Experimental paper

# Mild (34 °C) versus moderate hypothermia (24 °C) in a swine model of extracorporeal cardiopulmonary resuscitation

Alexandra M. Marquez [a,b,1,*], Marinos Kosmopoulos [b,c,1], Rajat Kalra [b,c], Tomaz Goslar [b], Deborah Jaeger [b], Christopher Gaisendrees [b], Alejandra Gutierrez [b,c], Gregory Carlisle [a], Tamas Alexy [b,c], Sergey Gurevich [b,c], Andrea M. Elliott [b,c], Marie E. Steiner [a,d], Jason A. Bartos [b,c], Davis Seelig [e], Demetris Yannopoulos [b,c]

## Abstract

**Background**: The role of hypothermia in post-arrest neuroprotection is controversial. Animal studies suggest potential benefits with lower temperatures, but high-fidelity ECPR models evaluating temperatures below 30 °C are lacking.

**Objectives**: To determine whether rapid cooling to 24 °C initiated upon reperfusion reduces brain injury compared to 34 °C in a swine model of ECPR.

**Methods**: Twenty-four female pigs had electrically induced VF and mechanical CPR for 30 min. Animals were cannulated for VA-ECMO and cooled to either 34 °C for 4 h (n = 8), 24 °C for 1 h with rewarming to 34 °C over 3 h (n = 7), or 24 °C for 4 h without rewarming (n = 9). Cooling was initiated upon VA-ECMO reperfusion by circulating ice water through the oxygenator. Brain temperature and cerebral and systemic hemodynamics were continuously monitored. After four hours on VA-ECMO, brain tissue was obtained for examination.

**Results**: Target brain temperature was achieved within 30 min of reperfusion (p = 0.74). Carotid blood flow was higher in the 24 °C without rewarming group throughout the VA-ECMO period compared to 34 °C and 24 °C with rewarming (p < 0.001). Vasopressin requirement was higher in animals treated with 24 °C without rewarming (p = 0.07). Compared to 34 °C, animals treated with 24 °C with rewarming were less coagulopathic and had less immunohistochemistry-detected neurologic injury. There were no differences in global brain injury score.

**Conclusions**: Despite improvement in carotid blood flow and immunohistochemistry detected neurologic injury, reperfusion at 24 °C with or without rewarming did not reduce early global brain injury compared to 34 °C in a swine model of ECPR.

**Keywords**: ECPR, ECMO, Cardiac arrest, Resuscitation, Hypothermia, Temperature

## Introduction

Cardiac arrest (CA) is a leading cause of death.[1–5] Extracorporeal cardiopulmonary resuscitation (ECPR) using veno-arterial extracorporeal membrane oxygenation (VA-ECMO) can improve outcomes in select patients refractory to conventional CPR alone.[6–8] Still, neurologically favorable survival is low, mainly driven by global anoxic brain injury.[7,9,10]

Temperature control in ECPR has not been evaluated in human and high-fidelity translational models. Clinical trials in conventional CA populations have not included ECPR patients.[11–16] ECPR is

characterized by long resuscitation durations, extreme metabolic derangements, and higher risk for severe post-cardiac arrest injury.[9] Hypothermia applied following ECPR may be uniquely advantageous because VA-ECMO facilitates rapid cooling and hemodynamic stabilization, mitigating side effects of lower target temperatures. Data from scant experimental, human observational, and registry studies suggest a potential benefit from hypothermia between 32 and 36 °C.[17–22] Whether there is additional benefit to colder temperature targets, particularly < 30 °C, remains unknown.

We conducted a randomized trial of mild (34 °C) versus moderate (24 °C) hypothermia with or without rewarming in a swine model of ECPR. We hypothesized that short-term histologic brain injury would be reduced in animals treated with moderate compared to mild hypothermia and in animals who were not rewarmed during the 4-hour post-arrest period.

## Methods

### Animal preparation

The University of Minnesota Institutional Animal Care and Use Committee approved all procedures and protocols in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals.*[23] Twenty-four female Yorkshire swine (*Sus scrofus domesticus*) underwent VF CA with mechanical CPR for 30 min followed by cannulation to VA-ECMO with therapeutic hypothermia to either 34 °C (n = 8), 24 °C with rewarming (n = 7), or 24 °C without rewarming (n = 9). The surgical preparation, anesthesia, and monitoring are described in the Supplemental Appendix. Briefly, swine (50–60 kg) were anesthetized and mechanically ventilated. Aortic and right atrial pressures were monitored by micromanometer tipped catheters (Millar Instruments, Houston, TX). Continuous brain and core temperature monitoring was established via a K-type thermocouple (ATB1, Fieldpiece, Orange, CA) inserted 2–3 cm into the brain parenchyma and an esophageal temperature probe (ESO-1, Physitemp, Clifton, NJ). A bidirectional Doppler flow probe was secured around the common carotid artery (TS420 Perivascular Flow Module, Transonic, Ithaca, NY). A second cut down to access the right carotid was used for the formalin flush at the end of the protocol. Sheaths were placed in the right femoral artery and right or left femoral vein to prepare for VA-ECMO.

### Model justification

We chose a VF out-of-hospital CA model with 30 min of CPR followed by VA-ECMO cannulation to model a clinically relevant scenario with a high likelihood of brain injury that has been shown to be mitigated by ECPR in the clinical setting.[6] Further, VA-ECMO enables rapid cooling upon reperfusion and facilitates hemodynamic stability. Swine models have been used extensively by the study team and others in the field due to similarities in CPR mechanics, central nervous system anatomy, and the relative ease of VA-ECMO cannulation compared to other species. Additional descriptions of our cardiac arrest and VA-ECMO models are available in prior publications.[24–27]

### Experimental protocol

#### CA and CPR

The experimental protocol is described in Fig. 1. Additional description of the CA and mechanical CPR protocol can be found in our prior publications.[28,29] Prior to CA, all animals received heparin (5000 U) to attain activated clotting time > 2x baseline. After 5 min of untreated VF, life support was initiated using a custom automated mechanical piston. Epinephrine (0.5 mg, IV bolus) was given every 5 min starting at minute 10 of the arrest for the duration of CPR. All animals received amiodarone (50 mg, IV bolus) and bicarbonate (50 mEq, IV bolus) at the end of CPR to mimic common clinical management in a prolonged arrest.

### VA-ECMO and hypothermia initiation

During the last 5 min of CPR, animals were cannulated for VA-ECMO. An additional bolus of heparin (2500 U) was given as needed to achieve an ACT goal of > 2x baseline. Stiff guidewires were advanced through femoral sheaths to the inferior vena cava and descending aorta and confirmed with fluoroscopy. Animals were cannulated with a 19-23F catheter in the femoral vein and a 13-15F catheter in the femoral artery (HLS cannulae, Maquet). After 30 min of CPR, cannulae were connected to a saline-primed VA-ECMO circuit (Getinge Cardiohelp, Sweden) circulating at 2–3 L/min of flow.

