ventricular fibrillation (VF). To compare values within groups, we used one-way repeated measures ANOVA for normal distributed variables and Friedman repeated measures ANOVA on ranks for non-normal distributed variables. Dunnett's test was used to compare all values obtained vs. 38°C baseline and also for values obtained during CPR vs. corresponding values after CPR for 15 min. Values are mean ± SD. *$p < 0.05$ vs. intragroup 38°C baseline; †$p < 0.05$ vs. intragroup 15 min of CPR at 27°C.

pressure during active decompression facilitating better filling of the heart between compressions. Mechanical chest compression devices most often offer ACD-CPR, as used in the present experiment, but guidelines do not consider ACD-CPR superior to good standard CPR (Wenzel et al., 2010).

From clinical practice and preclinical experiments, we know that the effectiveness of normothermic CPR to maintain blood flow rapidly deteriorates. In the present experiment, hemodynamics and organ blood flow were largely maintained during the first 2 h of hypothermic CPR. After that, a modest reduction in these variables was accompanied with a small increase in lactate production, but far from ending in a circulatory collapse as demonstrated in normothermic animals after 45 min of CPR.

Hemodynamics and Blood Flow

In pig models of normothermic CPR, hemodynamic and organ blood flow data are well documented after 5–10 min of CPR (Sunde et al., 1997, 1998; Langhelle et al., 2002; Steen et al., 2002). The present study evaluated the effects of CPR after 15 min, and the actual hemodynamic results are comparable to those reported by Carretero et al. (2010) after 15 min and by Steen et al. (2002) after only 5 min of CPR. Similar to our results, Gervais et al. (1997) reported almost complete cessation of myocardial and cerebral blood flow after 45 min of normothermic CPR.

Oxygen Transport

With compromised circulation, VO_2 will eventually become dependent on the DO_2. The O_2 extraction ratio (ER), the ratio between VO_2 and DO_2, can be used to demonstrate this physiologic mechanism. With limited O_2 supply, ER approaches a critical value, ER_{crit}, where tissue O_2 consumption is ruled by O_2 supply (Schumacker et al., 1987), reported to be 0.6–0.7 (Leach and Treacher, 1992) at normothermia. During hypothermia, the ER_{crit} is unchanged (∼0.65) (Schumacker et al., 1987), even with an increase in vascular resistance, which may override the physiologic local metabolic feedback to arterioles to regulate blood flow (Schumacker et al., 1987). During spontaneous circulation at 27°C, the reduced metabolism caused a significantly reduced VO_2 in parallel with the reduced DO_2. Importantly, we found that during the first 2 h of CPR at 27°C, despite the 74% reduction in CO, DO_2 was maintained at the same low level as during spontaneous circulation. Beyond 2 h of CPR, a significant fall in DO_2 took place, followed by a moderate, yet significant, increase in lactate production and a fall in SvO_2. For comparison, also in our previous pig experiment mentioned with 3 h of spontaneous circulation at 27°C, we found an elevation of ER to a level approaching ER_{crit} indicating a marginal O_2 supply during hypothermia, but this marginal supply was followed by a successful rewarming (Valkov et al., 2019).

Taken together, the actual values for lactate production, SvO_2, and ER demonstrate that 3 h of CPR at 27°C provides marginal, but sufficient, O_2 transport for aerobic metabolism in vital organs. Importantly, we also found that VO_2 was maintained during 3 h of CPR, indicating a use-dependent O_2 consumption. This is in essential contrast to normothermic CPR where DO_2 after 15 min was unable to provide aerobic metabolism, reflected by the significant elevation of ER exceeding the ER_{crit} value, in parallel with a substantial elevation in serum lactate and lowering of SvO_2.

LIMITATIONS

The porcine model was chosen in our experiment because of its previous use in exploring the effects of CPR, especially when using a compression device (Steen et al., 2002; Rubertsson and Karlsten, 2005). However, the incidence of fractures of the sternum and multiple costa after 3 h of CPR in all our animals clearly demonstrates a major limitation when applying equipment designed for humans. A multicenter survey has documented the absence of fatal injuries in patients after conventional CPR using a compression device (Smekal et al., 2014), but little is known about damage to the thoracic skeleton or to the heart of patients after prolonged mechanical chest compressions.

Potential Translational Value

From a clinical perspective, to date, the only possible way to treat patients with continuous CPR during evacuation and transport in an ambulance car is by using an automated chest compression device. Therefore, to treat accidental hypothermia patients with cardiac arrest in our catchment area, we aim to equip all car ambulances with automated compression devices. Further, the beneficial physiologic effects of prolonged CPR for cardiac arrest at reduced core temperatures, emerges to build a new basis with unknown potential for patient survival after the ensuing in-hospital rewarming. From clinical reports already listed, extracorporal circulation has evolved to become the method of choice for rewarming these patients. Preferentially, extra corporal membrane oxygenation (ECMO) is increasingly being used. By applying ECMO for rewarming, cardio/respiratory support can be continued during an ensuing ICU stay for days, if needed. This is to treat rewarming injury, which often manifests itself as cardiac dysfunction caused by hypothermia/rewarming, in addition to the unknown consequences of prolonged CPR to cause physical damage to the heart, lungs, and the thoracic skeleton. However, although effects of extracorporal rewarming to increase survival seems promising, more experimental and clinical work is needed to customize this method for rewarming patients from accidental hypothermia.

CONCLUSION

The results of this study support our working hypothesis and show the favorable effects of CPR when given at 27°C in comparison to normothermic CPR. The beneficial effects of CPR in providing a limited, but adequate, DO_2 during a 3 h period at 27°C, combined with an apparent patent peripheral circulatory function, make continuous CPR the most valuable prehospital intervention as a bridge for hypothermic patients to receive in-hospital rewarming.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

JN, TT, GS, and TK contributed to the conception and design. JN, SV, RM, TS, TN, and TK contributed to the completion of experiments and collection of data. TT, GS, TK, JN, SV, RM, and TS contributed to the data analysis and interpretation. JN, TT, and GS contributed to the drafting the manuscript for intellectual content. JN, SV, RM, TS, TK, TN, GS, and TT contributed to the revision of the manuscript.

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Paper 3

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Effects of Rewarming with Extracorporeal Membrane Oxygenation to Restore Oxygen Transport and Organ Blood Flow After Hypothermic Cardiac Arrest in a Porcine Model.

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Research

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Abstract

Background: We recently documented that cardiopulmonary resuscitation (CPR) for hypothermic cardiac arrest maintains cardiac output (CO) and mean arterial pressure (MAP) to the same reduced level during normothermia (38°C) vs. hypothermia (27°C). Furthermore, continuous CPR at 27°C maintains CO and MAP throughout a 3-h period, and provides O_2 delivery to support aerobic metabolism. The aim of the present study was to investigate the effects of extracorporeal membrane oxygenation (ECMO) rewarming to restore O_2 delivery and organ blood flow.

Methods: Eight male pigs were anesthetized and immersion cooled to 27°C. After induction of hypothermic cardiac arrest, CPR was started and continued for a 3-h period. Thereafter, the animals were rewarmed with ECMO. Organ blood flow was measured using microspheres.

Results: After cooling with spontaneous circulation to 27°C, MAP and CO were initially reduced to 66 and 44% of baseline, respectively. By 15 min after the onset of CPR, there was a further reduction in MAP and CO to 42 and 25% of baseline, respectively, which remained unchanged throughout the rest of 3-h CPR. During CPR, O_2 delivery and O_2 uptake ($\dot{V}O_2$) fell to critical low levels, but the simultaneous small increase in lactate and a modest reduction in pH, indicated the presence of maintained aerobic metabolism. Rewarming with ECMO restored MAP, CO, O_2 delivery, and blood flow to the heart and to parts of the brain, whereas flow to kidneys, stomach, liver and spleen remained significantly reduced.

