

# 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> delivery and O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>O (Siemens Servo 900D, Solna, Sweden). During ventilation, FIO<sub>2</sub> was adjusted to maintain PaO<sub>2</sub> > 10 kPa and alveolar ventilation was adjusted to maintain PaCO<sub>2</sub> at 4.5–6 kPa uncorrected for temperature ( $\alpha$ -stat). During ventricular fibrillation and CPR, FIO<sub>2</sub> 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  $\pm$  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  $\pm$  5% at a frequency of 100  $\pm$  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 place 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,

Dunedin, 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<sub>2</sub> 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<sub>2</sub> is blood O<sub>2</sub> saturation (%), Hb is haemoglobin (g/dl) determined in venous blood, and PO<sub>2</sub> is partial oxygen tension in blood (kPa). Global O<sub>2</sub> delivery was calculated as the product of CO and arterial O<sub>2</sub> content per kg body weight (ml/min/kg). Global O<sub>2</sub> uptake ( $\dot{V}O_2$ ) was calculated as the product of CO and the difference between arterial and venous O<sub>2</sub> content per kg body weight (ml/min/kg). Cerebral O<sub>2</sub> delivery was calculated as the product of cerebral blood flow in ml/100 g brain tissue and arterial O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> extraction rate was calculated as the ratio of corresponding O<sub>2</sub> uptake to O<sub>2</sub> 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<sub>2</sub> % 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  $88 \pm 11$  to  $76 \pm 7$  mmHg at 27 °C (-24%). After 15 min of CPR, MAP fell to  $38 \pm 8$  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  $3.6 \pm 1.1$  to  $1.2 \pm 0.4$  l/min (-56%). After 15 min of CPR, CO fell even further to  $0.9 \pm 0.2$  l/min (-75%) and remained at this reduced level during the remaining 3-h period of CPR.

### O<sub>2</sub> Transport and Extraction (Fig. 3A and C)

Global O<sub>2</sub> delivery was reduced significantly during cooling to 27 °C from  $15.2 \pm 4.4$  to  $6.8 \pm 1.9$  ml/min/kg (-55%). Similarly,  $\dot{V}O_2$  decreased during cooling to 27 °C from  $6.8 \pm 2.5$  to  $1.7 \pm 0.6$  ml/min/kg (-75%). After 15 min of CPR, O<sub>2</sub> delivery was further reduced to  $4.3 \pm 0.7$  (-72%), and  $\dot{V}O_2$  was reduced to  $2.2 \pm 0.5$  (-68%) ml/min/kg, and both O<sub>2</sub> delivery and  $\dot{V}O_2$  remained at these reduced levels throughout the remaining 3-h period of CPR. Global O<sub>2</sub> 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  $0.68 \pm 0.11$ ,

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<sub>2</sub> saturation (SvO<sub>2</sub>) (Table 1)

Table 1  
Plasma biochemical variables, and values for cerebral pressures

	38 °C	27 °C	27 °C <sub>15</sub> min	27 °C <sub>3-h</sub>	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 <sub>3</sub> <sup>-</sup> (mmol/l)	30 ± 3	27 ± 3	26 ± 1*	18 ± 2*	20 ± 2*	20 ± 3*
K <sup>+</sup> (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 <sub>2</sub> (kPa)	13 ± 5	16 ± 4	46 ± 28*	15 ± 13	73 ± 3*	66 ± 2*
PaCO <sub>2</sub> (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 <sub>2</sub> (%)	99 ± 2	99 ± 1	100 ± 0	82 ± 26	100 ± 0	100 ± 0
SvO <sub>2</sub> (%)	60 ± 11	78 ± 4	52 ± 15	21 ± 8*	68 ± 17	61 ± 17
SvO <sub>2</sub> 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<sub>3</sub><sup>-</sup>, bicarbonate; P<sub>a</sub>O<sub>2</sub>, arterial partial O<sub>2</sub> pressure; P<sub>a</sub>CO<sub>2</sub>, venous partial O<sub>2</sub> pressure; Sat<sub>a</sub>O<sub>2</sub>, arterial O<sub>2</sub> saturation; Sat<sub>v</sub>O<sub>2</sub>, venous O<sub>2</sub> 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<sub>2</sub> 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 <sub>15 min</sub>	27 °C <sub>3-h</sub>	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<sub>2</sub> transport and extraction (Fig. 3A-C)

Global O<sub>2</sub> delivery increased during ECMO rewarming but at 38 °C, it was still significantly reduced compared to baseline ( $15.2 \pm 4.4$  vs.  $9.4 \pm 3.0$  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 ( $6.8 \pm 2.5$  vs.  $4.3 \pm 1.1$  ml/min/100 g; -34%).