Immediately after reperfusion with VA-ECMO, animals were randomized in a 1:1 allocation to receive rapid cooling to 34 °C (mild hypothermia) or 24 °C (moderate hypothermia). Animals in the 34 °C group achieved target temperature by ambient room air with external cold packs if necessary, and 34 °C was maintained for the 4-hour post-CPR monitoring period. For animals cooled to 24 °C, ice water was circulated through the oxygenator. 24 °C was maintained for 1 h followed by rewarming by approximately 3.3 °C/hour over 3 h to 34 °C. Rewarming was achieved by titrating the temperature of the ice water bath, warm blankets, and forced-air warming (Bair Hugger, 3 M, Maplewood, MN). A third group was later added in a non-randomized fashion to receive 24 °C without rewarming. Treatment allocation was not blinded.

### Post-arrest/VA-ECMO management

After VA-ECMO flow initiation, animals that did not spontaneously achieve return of spontaneous circulation (ROSC) received up to 3 defibrillation attempts with 200–300 J. Defibrillation could also be attempted up to 3 times at each hour time point. Crystalloid (0.9% NS or albumin 5%), vasopressors (vasopressin 200 U/ml as infusion), and circuit RPMs were titrated to achieve mean arterial blood pressure (MAP) > 60 mmHg and VA-ECMO flow of 2–3 L/min. Arterial blood gases were obtained at 15 min, 30 min, and hourly for 4 h. Blood gases were managed using the alpha-stat method. Circuit delivery of oxygen and sweep gas were titrated to achieve animal $PaO_2$ 80–150 mmHg and $PaCO_2$ 35–45 mmHg. Sodium bicarbonate was not given to correct pH. Blood products were not administered. Propofol was titrated to achieve an appropriate level of sedation. ACTs were obtained at baseline, at the end of CPR, and hourly following VA-ECMO flow initiation. CBC, coagulation parameters, and thromboelastography were obtained at baseline, 1-hour, and 4-hours post-VA-ECMO flow. Clinical bleeding and thrombotic events were documented.

### Euthanasia and pathology assessment

Following 4 h of monitoring after VA-ECMO flow initiation, animals were euthanized with beuthanasia (100 mg/kg IV). Formalin (in a 10:1 tissue volume-to-formalin ratio) was injected into the brain via the accessed carotid artery. The whole brain was extracted via a vertex craniectomy.

A board-certified veterinary pathologist blinded to the treatment allocation performed pathology processing and analysis (comprehensive methods found in Supplemental Appendix). Briefly, brains were sectioned to capture distinct regions susceptible to acute ischemia–reperfusion injury: caudate nucleus, putamen, internal capsule, frontal cortex, hippocampus, and cerebellum.[30] Tissue sections were stained with hematoxylin and eosin (H&E) and scored on a scale of 1–5 (higher values indicating severity) according to previously published methods to provide a semi-quantitative assessment of edema, neuronal necrosis, neuronal and axonal degeneration, gliosis, inflammation, and infarction.[30,31]

The same regions were evaluated with immunohistochemistry (IHC) quantifying proteins identified in swine models of ischemia–reperfusion injury: glial fibrillary acidic protein, amyloid precursor protein, caspase-3, ionized calcium-binding adaptor molecule 1, and myelin basic protein.[32–35] Each IHC marker was analyzed separately by region and scored on a scale of 0–4 (higher values indicating marked immunoreactivity). Cumulative scores for histologic brain injury (global brain injury score) and IHC-detected injury were calculated by summing the equally weighted scores for each lesion type and IHC antibody.

### Outcomes and statistical analysis

The primary outcome was short-term global brain injury score (histopathology) obtained 4 h post-arrest. Secondary outcomes included: short-term survival, CBF, systemic hemodynamics, coagulopathy, and brain IHC. Sample size was calculated for two groups. A sample size of 8 animals in each group provided > 80% power to detect a 30% reduction in the mean total brain injury score between groups, using an effect size of 1.3 and SD of 4 (unpublished data from pilot animals).
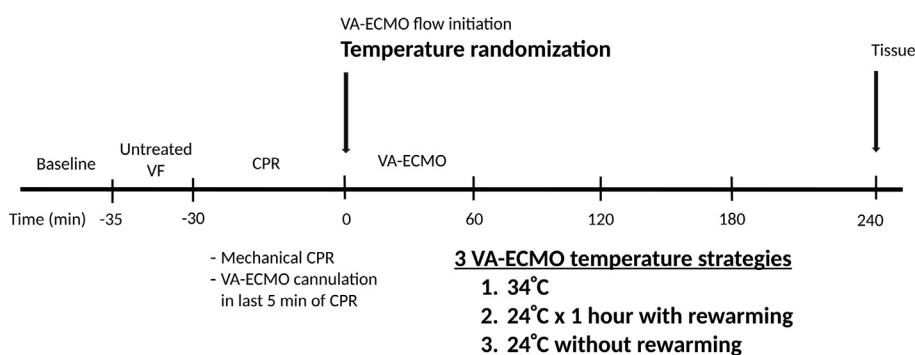


**Fig. 1 – Experimental protocol. Animals received CPR with mechanical chest compressions and standard advanced cardiac life support. VF indicates ventricular defibrillation; CPR, cardiopulmonary resuscitation; VA-ECMO, veno-arterial extracorporeal membrane oxygenation.**

### Table 1 – Pre-ECMO characteristics.

| Variable | 34 °C (n = 8) | 24 °C without rewarming (n = 9) | 24 °C with rewarming (n = 7) | P value |
|---|---|---|---|---|
| **Baseline** | | | | |
| Weight, kg | | | | |
| Systolic BP, mmHg | 108.77 ± 8.41 | 100.35 ± 7.82^ | 122.7 ± 3.65# | 0.046 |
| Diastolic BP, mmHg | 73.51 ± 6.41 | 71.59 ± 6.48 | 81.61 ± 1.82 | 0.243 |
| Mean arterial BP, mmHg | 85.73 ± 6.76 | 81.14 ± 8.09 | 95.47 ± 1.91 | 0.170 |
| Body temperature, °C | 37.09 ± 0.46 | 37.41 ± 0.23 | 37.44 ± 0.46 | 0.829 |
| Brain temperature, °C | 37.03 ± 0.40 | 37.29 ± 0.19 | 37.43 ± 0.32 | 0.752 |
| Arterial pH | 7.51 ± 0.01# | 7.57 ± 0.01* ^ | 7.50 ± 0.01# | <0.001 |
| Lactate | 1.22 ± 0.14 | 1.11 ± 0.21 | 1.25 ± 0.10 | 0.441 |
| **CPR end (30 min)** | | | | |
| Systolic BP, mmHg | 71.59 ± 5.41 | 65.83 ± 5.71 | 75.04 ± 3.14 | 0.382 |
| Diastolic BP, mmHg | 18.03 ± 2.68 | 15.15 ± 1.95^ | 24.02 ± 1.79# | <0.001 |
| Mean arterial BP, mmHg | 36.00 ± 3.28 | 35.49 ± 2.87 | 41.03 ± 1.89 | 0.205 |
| Coronary perfusion pressure, mmHg | 12.04 ± 2.94 | 6.40 ± 2.33^ | 15.56 ± 2.09# | 0.026 |
| Carotid blood flow (% baseline) | 22.42 ± 2.99 | 29.52 ± 8.61 | 35.80 ± 6.26 | 0.169 |
| Body temperature, °C | 37.13 ± 0.22^ | 37.22 ± 0.18^ | 37.96 ± 0.20* # | 0.026 |
| Brain temperature, °C | 37.65 ± 0.29 | 38.20 ± 0.11 | 38.14 ± 0.22 | 0.230 |
| Arterial pH | 7.37 ± 0.04# | 7.48 ± 0.07* ^ | 7.31 ± 0.07# | 0.018 |
| Lactate | 9.02 ± 0.61^ | 8.91 ± 0.54^ | 10.90 ± 0.72* # | 0.007 |

Comparison between the three groups at baseline and end of CPR. BP indicates blood pressure.