Conclusions: CPR for 3-h at 27°C with sustained lower levels of CO and MAP and maintained aerobic metabolism sufficient to support O_2 delivery. Rewarming with ECMO restores blood flow to the heart and brain, and creates a “shockable” cardiac rhythm. Thus, like continuous CPR, ECMO rewarming plays a crucial role in “the chain of survival” when resuscitating victims of hypothermic cardiac arrest.

Introduction

During the past decade, the overall mortality of accidental hypothermia patients has decreased from 52–80% in previous reports [1, 2] to the present 28–35% [3–6]. This favorable outcome is, however, closely linked to accidental hypothermia patients with maintained spontaneous circulation during rescue and rewarming, whereas survival rate of patients in hypothermic cardiac arrest during rescue is much lower. The recommended treatment of hypothermic cardiac arrest is rapid transfer of the patient under continuous cardiopulmonary resuscitation (CPR) to a hospital equipped for in-hospital rewarming using extracorporeal membrane oxygenation (ECMO) [7].

Case reports of accidental hypothermia patients in hypothermic cardiac arrest, also from our own hospital, have documented survival with favorable neurologic outcome [8–13]. The common denominator in these case reports is that they involve all elements of the “chain of survival”. Survival rates without neurologic impairment after ECMO rewarming ranges from 47 to 63% in different studies [8, 14–16]. Thus, there is potential to improve treatment of this hypothermic cardiac arrest patient group to

further lower their mortality rate. This view finds support by a recent survey reporting no change in survival rate over the last 30 years when using cardio-pulmonary bypass (CPB) to rewarm patients in hypothermic cardiac arrest [17]. After extracorporeal rewarming these patients often need cardio-pulmonary support, making ECMO the preferred rewarming method [16], which can be continued for days, if needed.

To improve the treatment of accidental hypothermia patients, it is important to systematically investigate the effects of all treatment modalities applied during rescue and transport. Optimally, this new knowledge should be collected when analyzing data from hypothermia registries of accidental hypothermia patients, but such registries are relatively new [18], and the volume of patient data is still limited. Therefore, detailed new information needs to be collected in preclinical animal experiments.

Accordingly, we have established a porcine model of accidental hypothermia and rewarming. In this model we previously have reported that after successful rewarming from 3-h at 27 °C with maintained spontaneous circulation, due to patency of adequate physiologic compensatory responses [19] blood flow distribution and O_2 availability was preserved in vital organs despite the existence of a significantly reduced CO [19, 20]. In this model we recently have documented that after 15 min of CPR for ventricular fibrillation at 38 °C vs. 27 °C, hemodynamic function, global O_2 delivery, and organ blood flow were generated at the same reduced levels irrespective of core temperature [21]. Furthermore, in contrast to the circulatory collapse that occurs by 45 min of CPR at 38 °C, continued CPR for up to 3-h at 27 °C provided adequate O_2 delivery to support aerobic metabolism in critical organs, apparently due to a patency of adequate physiologic compensatory responses even during these circumstances [21]. The reason for testing 3-h CPR is that our hospital is located in a scarcely populated catchment area, above the Arctic Circle, making evacuation and transportation time with air ambulance typically 3–4 h for patients with hypothermic cardiac arrest in need of in-hospital rewarming.

However, it remains to be determined if resuscitation by rewarming will lead to acute survival and restitution of function in vital organs. Our model of hypothermic cardiac arrest may benefit from hypothermia-induced reduction of metabolism during CPR, but during rewarming challenges related to “the post-cardiac arrest syndrome” may occur during reperfusion of organs after low flow ischemia [22]. Accordingly, the aim of the present study was to evaluate if spontaneous cardiac activity and O_2 delivery to critical organs can be re-established during ECMO rewarming following 3-h of continuous CPR at 27 °C in a porcine model.

Materials And Methods

Animals

This study on eight male pigs (24.5–33.0 kg) from a Norwegian stock (Noroc) was approved by the National Animal Research Authority and conform to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. The animals, which

received humane treatment in accordance with The Norwegian Animal Welfare Act, were penned for 3–5 days in our animal facility after arrival, fed twice daily and had free access to water at all times.

Anaesthesia and Instrumentation

The methods for hemodynamic monitoring, immersion cooling, and organ blood flow measurements in the porcine model have been previously reported in detail [19]. Briefly, the animals were fasted overnight and premedication was administered in the pen by an intramuscular bolus of ketamine 20 mg/kg, midazolam 30 mg and atropine 1 mg. After transfer to the research lab, venous access was established in an ear-vein and anaesthesia was induced by an intravenous bolus of fentanyl 10 µg/kg and pentobarbital sodium 10 mg/kg. Continuous anaesthesia was established with intravenous infusion of fentanyl 20 µg/kg/h, midazolam 0.3 µg/kg/h and pentobarbital sodium 4 mg/kg/h. Anaesthesia was discontinued when core body temperature reached 27 °C and re-introduced during the rewarming period.

After induction of anaesthesia, a primary tracheostomy was performed to secure the airway, and following intubation, the animals were ventilated with a positive end-expiratory pressure of 0 cm H_20 (Siemens Servo 900D, Solna, Sweden). During ventilation, FIO_2 was adjusted to maintain PaO_2 > 10 kPa and alveolar ventilation was adjusted to maintain $PaCO_2$ at 4.5–6 kPa uncorrected for temperature (α-stat). During ventricular fibrillation and CPR, FIO_2 was set to 1.0.

All vascular catheters were placed under ultrasound guidance. An 8-French sheath (Edwards Lifesciences, Irvine, CA, USA) was placed into the left femoral vein to ensure rapid establishment of continuous intravenous anaesthesia. A 7.5-French thermodilution catheter (Edwards Lifesciences, Irvine, CA, USA) was inserted through the sheath in the left femoral vein and advanced to the pulmonary artery. A thermodilution technique as described by Carretero and colleagues [23] was used to measure CO also during CPR. A 8-French Super Arrowflex (Arrow international Inc., Reading, PA, USA) sheath was placed in the left femoral artery and a 7.5-French catheter (Edwards Lifesciences, Irvine, CA, USA) was introduced into the aortic arch. A 10-French Super Arrowflex sheath (Arrow international Inc., Reading, USA) was placed in the right carotid artery, and a 6-French pigtail catheter (Cordis Corporation, Miami, FL, USA) was introduced into the left ventricle of the heart. A 3 mm flow probe (Cardiomed AS, Norway) was placed on the left carotid artery. An 18-gauge central venous catheter (Arrow international Inc., Reading, PA, USA) was placed retrograde into the left jugular bulb. Three 6-French sheaths (Cordis Corporation, Miami, FL, USA) were placed in the right jugular vein, right femoral vein and right femoral artery, respectively to allow for placement of ECMO cannulas during the last 30 min of CPR. A 3.5-French pressure catheter (SPR-524, Millar Instruments Inc., Houston, TX, USA) was introduced into the left hemisphere of the brain through a burr-hole in the skull. A 14-French urinary catheter was introduced into the bladder through a small incision in the abdomen. After instrumentation, a single dose of 5000 IU Heparin was given. The animals were allowed to stabilize for 45 min before starting the experimental protocol.