During rewarming, cerebral O<sub>2</sub> delivery and  $\dot{V}O_2$  (Fig. 3B) increased, but at 38 °C, they were both still reduced compared to baseline (cerebral O<sub>2</sub> delivery:  $4.41 \pm 2.14$  vs.  $2.11 \pm 2.23$ ; -50% and  $\dot{V}O_2$ :  $2.08 \pm 1.17$  vs.  $0.85 \pm 0.87$  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,  $7.39 \pm 0.1$  vs.  $7.55 \pm 0.05$ . Serum lactate levels (Table 1) were highest at 32 °C ( $5.8 \pm 2.59$ ), fell during rewarming to 38 °C, but were still significantly elevated compared to baseline ( $5.1 \pm 2.62$  vs.  $0.98 \pm 0.68$  mmol/l). After rewarming SvO<sub>2</sub> (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 <sub>3-h</sub>	RW 38 °C
<b>Brain</b>				
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)	-	-	-	-
<b>Heart</b>				
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*
<b>Kidney</b>				
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)	-	-	-	-
<b>Liver/pancreas</b>				
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. * <i>p</i> < 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<sub>2</sub> delivery and organ blood perfusion were reduced but that ECMO rewarming provided CO, MAP, and O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub> 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 device.

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<sub>2</sub> 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<sub>2</sub> transport and hypoxia in organs despite restitution of global O<sub>2</sub> transport. In our effort to reconstitute 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 shows 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 costal 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;  $\gamma$  GT:  $\gamma$ -glutamyl transferase; s-100 $\beta$ : soluble protein-100 $\beta$ ; 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