# p < 0.05 compared to 24 °C without rewarming.

* p < 0.05 compared to 34 °C.

^ p < 0.05 compared to 24 °C with rewarming. Values are mean ± SEM.

Continuous waveform and temperature data were captured every 250 ms, recorded in LabView, and then averaged into 5-minute data epochs. Normally distributed data were compared using analysis of variance (ANOVA) with Tukey post-hoc analysis for subgroup comparisons and reported as mean ± SEM. Non-normal data were compared using Kruskal-Wallis with Dunn's test for subgroup comparisons and reported as median with interquartile range. Categorical data are reported as relative frequencies (%). Dichotomous variables were evaluated using Fisher's exact test. Carotid blood flow was expressed as a percentage of baseline. Variables were compared at predetermined time points (e.g., end of CPR, hourly on VA-ECMO). Missing values were not imputed. Statistical analysis was completed using RStudio (RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. https://www.posit.co/).

## Results

Of 42 initial animal experiments, nine pre-randomization attritions and nine exclusions (Supplemental Fig. 1) resulted in 24 analyzed animals. Attrition occurred due to traumatic pulmonary hemorrhage
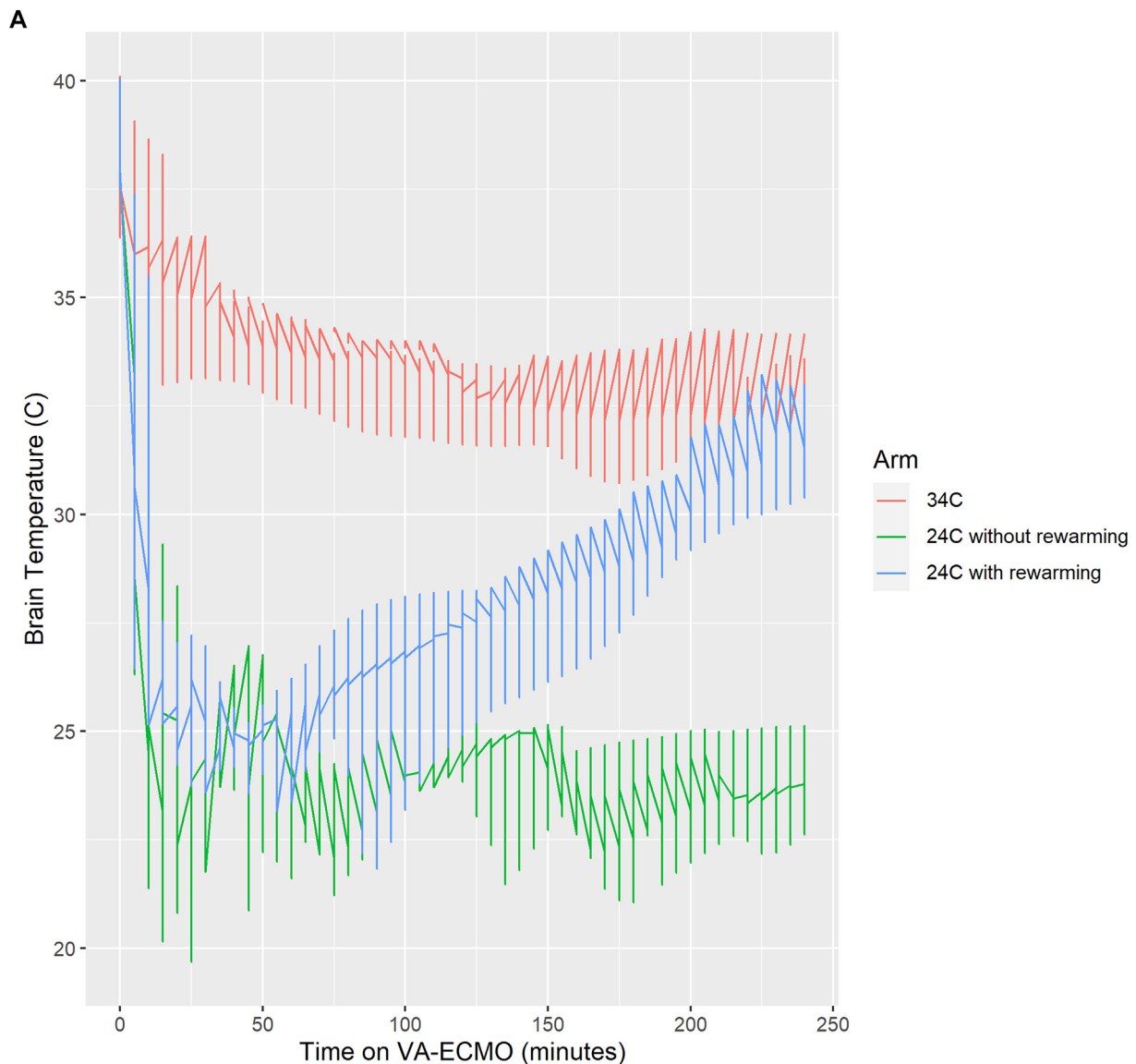