ECMO Circuit

The ECMO circuit was built with 3/8″ tubes for the main circuit and 1/4″ tubing for the cannulas. Dual venous tubes were used to provide venous blood for the oxygenator and heat/exchanger (Quadrox-I Adult, Maquet Cardiopulmonary AG, Hirrlingen, Germany), and the oxygenated blood was then pumped into the artery by a centrifugal pump (Rotaflow, Maquet Cardiopulmonary AG, Hirrlingen, Germany). The ECMO circuit was primed with 1000 ml ± 100 ml Ringer's lactate solution. Correct position of all guidewires and cannulas were verified by x-ray. The 6-French sheaths were used as ports to ensure rapid percutaneous placement of the ECMO cannulas. First a guidewire was inserted through the sheath, then the 6-French sheath was removed, and the flexible guidewire was replaced with a rigid guidewire (Amplatz super-stiff, Boston Scientific, Marlborough, MA, USA) via a guiding sheath. The vessels were then dilated in several steps up to 16-French. This was done on the right jugular vein, the right femoral vein and the right femoral artery. A 15-French x 18 cm ECMO cannula (Bio-Medicus, Medtronic Inc., Minneapolis, MN, USA) was inserted into the right jugular vein, and another into the right femoral artery. A 15-French x 50 cm venous ECMO cannula (Bio-Medicus, Medtronic Inc., Minneapolis, MN, USA) was inserted into the right femoral vein and advanced to the right atrium.

Experimental Protocol (Fig. 1)

After immersion cooling in ice water to a blood-temperature of 27 °C, hypothermic cardiac arrest was induced by stimulating the epicardial surface with an alternating current (5–20 mA, 6 Hz, and 30 V). To achieve this, a 15 cm long needle electrode was inserted in the epigastric area and directed towards the apex of the heart guided by suctioning of blood from the left ventricle. Hypothermic cardiac arrest was defined as the appearance of ventricular fibrillation on electrocardiogram simultaneous with absence of fluctuation in arterial pressure. After 90 s of hypothermic cardiac arrest, CPR was started using an automated chest compression device (LUCAS chest compression system, Physio-Control Inc., Lund, Sweden). Active decompression was achieved as the piston on this compression device was equipped with a suction cup to ensure a continuous compression/decompression mode with a duty cycle of 50 ± 5% at a frequency of 100 ± 5 compressions/min. Compression depth was 4–5 cm, and CPR was continued for 3 h. After 2.5 h of CPR, ECMO-instrumentation was started in order for all cannulas to be in placed at the 3-h mark. The animals were first rewarmed on ECMO (5 °C temperature gradient between core and arterial blood) to a blood temperature of 32 °C, and stabilized for 10 min, before blood sampling and recordings were performed. Electric cardioversion was attempted at 100 J up to three times. The animals were then rewarmed to a blood temperature of 38 °C (max heat exchanger temperature 38 °C), Thereafter, blood sampling and recordings were repeated and cardioversion was tried again. Based on the occurrence of multiple costae and sternal fractures made in our previous experiment after 3-h CPR [21], if cardioversion was unsuccessful after 3 shocks, a sternotomy was made to evacuate extravascular blood, followed by internal defibrillation (5–15 J). The experiment was then concluded and the animals euthanized.

Sampling

Mean arterial pressure (MAP), heart rate (HR), intracranial pressure (ICP), central venous pressure (CVP), and urinary output were recorded using PowerLAB 16/35 and LabChart software (ADInstruments,

Dunedine, New Zealand). CO was measured by thermodilution using 10 ml cold saline injected into the pulmonary artery catheter and recorded on a Vigilance monitor (Edwards Lifesciences, Irvine, CA, USA). All samplings were obtained at three core temperatures: baseline 38 °C, during cooling at 32° and at 27 °C. Samples were also obtained during CPR at 15, 60, 120 and 180 min, and during rewarming at 32° and 38 °C. At all sampling points, approximately 10 million stable isotope labelled 15 μm microspheres (BioPAL Inc., Worcester, MA, USA) were injected to determine organ blood flow. Differently labelled microspheres were used at all sampling points. During cooling and CPR, the microspheres were injected into the left ventricle through the pigtail catheter. During rewarming, the microspheres were injected through the injection port on the arterial ECMO cannula. Reference blood samples were drawn from the aortic arch (5 ml/min, 2 min) simultaneously with microsphere injections. Blood flow was determined in tissue samples from the brain, kidneys, liver, heart, small intestine, stomach and spleen based on a technique of neutron activation to analyse microsphere content as already described in details [19].

Calculations

Blood gases were analysed in arterial, central venous, and the jugular bulb samples using ABL800 FLEX (Radiometer medical, Copenhagen, Denmark). O_2 content (ml/100 ml) values (arterial, central venous, and jugular bulb) was calculated according to the formula: $SaO_2 \times Hb \times (1.34 \times 10^{-2}) + 0.0031 \times PO_2 \times 7.5$, where SaO_2 is blood O_2 saturation (%), Hb is haemoglobin (g/dl) determined in venous blood, and PO_2 is partial oxygen tension in blood (kPa). Global O_2 delivery was calculated as the product of CO and arterial O_2 content per kg body weight (ml/min/kg). Global O_2 uptake ($\dot{V}O_2$) was calculated as the product of CO and the difference between arterial and venous O_2 content per kg body weight (ml/min/kg). Cerebral O_2 delivery was calculated as the product of cerebral blood flow in ml/100 g brain tissue and arterial O_2 content (ml/min/100 g). Cerebral $\dot{V}O_2$ was calculated as the product of cerebral blood flow in ml/100 g brain tissue and the difference between arterial and jugular bulb O_2 content (ml/min/100 g). Cerebral blood flow values were calculated as mean of pooled data of left and right brain blood flow (temporal lobes and cerebellum). Global and cerebral O_2 extraction rate was calculated as the ratio of corresponding O_2 uptake to O_2 delivery values. Cerebral perfusion pressure was calculated as a difference between MAP and CVP.

Biochemistry

The selection of biomarkers to monitor organ function and to detect organ injury were based on a previous report [24]. Alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP), amylase, total bilirubin, creatinine, lipase, urea, and γ-glutamyl transferase (γ GT) were analysed by a colorimetric method in plasma samples using a Cobas 8000 analyser (Roche Diagnostics GmbH, Mannheim, Germany). Porcine soluble protein-100β (s-100β), porcine adrenomedullin (ADM), porcine activin A, porcine neuron-specific enolase (NSE), porcine glial fibrillary acidic protein (GFAP), porcine ubiquitin carboxyl terminal hydrolase L1 (UCHL1), porcine creatine kinase MB isoenzyme (CK-MB), and porcine cardiac troponin T type 2 (cTn-T) were analysed in plasma samples using ELISA kits (MyBioSource Inc., San Diego, CA, USA, and Nordic BioSite AB, Täby, Sweden).

Statistics

Statistical analysis was performed using Sigma Plot statistical software version 14 (Systat Software Inc. (SSI), Richmond, CA, USA). Normal distribution was assessed using the Shapiro-Wilk test. Intragroup comparisons were performed by one-way repeated measures ANOVA for normal distributed variables, and Friedman repeated measures ANOVA on ranks for non-normal distributed variables. Where significant differences were found, Dunnett's test was used to compare values within group vs. baseline. The level of significance was set at $p < 0.05$. Data are presented as means and SD.

Results

All animals had spontaneous circulation during cooling to 27 °C, and hemodynamic variables and electrocardiogram were continuously monitored. At predetermined core temperatures, arterial blood gases, central venous- and jugular bulb O_2 % saturation, and organ blood flow were determined during cooling, after 3-h of CPR at 27 °C, and after rewarming.