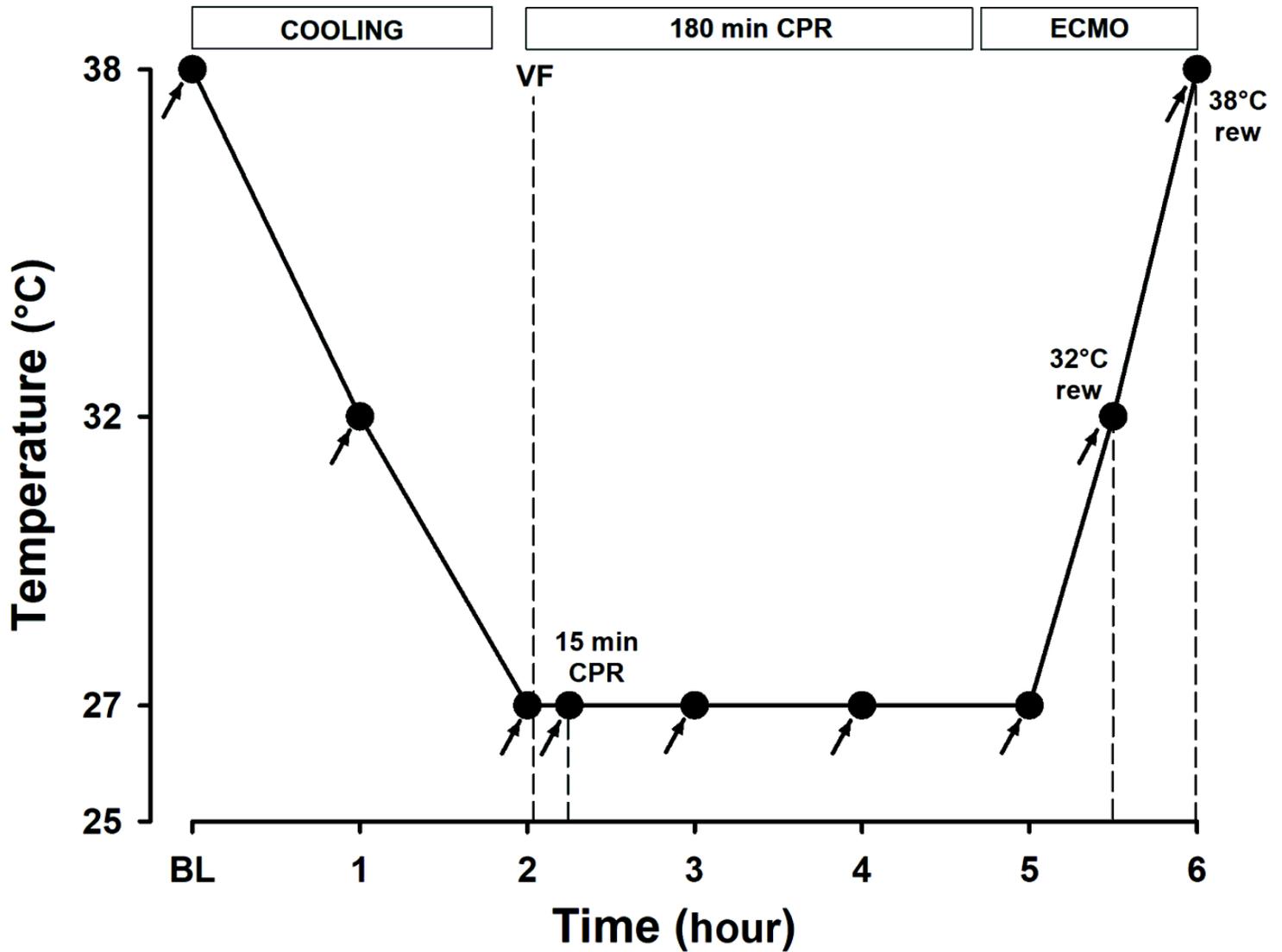
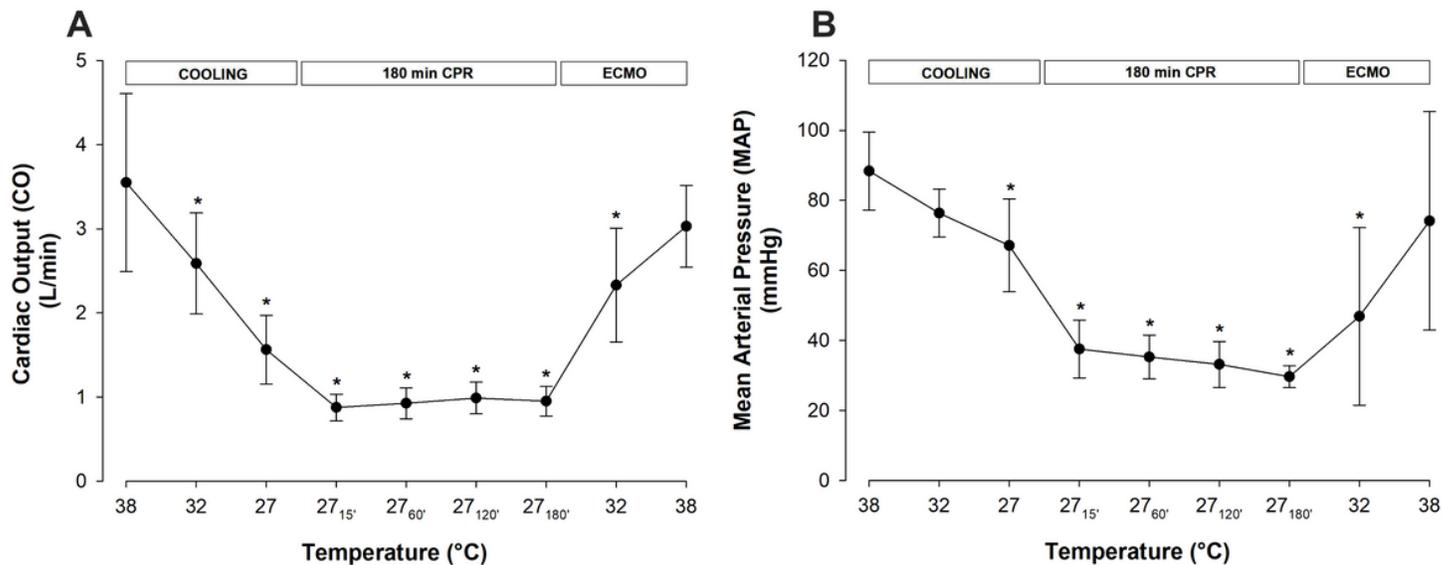