**Fig. 2 – Brain and esophageal temperatures during VA-ECMO. (A) Brain and (B) esophageal temperatures are displayed throughout the VA-ECMO period for 34 °C (pink), 24 °C without rewarming (green), and 24 °C with rewarming (blue) experimental groups. Error bars represent SEM. In the 24 °C with rewarming group, animals were rewarmed at a rate of three degrees per hour to reach the mild hypothermia range (32-34°) by the end of the 4-hour monitoring period. VA-ECMO indicates veno-arterial extracorporeal membrane oxygenation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)**
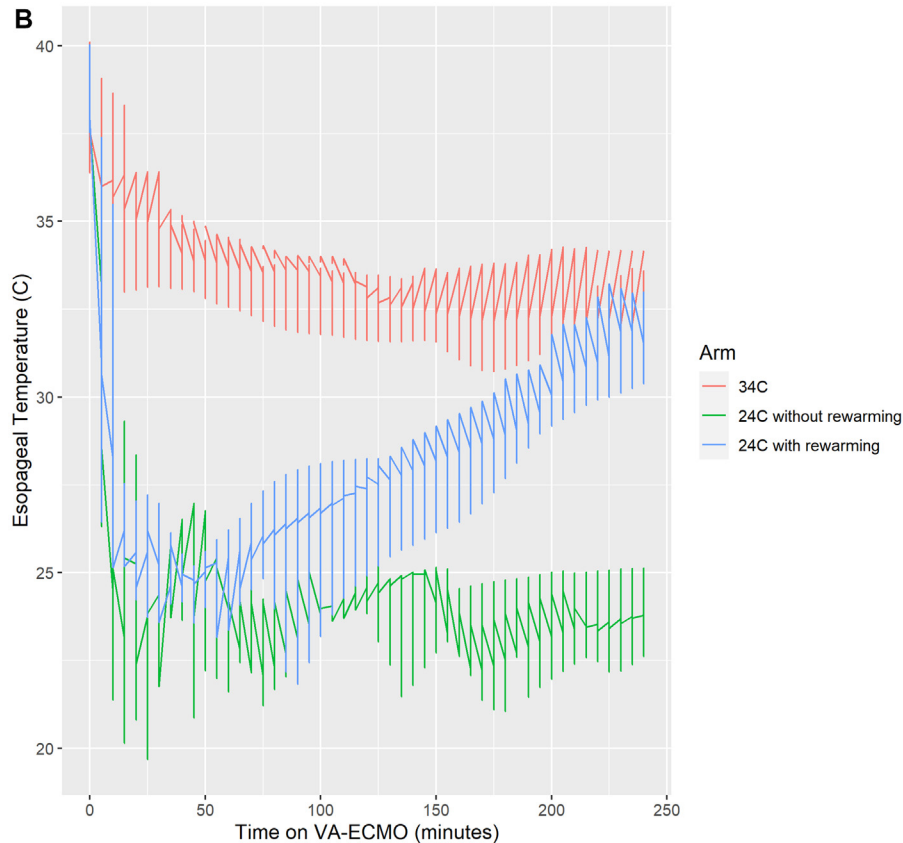
Fig 2. *(continued)*

(n = 5) and failed cannulation (n = 4). Animals were excluded post-randomization for VA-ECMO circuit failure (n = 1) and refractory hypotension with inability to extract the brain (n = 1). To isolate the confounding generated by poor quality CPR from the effects of hypothermia, Grubb's test for outliers was performed on the end of CPR coronary perfusion pressure (CPP), resulting in the additional exclusion of seven animals. Post-randomization exclusions due to poor CPR hemodynamics occurred prior to unblinding the hemodynamic analysis.

### Baseline and CPR

Baseline and CPR characteristics are described in Table 1. Baseline hemodynamics were similar. During CPR, 24 °C with rewarming animals had higher CPP ($15.67 \pm 2.09$ vs $12.04 \pm 2.94$ in 34 °C vs $6.4 \pm 2.3$ in 24 °C without rewarming, $p = 0.026$) and higher lactic acid at the end of CPR ($10.90 \pm 0.72$ vs $9.02 \pm 0.61$ in 34 °C vs $8.91 \pm 0.54$ in 24 °C without rewarming, $p = 0.007$).

### VA-ECMO reperfusion period

Brain and esophageal temperatures are displayed in Fig. 2. The mean time to target brain temperature was 27.9 min in the 34 °C group, 21.9 min in the 24 °C without rewarming group, and 28.3 min in the 24 °C with rewarming group ($p = 0.74$).

Post-arrest systemic and cerebral hemodynamics are represented in Fig. 3. MAP was significantly higher in the 24 °C without rewarming group throughout the VA-ECMO period compared to the 34 °C and 24 °C with rewarming groups. The 24 °C with rewarming group exhibited higher MAP than the 34 °C group through the third hour of reperfusion. Animals treated with 24 °C without rewarming required higher cumulative doses of vasopressin to achieve adequate perfusion with a mean of $78 \pm 29.0$ total units compared to $51.4 \pm 22.7$ in 34 °C and $56.7 \pm 8.2$ in 24 °C with rewarming animals ($p = 0.07$).

At the end of reperfusion, all animals in the 34 °C group achieved ROSC compared to 75% of the animals in the 24 °C with rewarming group and none in the 24 °C without rewarming group ($p < 0.001$).

CBF was significantly higher for both the 24 °C with and without rewarming groups when compared to the 34 °C group in the first and second hour of VA-ECMO reperfusion. In the third and fourth hours of reperfusion, the 24 °C without rewarming group maintained significantly higher CBF than the 34 °C group.

Lactate was similar between the three groups for the whole study (Supplemental Fig. 2). There were no differences in arterial pH between groups throughout the VA-ECMO period.

### Brain injury

Overall, there was no statistically significant difference in the global brain injury score between groups; 10 (8, 13.5) at 34 °C vs 8 (6, 10) at 24 °C without rewarming vs 6 (5, 9.5) at 24 °C with rewarming (Fig. 4, Supplemental Table 1). Animals treated with 24 °C without rewarming had less severe total IHC score with median (IQR) of 40.5 (39.5, 41) compared to 56 (49.5, 59.8) in 34 °C animals (Table 2,

Supplemental Table 2). This finding was driven mainly by differences in the caudate nucleus and frontal cortex. There were no differences between 34 °C and 24 °C with rewarming.

### Coagulation

There were no major clinical bleeding or thrombotic events. Coagulation data are summarized in Supplemental Table 3. Animals treated with 24 °C without rewarming had significantly higher levels of fibrinogen at 4 h compared to those treated with 34 °C (146.2 ± 23.7 vs 110.0 ± 37.2, p = 0.039), with no differences compared to 24 °C with rewarming animals. Antithrombin III levels were significantly lower in 34 °C animals compared to 24 °C without rewarming animals (67.8 ± 9.0 vs 50.1 ± 16.7, p = 0.02) at 4 h post-arrest. There were no differences in ACT, R and K times, activated prothrombin time, d-dimer, or INR at any time. Hematocrit, hemoglobin, platelets, and lactate dehydrogenase were also similar throughout the study.

## Discussion

In this swine model of ECPR, moderate hypothermia to 24 °C initiated rapidly upon reperfusion improved CBF but did not significantly reduce short-term histologic brain injury compared to mild hypothermia to 34 °C. Animals cooled to 24 °C demonstrated modest reduction in immunohistochemical markers of axonal injury, apoptosis, and neuroinflammation compared to animals treated with 34 °C and 24 °C with rewarming. However, cooling to 24 °C led to more intensive vasopressor support and a lower likelihood of successful defibrillation. Our findings suggest that rapid reperfusion with 24 °C is unlikely to be markedly advantageous over mild hypothermia following ECPR, but our study is limited by short-term endpoints.