CO was measured using two different methods; thermodilution and by microspheres. After comparing individual CO values at the different measuring points a high level of correlation (r = 0.96) was demonstrated by using the two different techniques of measuring CO, values measured by thermodilution were in average 6–10% higher than with microspheres.

Immersion Cooling and 3-h CPR at 27 °C

Hemodynamics (Fig. 2A and B)

All statistical comparisons are made in reference to individual baseline (38 °C) values. Cooling reduced MAP significantly from 88 ± 11 to 76 ± 7 mmHg at 27 °C (-24%). After 15 min of CPR, MAP fell to 38 ± 8 mmHg (-58%), and remained at this reduced level throughout the remaining 3-h period of CPR. Similarly, after cooling to 27 °C, CO fell significantly from 3.6 ± 1.1 to 1.2 ± 0.4 l/min (-56%). After 15 min of CPR, CO fell even further to 0.9 ± 0.2 l/min (-75%) and remained at this reduced level during the remaining 3-h period of CPR.

O_2 Transport and Extraction (Fig. 3A and C)

Global O_2 delivery was reduced significantly during cooling to 27 °C from 15.2 ± 4.4 to 6.8 ± 1.9 ml/min/kg (-55%). Similarly, $\dot{V}O_2$ decreased during cooling to 27 °C from 6.8 ± 2.5 to 1.7 ± 0.6 ml/min/kg (-75%). After 15 min of CPR, O_2 delivery was further reduced to 4.3 ± 0.7 (-72%), and $\dot{V}O_2$ was reduced to 2.2 ± 0.5 (-68%) ml/min/kg, and both O_2 delivery and $\dot{V}O_2$ remained at these reduced levels throughout the remaining 3-h period of CPR. Global O_2 extraction ratio ($\dot{V}O_2/O_2$ delivery) was significantly reduced by cooling to 27 °C, and after 60 min of CPR, extraction ratio reached 0.68 ± 0.11,

the reported critical extraction ratio necessary to provide aerobic metabolism [25]. Due to the stable CO throughout the 3-h period of CPR period, extraction ratio remained at this elevated level.

Arterial lactate, pH, and central venous O_2 saturation (SvO_2) (Table 1)

Table 1
Plasma biochemical variables, and values for cerebral pressures

	38 °C	27 °C	27 °C$_{15}$ min	27 °C$_{3-h}$	RW 32 °C	RW 38 °C
pH	7.55 ± 0.05	7.42 ± 0.03*	7.4 ± 0.02*	7.20 ± 0.08*	7.27 ± 0.11*	7.39 ± 0.1*
Hb (g/dL)	8.2 ± 1.1	8.7 ± 1.2	9.2 ± 1.3	7.9 ± 0.9	5.5 ± 1.3*	5.3 ± 1.6*
Hct (%)	27 ± 2	27 ± 4	29 ± 4	25 ± 4	16 ± 4*	17 ± 5*
Lactate (mmol/l)	1.0 ± 0.7	0.5 ± 0.1	0.9 ± 0.3	5.2 ± 2.0	5.8 ± 2.6*	5.1 ± 2.6
BE (mmol/l)	5.6 ± 2.7	3.3 ± 3.5	2.2 ± 1.6*	-6.3 ± 2.4*	-8.9 ± 4.0*	-8.2 ± 3.3*
HCO_3- (mmol/l)	30 ± 3	27 ± 3	26 ± 1*	18 ± 2*	20 ± 2*	20 ± 3*
K^+ (mmol/l)	3.3 ± 0.4	2.6 ± 0.4	3.1 ± 0.3	5.3 ± 1.5*	4.5 ± 0.4	4.5 ± 0.7
PaO_2 (kPa)	13 ± 5	16 ± 4	46 ± 28*	15 ± 13	73 ± 3*	66 ± 2*
$PaCO_2$ (kPa)	4.4 ± 0.3	5.8 ± 0.8	5.8 ± 0.6	7.5 ± 1.5*	5.2 ± 1.9	3.7 ± 0.9
SaO_2 (%)	99 ± 2	99 ± 1	100 ± 0	82 ± 26	100 ± 0	100 ± 0
SvO_2 (%)	60 ± 11	78 ± 4	52 ± 15	21 ± 8*	68 ± 17	61 ± 17
SvO_2 jug.bulb (%)	58 ± 12	85 ± 13*	66 ± 21	26 ± 12*	59 ± 18	66 ± 12
CVP (mmHg)	6 ± 1	5 ± 2	19 ± 17*	14 ± 7	13 ± 6	15 ± 4
ICP (mmHg)	14 ± 3	13 ± 6	21 ± 5*	17 ± 4	17 ± 4	22 ± 7*
CPP (mmHg)	82 ± 11	62 ± 12	17 ± 9*	13 ± 5*	32 ± 21*	52 ± 32

Hct, hematocrit; BE, base excess; HCO_3-, bicarbonate; P_aO_2, arterial partial O_2 pressure; P_aCO_2, venous partial O_2 pressure; Sat_aO_2, arterial O_2 saturation; Sat_vO_2, venous O_2 saturation; CVP, central venous pressure; ICP, intra-cerebral pressure; CPP, cerebral perfusion pressure (CPP = MAP − CVP). n = 8, values are mean and SD. *$p < 0.05$ statistically significantly different from baseline value.

Cooling to 27 °C and the 3-h period of CPR caused an almost linear reduction in pH from 7.55 ± 0.05 to 7.20 ± 0.08, simultaneously with an increase in serum lactate from 0.98 ± 0.68 to 5.18 ± 1.96 mmol/l.

These changes in pH and lactate took place while SvO_2 fell from 57 ± 12 to 21 ± 5%.

Organ Blood flow (Fig. 4A-E, Table 2)

Table 2
Organ blood flow (ml/min/100 g)

	38 °C	27 °C	27 °C$_{15\ min}$	27 °C$_{3-h}$	RW 32 °C	RW 38 °C
Heart	153 ± 78	71 ± 28*	24 ± 15*	23 ± 20*	82 ± 46	107 ± 68
Left temp. lobe	35 ± 17	12 ± 7*	12 ± 6*	6 ± 9*	20 ± 26	17 ± 20
Right temp. lobe	32 ± 15	16 ± 5*	10 ± 4	3 ± 4*	17 ± 14*	14 ± 10*
Left cerebellum	42 ± 21	14 ± 5*	14 ± 3*	6 ± 6*	29 ± 24	27 ± 21
Right cerebellum	41 ± 24	16 ± 7*	12 ± 5*	5 ± 4*	27 ± 22	31 ± 28
Left kidney	242 ± 74	138 ± 37*	39 ± 28*	9 ± 7*	83 ± 41*	132 ± 56*
Right kidney	281 ± 96	157 ± 47*	44 ± 31*	9 ± 5*	95 ± 26*	134 ± 66*
Liver	112 ± 61	113 ± 92	20 ± 19*	5 ± 5*	19 ± 14*	54 ± 49*
Stomach	34 ± 13	47 ± 20	15 ± 15	3 ± 3*	12 ± 7*	20 ± 13
Small intestine	40 ± 15	49 ± 19	17 ± 12	8 ± 4*	28 ± 13	36 ± 40
Spleen	231 ± 77	78 ± 28*	19 ± 16*	4 ± 1*	26 ± 11*	35 ± 32*

n = 8, values are mean and SD. *$p < 0.05$ statistically significantly different from baseline value.