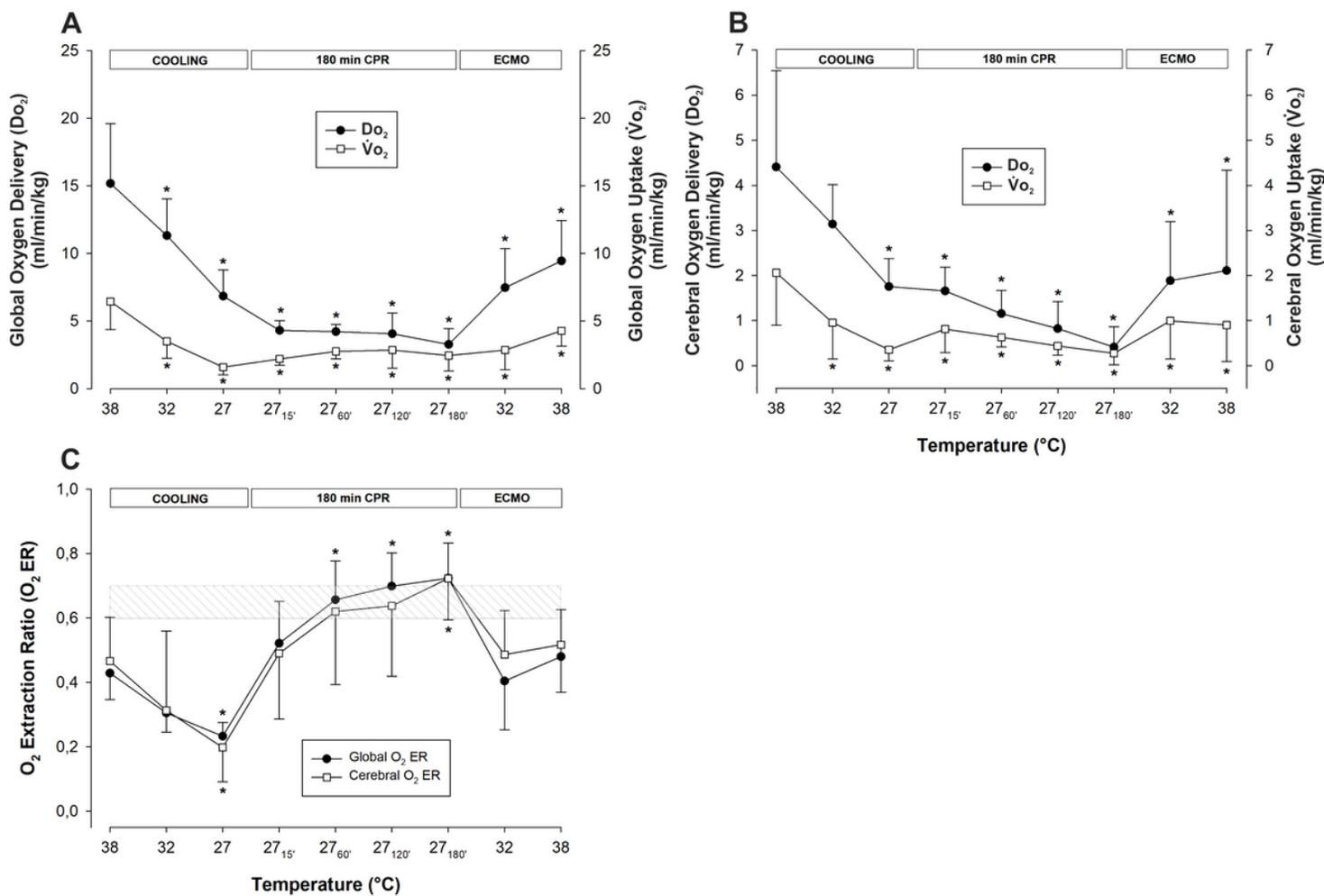
Figure 1

Experimental protocol. Arrows depict time points for hemodynamic measurements, blood sampling, and microsphere administration.