To our knowledge, this is the first study evaluating hypothermic temperatures < 30° in a large-animal model of ECPR. Prior ECPR studies demonstrate neurologic benefits with therapeutic hypothermia between 30–34 °C, compared to normothermia.[17–20,30] We did not observe clear benefit or harm in terms of neurologic injury among animals cooled to 24 °C, compared to mild hypothermia. Total histologic injury 4 h post-arrest was decreased in both 24 °C groups compared to 34 °C (p = 0.22) but did not reach significance, and was not affected by rewarming. Total IHC score was significantly lower in 24 °C animals compared to 34 °C and 24 °C with rewarming, although not all markers assessed showed differences. While immunostaining can detect early signals of axonal injury, apoptosis, neuroinflammation, and myelination, injury visualized by H&E may not be as pronounced until 24–72 h following CA.[36,37] Tissue evaluation at a later time point may have revealed larger differences between groups.[38]

In this study, CBF was significantly increased among animals cooled to 24 °C, and this effect was not entirely explained by differences in systemic hemodynamics. Cooling-induced vasodilation of
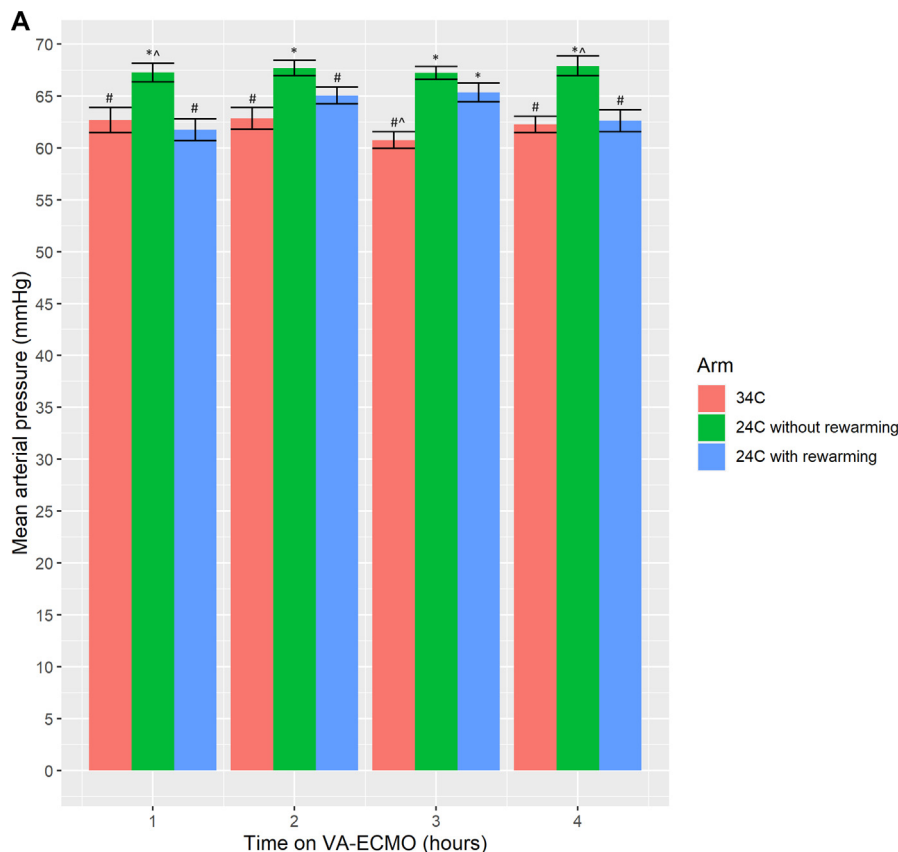


Fig. 3 – Systemic and cerebral hemodynamics during VA-ECMO. (A) Mean arterial pressure and (B) carotid blood flow compared at hours 1, 2, 3, and 4 during VA-ECMO for 34 °C (pink), 24 °C without rewarming (green), and 24 °C with rewarming (blue) experimental groups. Data are presented as mean ± SEM. *p < 0.05 compared to 34 °C; # p < 0.05 compared to 24 °C without rewarming; ^p < 0.05 compared to 24 °C with rewarming. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**B**
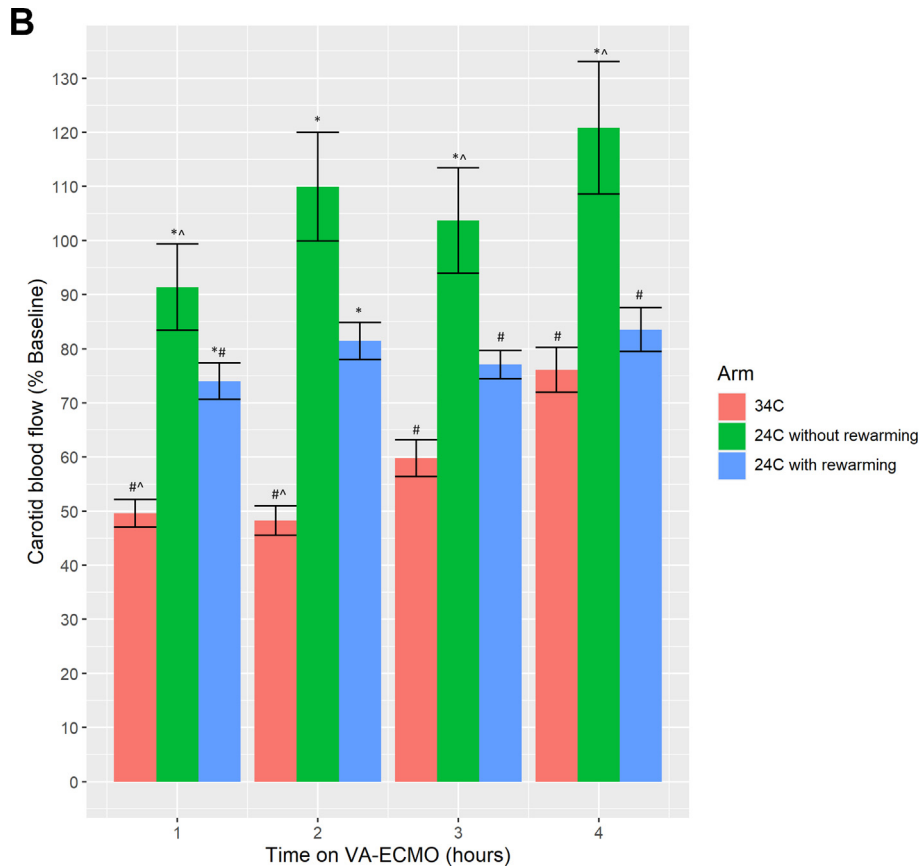


Fig 3. *(continued)*

large vessels, including the carotid artery, is thought to be mediated by a temperature-dependent reduction of calcium influx into smooth muscle cells.[39] However, the effect of hypothermia on the cerebral microcirculation is disputed. Hypothermia may increase microcirculatory blood flow by enhancing endothelial health, namely reducing apoptosis of microvascular endothelial cells and fibrin deposition in capillary vessels, and improving balance of prostanoids.[40–42] By reducing the metabolic rate, hypothermia may also improve the mismatch between supply and demand.[43,44] However, a swine model of cardiopulmonary bypass found that cerebral blood flow decreased concomitantly with increased cerebral vascular resistance as animals were cooled from 37 to 18°C.[45] These opposing findings are challenging to reconcile, but may be due to differences in model, method of cooling, use of anticoagulation, measurement technique (whole brain versus regional), and potential loss of cerebral blood flow autoregulation following CA. This is relevant because the brain experiences heterogeneous reperfusion, with some areas remaining relatively ischemic despite return of circulation (i.e., "no-reflow" phenomenon).[46] More work is needed to understand the effects of hypothermia on macro and microcirculation following ECPR.