Compared to baseline, myocardial blood flow (Fig. 4A) was significantly reduced (-54%) after cooling to 27 °C. After ventricular fibrillation and 15 min CPR, myocardial blood flow was further reduced (-85%), and remained at this reduced level during the remaining 3-h period of CPR. After cooling to 27 °C, blood flow in the temporal lobes (Fig. 4B) was significantly reduced (left lobe − 65%, and right lobe − 51%). After 15 min of CPR, there was a further reduction in blood flow to the left and right temporal lobe, -64% and − 68%, respectively, and after 3-h of CPR, blood flow to the left and the right temporal lobes were reduced to -83% and − 91% of baseline, respectively. Compared to baseline at 38 °C, cooling to 27 °C significantly reduced blood flow to the left (-67%) and right (-61%) cerebellar hemispheres (Fig. 4C), but CPR for 15 min did not lead to any further reduction in blood flow. However, after 3-h of CPR, blood flow to the left (-87%) and the right (-88%) cerebellar hemispheres was further reduced. Abdominal organs showed a varying reductions in blood flow during cooling, as well as during 3-h period of CPR (Fig. 4D-F). After cooling to 27 °C, blood flow to the stomach and small intestine increased by + 38% and + 23%, respectively (Fig. 4D). However, after 3-h of CPR, blood flow to the stomach and small intestine was severely reduced by -90% and − 79% of baseline, respectively. Cooling to 27 °C significantly reduced blood flow to the right (-44%) and left (-43%) kidneys (Fig. 4E), and renal blood flow was almost completely shut off after 3-h of CPR (-97%, and − 96%, respectively). Liver blood flow was unaltered after cooling to 27 °C, whereas blood flow

to the spleen was reduced (-66%) (Fig. 4F). Both organs had severely impaired blood flow during CPR, and after 3-h, blood flow was reduced by -97% in the liver and − 99% in the spleen compared to baseline at 38 °C.

ECMO rewarming

The mean time required to rewarm blood temperature to 32 °C was 17 min with an additional 45 min to rewarm blood temperature to 38 °C. Ringer acetate was added to the ECMO circuit to maintain venous access pressure above − 100 mmHg.

Return of cardiac rhythm

All 8 pigs achieved sinus rhythm on ECMO, one spontaneously at 29 °C, two after cardioversion at 32 °C, and two after cardioversion at 38 °C. Three animals resumed a sinus rhythm only after sternotomy and pericardiotomy, to evacuate blood congesting in the mediastinum and in pericardium, before internal cardioversion at 38 °C.

Blood flow, pressure, and rewarming rate during ECMO

In the ECMO circuit, CO and MAP (Fig. 2A and B) were adjusted to mimic hemodynamic characteristics of the individual animal during cooling. Statistical comparisons were based on comparisons to individual baseline (38 °C) values. At 27 °C, the circuit was started at a flow of 1 l/min, gradually increased to 2.0–2.5 l/min at 32 °C, and finally 3.0–3.5 l/min at 38 °C. No vasoactive pharmacologic intervention was required to adjust pressure generation during rewarming. In each experiment, total volume of Ringer acetate added varied between 2,000–6,000 ml (mean 3,750 ml).

O_2 transport and extraction (Fig. 3A-C)

Global O_2 delivery increased during ECMO rewarming but at 38 °C, it was still significantly reduced compared to baseline (15.2 ± 4.4 vs. 9.4 ± 3.0 ml/min/100 g; -38%). Similarly, global $\dot{V}O_2$ increased during rewarming, but at 38 °C, it was still reduced compared to baseline (6.8 ± 2.5 vs. 4.3 ± 1.1 ml/min/100 g; -34%).

During rewarming, cerebral O_2 delivery and $\dot{V}O_2$ (Fig. 3B) increased, but at 38 °C, they were both still reduced compared to baseline (cerebral O_2 delivery: 4.41 ± 2.14 vs. 2.11 ± 2.23; -50% and $\dot{V}O_2$: 2.08 ± 1.17 vs. 0.85 ± 0.87 ml/min/100 g; -56%). Both global and cerebral extraction ratio (Fig. 3C) fell to values < 0.7 during rewarming but at 38 °C, they were unchanged compared to baseline values.

After rewarming arterial pH levels (Table 1) returned to baseline, 7.39 ± 0.1 vs. 7.55 ± 0.05. Serum lactate levels (Table 1) were highest at 32 °C (5.8 ± 2.59), fell during rewarming to 38 °C, but were still significantly elevated compared to baseline (5.1 ± 2.62 vs. 0.98 ± 0.68 mmol/l). After rewarming SvO_2 (Table 1) was restored to 60%.

Organ Blood flow (Fig. 4A-E, and Table 2)

Rewarming elevated blood flow to all organs, but statistical analyses showed that blood flow returned to baseline in only a few organs. Myocardial blood flow was restored after rewarming. Similarly, blood flow to the left temporal lobe was restored, whereas blood flow to the right temporal lobe was still significantly reduced (-56%). Rewarming restored blood flow to both cerebellar hemispheres. In most abdominal organs, rewarming only led to partial restoration of blood flow. Blood flow to the stomach and small intestine was restored, whereas, compared to baseline, blood flow remained significantly reduced in the liver (-52%) and spleen (-82%). Compared to baseline 38 °C, renal blood flow was significantly reduced after rewarming in both the left (-45%), and right (-53%) kidney.

Plasma biomarkers (Table 3)

Table 3
Serum biomarkers for organ function and organ injury

ORGAN	38 °C	27 °C	27 °C$_{3-h}$	RW 38 °C
Brain				
S100β (pg/ml)	64 ± 44	40 ± 28	85 ± 56	89 ± 53
UCHL1 (pg/ml)	91 ± 12	108 ± 48	92 ± 9	95 ± 15
GFAP (pg/ml)	8.8 ± 5.6	8.4 ± 5.1	9.8 ± 4.9	14.7 ± 8.0*
NSE (ng/ml)	-	-	-	-
Heart				
CK-MB (ng/ml)	1.7 ± 1.9	2.0 ± 2.1	1.0 ± 0.8	1.2 ± 1.0
Troponin T (pg/ml)	65.8 ± 51.2	54.1 ± 47.2	47.4 ± 39*	49.2 ± 46.5*
Kidney				
Carbamide (mmol/l)	1.6 ± 0.3	2.0 ± 0.5	2.4 ± 0.6*	2.2 ± 0.7*
Creatinine (μmol/l)	57.1 ± 7.8	49.3 ± 11.3*	61.6 ± 12.5	47.4 ± 9.9*
Activin-A (pg/ml)	-	-	-	-
Liver/pancreas				
ASAT (U/l)	40.6 ± 5.3	51.4 ± 9.6	264.0 ± 81.6*	374.1 ± 226.5*
ALAT (U/l)	72.6 ± 16.8	67.2 ± 15.1*	70.4 ± 12.6	55.7 ± 12.1*
γ-GT (U/l)	31.1 ± 9.4	27.1 ± 6.8	22.8 ± 4.8*	15.4 ± 5.2*
Amylase (U/l)	1744 ± 562	1591 ± 478	1359 ± 466*	879 ± 352*
Lipase (U/l)	-	-	-	-
Bilirubin (μmol/l)	-	-	-	-
ALP (U/l)	-	-	-	-

UCHL1, Ubiquitin Carboxyl-terminal Esterase-L1; GFAP, Glial Fibrillary Acidic Protein; NSE, Neuron-Specific Enolase; CK-MB, Creatine Kinase-Muscle/Brain isozyme; ASAT, Aspartate Aminotransferase; ALAT, Alanine; Aminotransferase; γ-GT, γ-Glutamyl Transpeptidase; ALP, Alkaline Phosphatase. n = 8, values are mean and SD. *$p < 0.05$ statistically significantly different from baseline value. - indicate serum level below lower level of detection.