**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 ( $\dot{V}O_2$ ). (B) Cerebral oxygen delivery, and cerebral oxygen uptake ( $\dot{V}O_2$ ). (C) Global and cerebral oxygen extraction ratio. n=8, values are mean  $\pm$  SD. \*p < 0.05 statistically significantly different from baseline value. Striated area indicates critical level of extraction ratio.

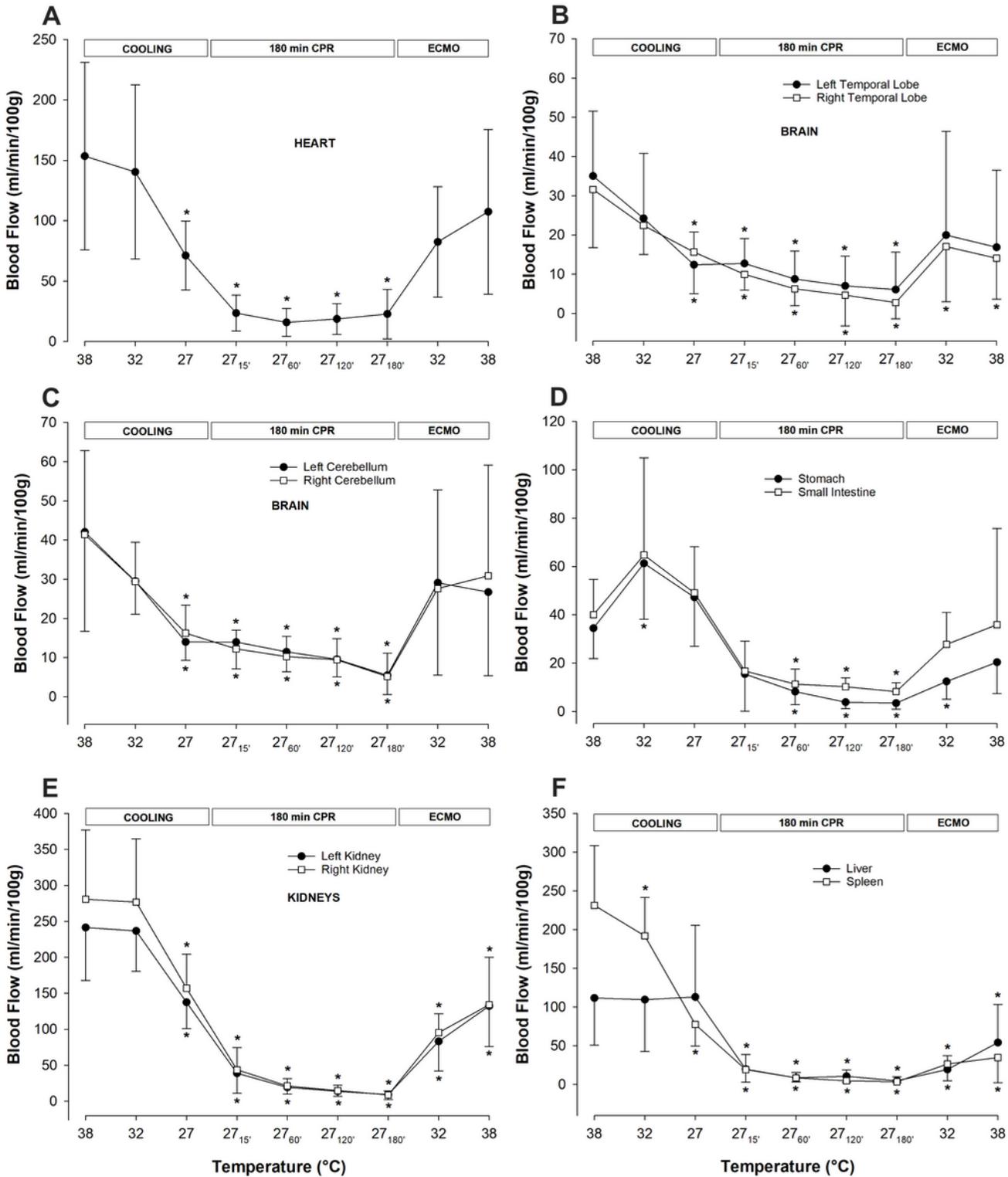


Figure 4

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  $\pm$  SD. \*p < 0.05 statistically significantly different from baseline value.