In this study, animals treated with 24 °C without rewarming required a higher cumulative dose of vasopressin (p = 0.07) to maintain a perfusing blood pressure. Animal studies in cardiac surgery models have shown that hypothermia decreases vascular response to vasopressors.[47] It also follows that animals treated with 24 °C without rewarming were not able to defibrillate successfully into a perfusing rhythm. Clinically, prolonged absence of cardiac contractility poses a risk for intracardiac thrombus. This risk may be mitigated

by continuous therapeutic anticoagulation, which was maintained in our animals throughout the study.

Coagulopathy is a known complication of therapeutic hypothermia and is also common in patients treated with ECPR.[48] We performed a battery of coagulation testing in our animals. Unsurprisingly, all three ECPR groups in this study developed a consumptive coagulopathy. However, animals cooled to 24 °C without rewarming had less severe derangement, with significantly higher levels of fibrinogen and antithrombin III than their 34 °C counterparts. The mechanism underlying this observation deserves further study. Notably, the severity of coagulopathy is associated with worse outcomes in human studies of ECPR, namely major bleeding and death.[48,49] Overall, our data corroborate previous findings of coagulopathy following prolonged cardiac arrest and ECPR while casting doubt on the dogma that colder temperatures necessarily worsen coagulation.

This study had limitations. First, we did not compare hypothermia to normothermia. Second, experimental blinding was not possible due to the clearly visible effects of moderate hypothermia. However, hemodynamics and histopathology were subject to blinded assessment. Third, despite consistent methods of CPR, animals within groups had more variability in CPR hemodynamics than we anticipated. We excluded animals with the lowest CPP at the end of CPR, as differences in CPR perfusion can confound post-resuscitation care. However, CPP did not correlate with lactate at the end of CPR. Although VA-ECMO management was highly protocolized, there were differences in hemodynamics and vasopressor requirement. An important limitation is that we were unable to include
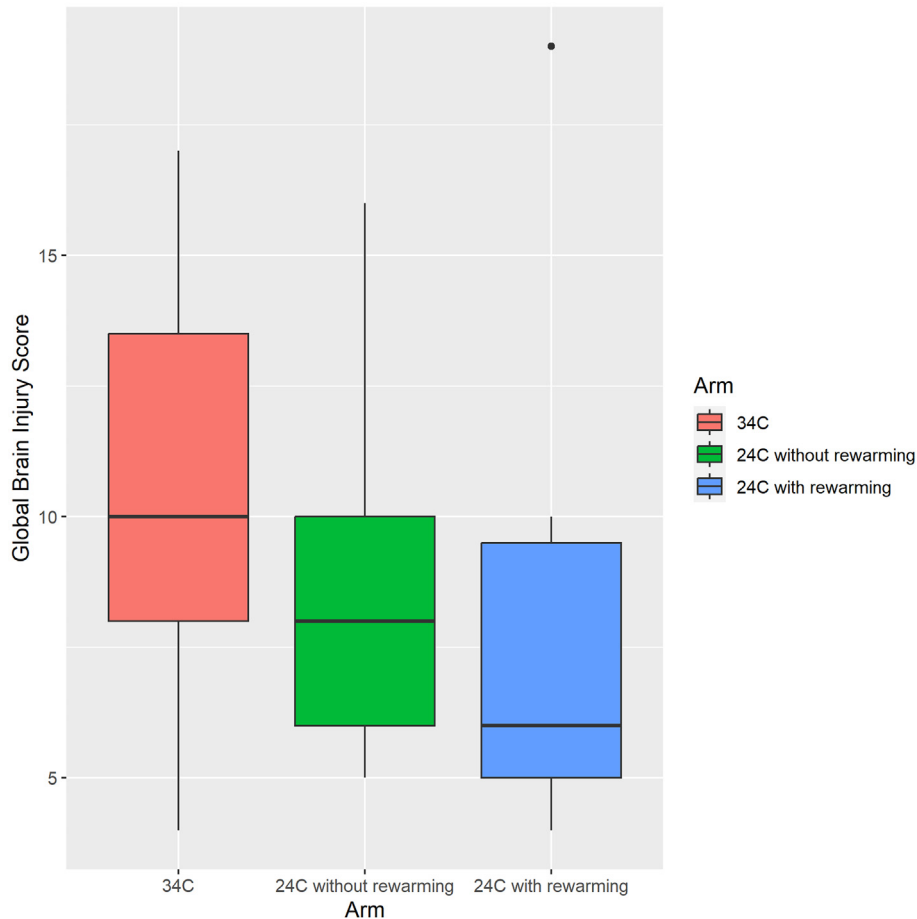
**Fig. 4 – Global brain injury score at 4 h post-cardiac arrest. There were no significant differences (p = 0.21) in global brain injury at 4 h following cardiac arrest across experimental groups treated with VA-ECMO at 34 °C (pink), 24 °C without rewarming (green), or 24 °C with rewarming (blue). Boxplot horizontal lines indicate median global brain injury scores, boxes represent the 25th and 75th percentiles, whiskers represent the 5th and 95th percentiles, and the dot indicates the outlier. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)**

**Table 2 – Immunostaining characteristics at 4 h following cardiac arrest.**

| IHC Antibody | 34 °C (n = 8) | 24 °C without rewarming (n = 9) | 24 °C with rewarming (n = 7) |
|---|---|---|---|
| APP | **13 (12.9, 13.3)[#]** | **7.5 (7, 8.5)[*,^]** | **12.5 (12, 13)[#]** |
| Caspase-3 | 12 (9.6, 14) | **9.5 (9, 10.5)[^]** | **12.3 (11.3, 12.9)[#]** |
| GFAP | **8.3 (5.8, 10.9)[#]** | **3 (3, 3.5)[*,^]** | **7.3 (6.5, 9.1)[#]** |
| IBA-1 | 13.3 (11, 14.6) | 11.5 (10.5, 13) | 8.5 (8, 9.4) |
| MBP | 7.3 (6.3, 9) | 8.5 (8, 9) | 6.5 (5.1, 10.5) |
| Total IHC score | **56 (49.8. 59.8)[#]** | **40.5 (39.5, 41)[*]** | 45.3 (42.4, 53.4) |

Intensity of IHC antibody immunostaining was evaluated by brain region (caudate nucleus, putamen, internal capsule, frontal cortex, hippocampus, and Purkinje cells of the cerebellum), scored on a scale of 0–4, and summed. IHC indicates immunohistochemistry; APP, amyloid precursor protein; GFAP, glial fibrillary acidic protein; IBA-1, ionizing calcium-binding adaptor molecule; MBP, myelin basic protein.

[#] p < 0.05 compared to 24 °C without rewarming.

[*] p < 0.05 compared to 34 °C.

[^] p < 0.05 compared to 24 °C with rewarming. Values are median (interquartile range).

ECMO flow data due to substantial missingness (10 of 24 animals) from a storage issue. Fourth, we evaluated brief durations of hypothermia with short-term histopathologic and immunohistochemical outcomes. Our scoring system was validated for a 72-hour endpoint, and injury was highly variable within groups. These factors could lead to detection of a less severe brain injury on pathology and thus underpower the study. Future studies could measure longer durations of hypothermia, slower rewarming speeds, and clinical endpoints such as neurologic exams or neuroimaging. However, clinical outcomes are challenging to obtain with an ECPR model in which

there is a high amount of instrumentation and ethical concerns about the surviving animals.