Significant (6–10 fold) increase in ASAT appeared after 3-h CPR and remained after rewarming. Also level of glial fibrillary acidic protein (GFAP) was increased after rewarming.

Discussion

This experiment demonstrates that following ventricular fibrillation at 27 °C (hypothermic cardiac arrest) and 3-h of CPR, O_2 delivery and organ blood perfusion were reduced but that ECMO rewarming provided CO, MAP, and O_2 delivery to support global aerobic organ metabolism. After rewarming to 38 °C, organ blood flow was unequally restored, but with an apparent preference to essential parts of the brain and heart indicating the patency of autonomic blood flow regulation to support O_2 delivery to critical organs. The results of this study also demonstrate that 3-h of continues CPR at 27 °C maintained MAP, CO, and blood flow to the brain, heart, liver, and spleen at the same reduced level, with adequate O_2 delivery to enable aerobic metabolism. In this respect, the findings of the present study are consistent with those of a previous study [21].

Resuscitation during hypothermia and normothermia

The results of the present study confirm that after ECMO rewarming, prognostic outcome markers are favourable as compared to survivors of normothermic cardiac arrest [26]. These markers include higher pH, low level of plasma lactate, and a shockable cardiac rhythm. In fact, the low core temperature present in hypothermic cardiac arrest, may provide protective effects that would mitigate the complex pathophysiologic processes created by the actual prolonged low-flow condition. Protective mechanisms created by low temperatures relate to the general slowing of enzymatic activities particularly those that are ATP-dependent. On the other hand, the protective effects of hypothermia may be partly offset by harmful effects created during exposure to low core temperature in the absence of ischemia or hypoxia, which may cause end-organ dysfunction. We previously have documented hypothermia-rewarming induced cardiac dysfunction in both *in vivo* and *in vitro* models [20, 27–31]. Underlying pathophysiologic mechanisms include derangement in metabolism and calcium homeostasis [20, 27, 28, 32], elevated protein kinase A levels with increased phosphorylation in myocardial contractile proteins [30, 31, 33], and reactive oxygen species formation [34]. Likewise, after rewarming, we have documented derangements in renal [35] and nervous tissue morphology [36]. Severity of these different pathophysiologic elements are closely related to duration and level of the hypothermic exposure with similarities to what takes place during normothermic low flow ischemia.

Reperfusion

Rewarming from hypothermia and reperfusion after ischemia share the same treatment strategy; to restore blood flow at the macro-vascular level in an attempt to optimize blood flow at the micro-vascular level to minimize end organ dysfunction. However, alterations in micro-vascular function frequently occur in critically ill patients and with clear implications to development of organ failure. Although reperfusion is the ultimate constituent when resuscitating an ischemic or hypoxic organ as during prolonged cardiac arrest, we still have limited knowledge about how the different organs respond to reperfusion. However, all reperfused organs are exposed to complex pathophysiologic processes causing uneven alterations in organ function, collectively termed the post-cardiac arrest syndrome [22]. We still do not understand how different organs respond to reperfusion after prolonged periods of ischemia or limited blood flow, as following hypothermic cardiac arrest. However, considerable variability in metabolic responses in critical

organs was recently reported to take place after 30 min of cardiac arrest, as well as after CPB, in an experimental model of cardiac arrest during normothermia [37].

Restitution of organ blood flow after rewarming

In the present experiment ECMO rewarming restored MAP and CO in parallel with a return to well below critical value for extraction ratio (0.6–0.7) [25], return of SvO_2, whereas global $\dot{V}O_2$ remained reduced. The reduced global $\dot{V}O_2$ may well be a mirror image of reduced organ function as heterogeneity in the recovery of organ blood flow was evident in most organs investigated.

The brain is the organ most sensitive to ischemic injury and is therefore the limiting organ for survival after cardiac arrest in general [38]. We observe that cerebral O_2 delivery and $\dot{V}O_2$ were both significantly reduced in parallel with a reduction in extraction ratio to far below critical levels. Also, if we compare to human data after cardiac arrest, a patent autoregulation of cerebral blood flow after reperfusion is suggested if decreased cerebral blood flow is matched to decreased $\dot{V}O_2$ [39]. Deliberate hypothermic cardiac arrest is used for repair of complex cardiovascular conditions, and for cerebral protection the safe use of cardiac arrest for up to 60 min at 8–13 °C [40–42] has been documented. In the present experiment biomarkers of brain injury disclose no pathological changes as GFAP and UCHL1 (Table 3), both highly selective for CNS injury [24], are within normal control levels in pigs [43].

Myocardial blood flow was normalized after the return of spontaneous electro-mechanic activity despite that external heart work was reduced as global circulation was provided by the ECMO circuit indicating the occurrence of reactive hyperaemia. Biomarkers of cardiac injury, CK-MB and Troponin T, were both within normal levels. The significant increase in ASAT is most probably caused by trauma of the thoracic muscles from the automated compression devise.

Reduced renal blood flow appears to be a consequence of the well-documented physiologic mechanisms that compensates for a sudden drop in MAP and/or CO as during cardiac arrest. The biomarker activin-A, also reported to be increased during acute renal failure, was beyond detection levels in our experiment. Reduced blood flow to the spleen can be observed after circulatory shock secondary to emptying stored erythrocytes into the blood stream as a compensatory mechanism [44]. However, the immediate restoration of blood flow to the small intestine and stomach, while liver blood flow was significantly reduced, is more difficult to interpret.

Adequacy of extracorporeal rewarming for macro- and micro-vessel reperfusion

The recommended treatment for hypothermic cardiac arrest patients is rapid transfer under continuous CPR to a hospital capable of rewarming by use of extracorporeal circulation [7]. The safe use of extracorporeal circulation, routinely applied as CPB during cardiac surgery, is supported by extensive preclinical and clinical research over the past 60 years. This would suggest that extracorporeal circulation/CPB would also be safe for rewarming accidental hypothermia patients. However, a comprehensive preclinical study is lacking. Obvious differences between cardiac surgery patients and

accidental hypothermia patients include the way cooling takes place, duration of the hypothermic insult, and patency of O_2 transport during the insult, factors that also may warrant different approaches for the use of extracorporeal circulation for rewarming.

Restitution of capillary flow is a key element when rewarming accidental hypothermia patients with extracorporeal circulation. Even after exposure to short-term hypothermia with maintained spontaneous circulation, intravascular erythrocyte aggregation has been reported [45], and other studies have documented that the size of intravascular erythrocyte aggregates during hypothermia was inversely related to blood flow [46]. These changes create a heterogeneous micro-vascular blood flow with perfused capillaries in close vicinity to non-perfused capillaries, which subsequently may cause alterations in tissue O_2 transport and hypoxia in organs despite restitution of global O_2 transport. In our effort to restitute systemic hemodynamic function, the micro-vascular hemodynamic function may suffer, a fact that underlines the existence of an uncoupling between macro and micro-vascular circulation [47]. Another important factor to compromise capillary integrity during hypothermia with spontaneous circulation is that increased extravasation of plasma from the intravascular to the interstitial space [48, 49] regularly takes place, and this extravasation is substantially increased when applying extracorporeal circulation for rewarming [49]. Extracorporeal circulation has evolved to become the method of choice for rewarming patients with hypothermic cardiac arrest. However, a recent review has documented impaired micro-vascular integrity as a consequence of CPB during cardiac surgery [50]. By use of sublingual micro-circulatory measurements, numerous reports have documented impaired micro-circulatory perfusion with subsequent reduction of functional capillary density, and these changes may last 24 h after CPB [50]. The reduction in functional capillary density after CPB shear great similarities with those taking place during hypothermia with spontaneous circulation mentioned above.