## Conclusion

This large animal study of ECPR using short-term endpoints suggests that reperfusion with moderate hypothermia to 24 °C is not clearly beneficial over 34 °C and requires more intensive hemodynamic management. Moderate hypothermia to 24 °C produces higher carotid blood flow, a modest improvement in immunohistochemical markers of brain injury, and less severe coagulopathy but the clinical relevance is unclear.

## CRediT authorship contribution statement

**Alexandra M. Marquez:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marinos Kosmopoulos:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rajat Kalra:** Writing – review & editing, Methodology. **Tomaz Goslar:** Writing – review & editing, Methodology. **Deborah Jaeger:** Writing – review & editing, Methodology, Investigation. **Christopher Gaisendrees:** Writing – review & editing, Methodology, Investigation. **Alejandra Gutierrez:** Writing – review & editing, Methodology. **Gregory Carlisle:** Writing – review & editing, Methodology. **Tamas Alexy:** Writing – review & editing, Methodology. **Sergey Gurevich:** Writing – review & editing, Methodology. **Andrea M. Elliott:** Writing – review & editing, Methodology. **Marie E. Steiner:** Writing – review & editing, Methodology. **Jason A. Bartos:** Writing – review & editing, Methodology. **Davis Seelig:** Writing – review & editing, Methodology, Investigation. **Demetris Yannopoulos:** Writing – review & editing, Supervision, Methodology, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

## Funding

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.resplu.2024.100745.

## Author details

[a]Division of Pediatric Critical Care Medicine, Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA [b]Center for Resuscitation Medicine, University of Minnesota School of Medicine, Minneapolis, MN, USA [c]Cardiovascular Division, Department of Medicine, University of Minnesota, Minneapolis, MN, USA [d]Division of Pediatric Hematology and Oncology, Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA [e]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, Minneapolis, MN, USA

## REFERENCES

1. Virani SS, Alonso A, Benjamin EJ, et al. Heart disease and stroke statistics-2020 update: a report from the American heart association. Circulation 2020;141:E139–596. https://doi.org/10.1161/CIR.0000000000000757.

2. Andersen LW, Holmberg MJ, Berg KM, Donnino MW, Granfeldt A. In-hospital cardiac arrest: a review. JAMA 2019;321:1200–10. https://doi.org/10.1001/JAMA.2019.1696.

3. Knudson JD, Neish SR, Cabrera AG, et al. Prevalence and outcomes of pediatric in-hospital cardiopulmonary resuscitation in the United States. Crit Care Med 2012;40:2940–4. https://doi.org/10.1097/CCM.0b013e31825feb3f.

4. Holmberg MJ, Wiberg S, Ross CE, et al. Trends in survival after pediatric in-hospital cardiac arrest in the United States. Circulation 2019. https://doi.org/10.1161/circulationaha.119.041667.

5. Holmberg MJ, Ross CE, Fitzmaurice GM, et al. Annual incidence of adult and pediatric in-hospital cardiac arrest in the United States. Circ Cardiovasc Qual Outcomes 2019;12:e005580. https://doi.org/10.1161/CIRCOUTCOMES.119.005580.

6. Yannopoulos D, Bartos J, Raveendran G, et al. Advanced reperfusion strategies for patients with out-of-hospital cardiac arrest and refractory ventricular fibrillation (ARREST): a phase 2, single centre, open-label, randomised controlled trial. Lancet 2020;396:1807–16. https://doi.org/10.1016/S0140-6736(20)32338-2.

7. Belohlavek J, Yannopoulos D, Smalcova J, et al. Intraarrest transport, extracorporeal cardiopulmonary resuscitation, and early invasive management in refractory out-of-hospital cardiac arrest: an individual patient data pooled analysis of two randomised trials. EClinicalMedicine 2023:59. https://doi.org/10.1016/J.ECLINM.2023.101988.

8. Belohlavek J, Smalcova J, Rob D, et al. Effect of intra-arrest transport, extracorporeal cardiopulmonary resuscitation, and immediate invasive assessment and treatment on functional neurologic outcome in refractory out-of-hospital cardiac arrest: a randomized clinical trial. JAMA 2022;327:737–47. https://doi.org/10.1001/JAMA.2022.1025.

9. Bartos JA, Grunau B, Carlson C, et al. Improved survival with extracorporeal cardiopulmonary resuscitation despite progressive metabolic derangement associated with prolonged resuscitation. Circulation 2020:877–86. https://doi.org/10.1161/CIRCULATIONAHA.119.042173.

10. Scquizzato T, Bonaccorso A, Consonni M, et al. Extracorporeal cardiopulmonary resuscitation for out-of-hospital cardiac arrest: A systematic review and meta-analysis of randomized and propensity score-matched studies. Artif Organs 2022;46:755–62. https://doi.org/10.1111/AOR.14205.

11. The Hypothermia after Cardiac Arrest Study Group. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. NEJM 2009;346:549–56. https://doi.org/10.1056/NEJMOA012689.

12. Lascarrou J-B, Merdji H, Le Gouge A, et al. Targeted temperature management for cardiac arrest with nonshockable rhythm. N Engl J

Med 2019;381:2327–37. https://doi.org/10.1056/NEJMOA1906661/SUPPL_FILE/NEJMOA1906661_DATA-SHARING.PDF.

13. Dankiewicz J, Cronberg T, Lilja G, et al. Hypothermia versus Normothermia after out-of-hospital cardiac arrest. N Engl J Med 2021;384:2283–94. https://doi.org/10.1056/NEJMOA2100591/SUPPL_FILE/NEJMOA2100591_DATA-SHARING.PDF.

14. Moler FW, Silverstein FS, Holubkov R, et al. Therapeutic hypothermia after in-hospital cardiac arrest in children. N Engl J Med 2017;376:318–29. https://doi.org/10.1056/NEJMOA1610493/SUPPL_FILE/NEJMOA1610493_DISCLOSURES.PDF.

15. Le May M, Osborne C, Russo J, et al. Effect of moderate vs mild therapeutic hypothermia on mortality and neurologic outcomes in comatose survivors of out-of-hospital cardiac arrest: The CAPITAL CHILL randomized clinical trial. JAMA 2021;326:1494–503. https://doi.org/10.1001/JAMA.2021.15703.

16. Soar J, Nolan J, Andersen L, et al. Temperature Management in Adult Cardiac Arrest Consensus on Science with Treatment Recommendations. [Internet] 2021.

17. Han F, Boller M, Guo W, et al. A rodent model of emergency cardiopulmonary bypass resuscitation with different temperatures after asphyxial cardiac arrest. Resuscitation 2010;81:93–9. https://doi.org/10.1016/J.RESUSCITATION.2009.09.018.

18. Zhang B, Gu Q, Chen X, et al. Temperature variability does not attenuate the beneficial effects of therapeutic hypothermia on cellular apoptosis and endoplasmic reticulum stress in the cerebral cortex of a swine cardiac arrest model. Neurocrit Care 2021;34:769–80. https://doi.org/10.1007/S12028-020-01083-2/FIGURES/6.