Taken together, this information points at alterations in capillary integrity taking place during accidental hypothermia, most likely due to spontaneous circulation with low CO and perfusion pressure, and the compromised microcirculatory function may be prolonged and even aggravated by adding extracorporeal circulation for rewarming. Therefore, future aim must be to establish a refined extracorporeal circulation system for rewarming, using CPB or ECMO, which has the ability to support micro-vascular integrity rather than prolong micro-vascular dysfunction. Based on promising clinical reports [16] the use of ECMO for rewarming from accidental hypothermia has been recommended as ECMO can also be continued after rewarming for cardio/respiratory support for days, if needed [16, 51].

Limitations. Perhaps as a consequence of using an automated chest compression device designed for human CPR [52], the use in our pig model resulted in multiple costa and sternum fractures in all animals. Furthermore, as a consequence of these fractures, blood congesting in the mediastinum and in pericardium necessitated surgical evacuation to manage cardioversion in three out of eight animals during rewarming. This may well be due to the prolonged 3-h period of CPR, although fatal injuries in human patients have not been documented [53] after conventional CPR using a compression device [54]. To determine organ blood flow, microspheres were injected into the left ventricle during cooling and CPR, but injected directly into a port on the arterial cannula during ECMO rewarming. This may have altered the

way microspheres were introduced into the circulation, which may have had impact on blood flow measurements.

Conclusions

This study shows that ECMO rewarming following 3-h of CPR at 27 °C restores hemodynamics and partially or fully re-establishes blood flow to critical organs. Our results showing normal pH albeit elevated lactate levels, absence of hyperkaliaemia, and restored cardiac electro-mechanical activity, support the conclusion that aerobic metabolism is at least partially sustained during hypothermic CPR and ECMO rewarming. Based on these findings, it is pertinent to advocate for continued prehospital CPR during transport of victims of severe accidental hypothermia to centres equipped for rewarming using extracorporeal circulation. Clinical reports favour the use of ECMO rewarming, but extensive research work is needed to optimize the use of extracorporeal circulation techniques for the rewarming of accidental hypothermia patients.

Abbreviations

CPR: cardiopulmonary resuscitation; HCA: hypothermic cardiac arrest; CO: cardiac output; MAP: mean arterial pressure; DO_2: O_2 delivery; ECMO: extracorporeal membrane oxygenation; ER: extraction rate; CPB: cardio-pulmonary bypass; VF: ventricular fibrillation; HR: heart rate; ICP: intracranial pressure; CVP: central venous pressure; VO_2: O_2 consumption; CPP: cerebral perfusion pressure; ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase; ALP: alkaline phosphatase; γ GT: γ-glutamyl transferase; s-100β: soluble protein-100β; ADM: adrenomedullin; NSE: neuron-specific enolase; GFAP: glial fibrillary acidic protein; UCHL1: ubiquitin carboxyl terminal hydrolase L1; CK-MB: creatine kinase MB isoenzyme; SvO_2: central venous O_2 saturation; PKA: protein kinase A; ROS: reactive oxygen species; ECC: extracorporeal circulation.

Declarations

Ethics approval and consent to participate

This study was approved by the National Animal Research Authority and conform to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

Consent for publication

Not applicable.

Availability of data and materials

All data are available upon reasonable request to the authors.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conception and design: JHN, TT, GCS, TS, TN, and TVK. Completion of experiments and collection of data: JHN, TS, SV, RM, TS, BH, and TVK. Data analysis and interpretation: TT, GCS, JHN, TVK, SV, RM and TS. Drafting the manuscript for intellectual content: JHN, TT, TS, and GCS. Revision of the manuscript: JHN, TS, SV, RM, BH, TVK, TN, GCS and TT.

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Figures

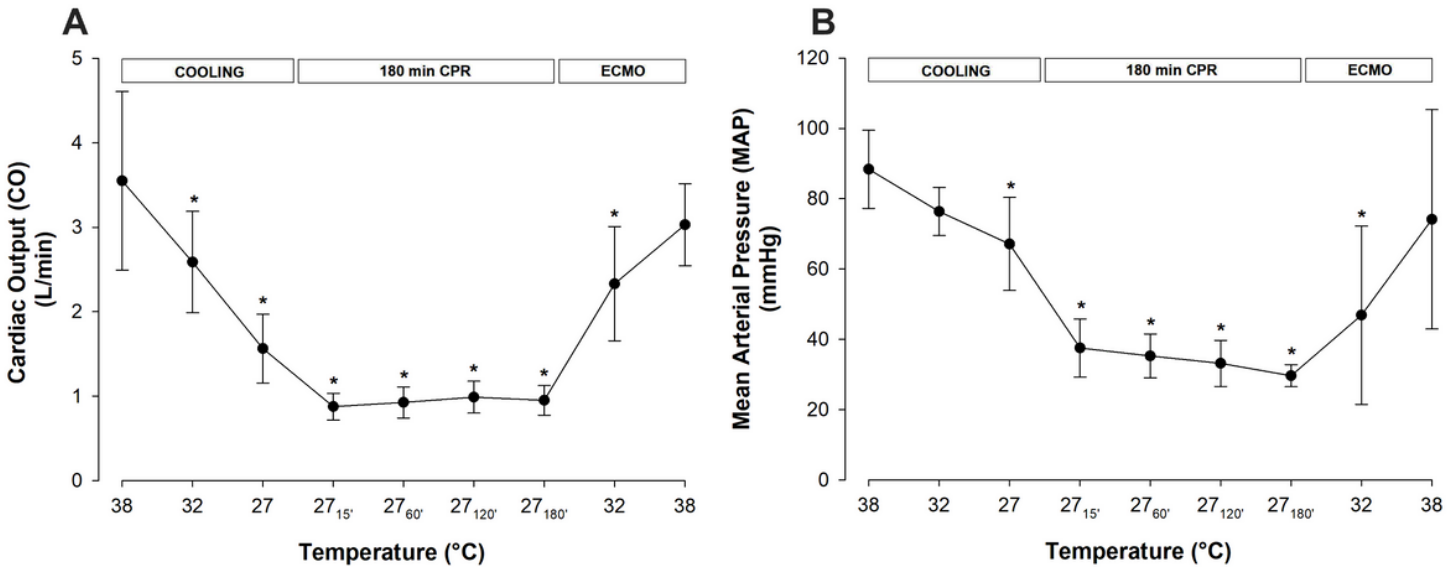

Figure 2

Measurements of hemodynamic function during cooling, 3-h CPR at 27°C, and ECMO rewarming. (A) Cardiac output. (B) Mean arterial pressure. n=8, values are mean ± SD. *p < 0.05 statistically significantly different from baseline value.

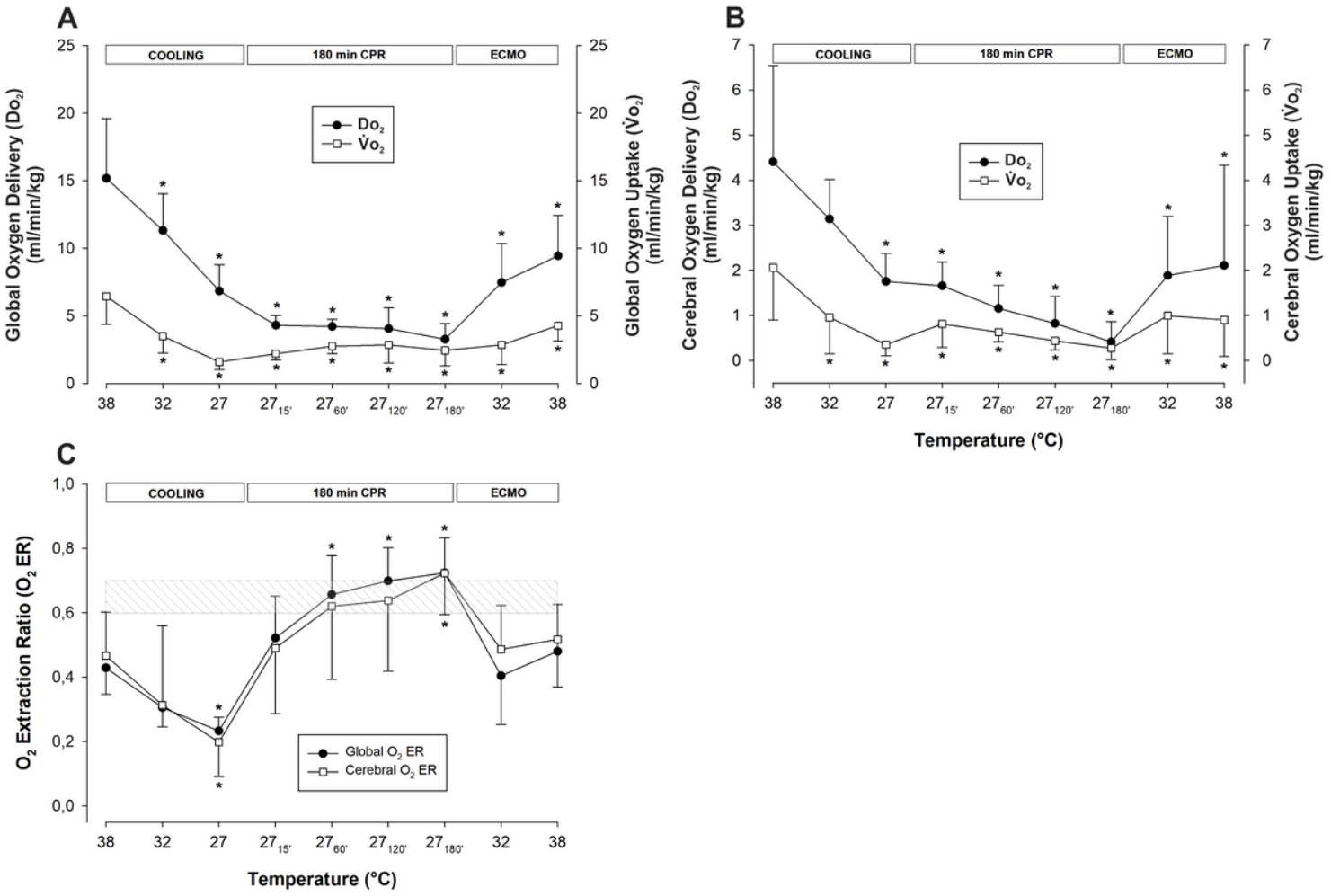

Figure 3

Global and cerebral oxygen delivery, uptake and oxygen extraction ratio. (A) Global oxygen delivery, and global oxygen uptake (V̇O2). (B) Cerebral oxygen delivery, and cerebral oxygen uptake (V̇O2). (C) Global and cerebral oxygen extraction ratio. n=8, values are mean ± SD. *p < 0.05 statistically significantly different from baseline value. Striated area indicates critical level of extraction ratio.

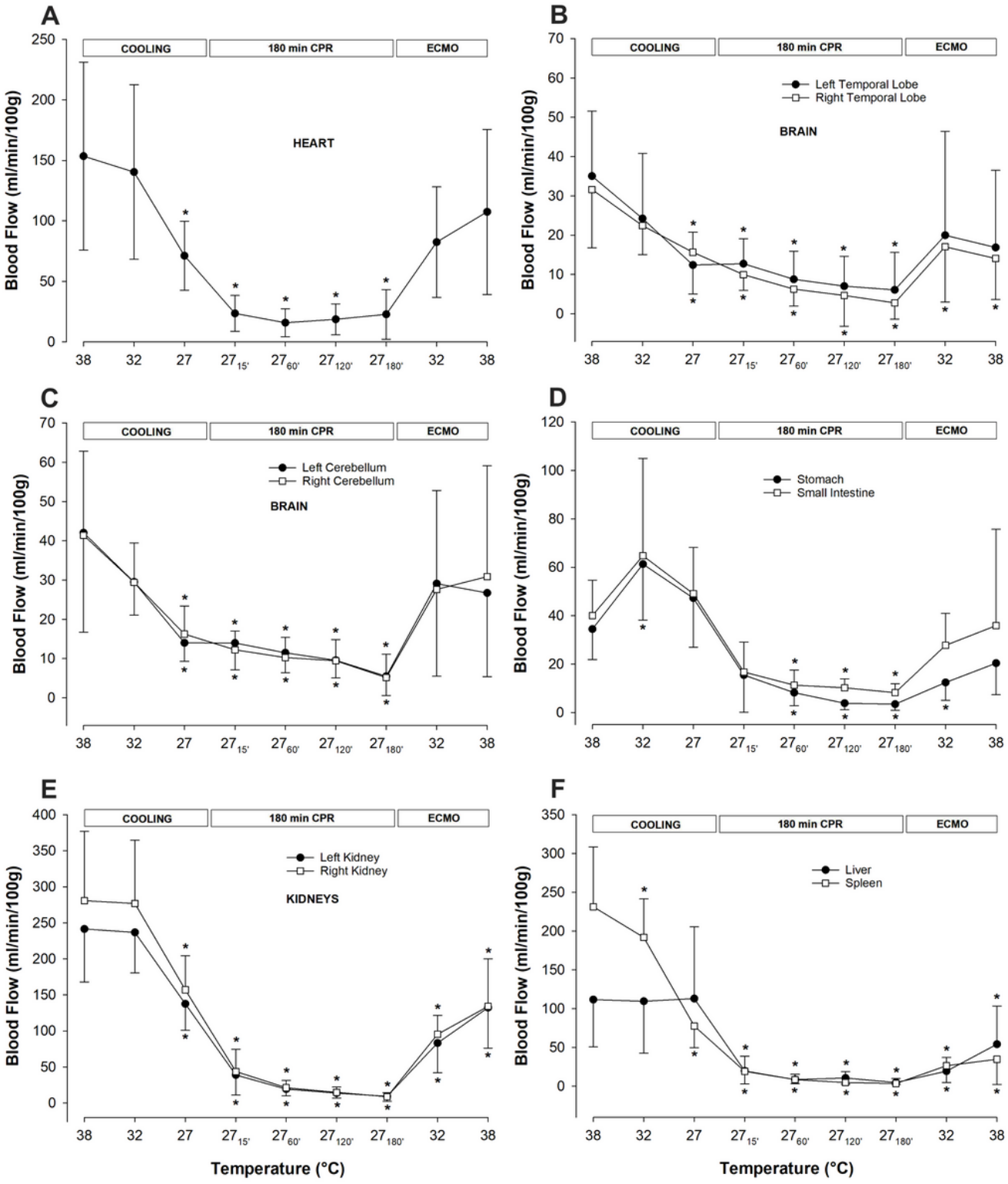

Figure 4

Regional blood flow during cooling, 3-h CPR at 27°C, and ECMO rewarming. (A) Myocardial blood flow. (B) Blood flow in left and right temporal lobes. (C) Blood flow in left and right cerebellar hemispheres. (D) Blood flow in stomach and small intestine. (E) Renal blood flow. (F) Blood flow in liver and spleen. n=8, values are mean ± SD. *p < 0.05 statistically significantly different from baseline value.