19. Ao H, Tanimoto H, Yoshitake A, Moon JK, Terasaki H. Long-term mild hypothermia with extracorporeal lung and heart assist improves survival from prolonged cardiac arrest in dogs. Resuscitation 2001;48:163–74. https://doi.org/10.1016/S0300-9572(00)00252-5.

20. Foerster K, Benk C, Beyersdorf F, et al. Twenty minutes of normothermic cardiac arrest in a pig model: the role of short-term hypothermia for neurological outcome. Perfusion (United Kingdom) 2018;33:270–7. https://doi.org/10.1177/0267659117742478/ASSET/IMAGES/LARGE/10.1177_0267659117742478-FIG3.JPEG.

21. Pang PYK, Wee GHL, Huang MJ, et al. Therapeutic hypothermia may improve neurological outcomes in extracorporeal life support for adult cardiac arrest. Heart Lung Circ 2017;26:817–24. https://doi.org/10.1016/j.hlc.2016.11.022.

22. Nakashima T, Ogata S, Noguchi T, et al. Association of intentional cooling, achieved temperature and hypothermia duration with in-hospital mortality in patients treated with extracorporeal cardiopulmonary resuscitation: An analysis of the ELSO registry. Resuscitation 2022;177:43–51. https://doi.org/10.1016/J.RESUSCITATION.2022.06.022.

23. National Research Council (US). Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011.

24. Yannopoulos D, Matsuura T, Schultz J, Rudser K, Halperin HR, Lurie KG. Sodium nitroprusside enhanced cardiopulmonary resuscitation improves survival with good neurological function in a porcine model of prolonged cardiac arrest. Crit Care Med 2011;39:1269–74. https://doi.org/10.1097/CCM.0B013E31820ED8A6.

25. Yannopoulos D, McKnite S, Aufderheide TP, et al. Effects of incomplete chest wall decompression during cardiopulmonary resuscitation on coronary and cerebral perfusion pressures in a porcine model of cardiac arrest. Resuscitation 2005;64:363–72. https://doi.org/10.1016/J.RESUSCITATION.2004.10.009.

26. Schultz J, Segal N, Kolbeck J, et al. Sodium nitroprusside enhanced cardiopulmonary resuscitation prevents post-resuscitation left ventricular dysfunction and improves 24-hour survival and neurological function in a porcine model of prolonged untreated ventricular fibrillation. Resuscitation 2011:S35–40. https://doi.org/10.1016/S0300-9572(11)70149-6.

27. Voicu S, Sideris G, Dillinger JG, et al. Synchronized pulsatile flow with low systolic output from veno-arterial extracorporeal membrane oxygenation improves myocardial recovery after experimental cardiac arrest in pigs. Artif Organs 2018;42:597–604. https://doi.org/10.1111/AOR.13089.

28. Sebastian PS, Kosmopoulos MN, Gandhi M, et al. Closed-loop machine-controlled CPR system optimises haemodynamics during prolonged CPR. Resusc plus 2020;:3. https://doi.org/10.1016/J.RESPLU.2020.100021.

29. Ripeckyj A, Kosmopoulos M, Shekar K, et al. Sodium nitroprusside-enhanced cardiopulmonary resuscitation improves blood flow by pulmonary vasodilation leading to higher oxygen requirements. JACC Basic Transl Sci 2020;5:183–92. https://doi.org/10.1016/J.JACBTS.2019.11.010.

30. Högler S, Sterz F, Sipos W, et al. Distribution of neuropathological lesions in pig brains after different durations of cardiac arrest. Resuscitation 2010;81:1577–83. https://doi.org/10.1016/J.RESUSCITATION.2010.07.005.

31. Behringer W, Safar P, Wu X, et al. Survival without brain damage after clinical death of 60–120 mins in dogs using suspended animation by profound hypothermia. Crit Care Med 2003;31:1523–31. https://doi.org/10.1097/01.CCM.0000063450.73967.40.

32. Lafrenaye AD, Todani M, Walker SA, Povlishock JT. Microglia processes associate with diffusely injured axons following mild traumatic brain injury in the micro pig. J Neuroinflammation 2015;12. https://doi.org/10.1186/S12974-015-0405-6.

33. Ruggeri L, Nespoli F, Ristagno G, et al. Esmolol during cardiopulmonary resuscitation reduces neurological injury in a porcine model of cardiac arrest. Sci Rep 2021;11. https://doi.org/10.1038/S41598-021-90202-W.

34. Gagno G, Ferro F, Fluca AL, et al. From brain to heart: possible role of amyloid-β in ischemic heart disease and ischemia-reperfusion injury. Int J Mol Sci 2020;21:1–15. https://doi.org/10.3390/IJMS21249655.

35. Sharma HS, Miclescu A, Wiklund L. Cardiac arrest-induced regional blood-brain barrier breakdown, edema formation and brain pathology: A light and electron microscopic study on a new model for neurodegeneration and neuroprotection in porcine brain. J Neural Transm 2011;118:87–114. https://doi.org/10.1007/S00702-010-0486-4/TABLES/5.

36. Pulsinelli WA, Brierley JB, Plum F. Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol 1982;11:491–8. https://doi.org/10.1002/ANA.410110509.

37. Abe K, Aoki M, Kawagoe J, et al. Ischemic delayed neuronal death. A mitochondrial hypothesis. Stroke 1995;26:1478–89. https://doi.org/10.1161/01.STR.26.8.1478.

38. Polderman KH. Mechanisms of action, physiological effects, and complications of hypothermia. Crit Care Med 2009;37. https://doi.org/10.1097/CCM.0B013E3181AA5241.

39. Mustafa S, Thulesius O. Cooling-Induced Carotid Artery Dilatation. Stroke 2002;33:256–60. https://doi.org/10.1161/HS0102.101545.

40. Aibiki M, Maekawa S, Yokono S. Moderate hypothermia improves imbalances of thromboxane A2 and prostaglandin I2 production after traumatic brain injury in humans. Crit Care Med 2000;28:3902–6. https://doi.org/10.1097/00003246-200012000-00029.

41. Hu CL, Wen J, Liao XX, et al. Effects of therapeutic hypothermia on coagulopathy and microcirculation after cardiopulmonary resuscitation in rabbits. Am J Emerg Med 2011;29:1103–10. https://doi.org/10.1016/J.AJEM.2010.07.016.

42. Yu H, Wang L, Zhang H, et al. Effect of mild hypothermia on cerebral microcirculation in a murine cardiopulmonary resuscitation model. Microcirculation 2019;26:e12537. https://doi.org/10.1111/MICC.12537.

43. Busija DW, Leffler CW. Hypothermia reduces cerebral metabolic rate and cerebral blood flow in newborn pigs. Https://DoiOrg/101152/

Ajpheart19872534H869. 1987; 253. doi: 10.1152/
AJPHEART.1987.253.4.H869.

44. Strauch JT, Spielvogel D, Haldenwang PL, et al. Impact of
hypothermic selective cerebral perfusion compared with hypothermic
cardiopulmonary bypass on cerebral hemodynamics and
metabolism. Eur J Cardiothorac Surg 2003;24:807–16. https://doi.
org/10.1016/S1010-7940(03)00517-7.

45. Ehrlich MP, McCullough JN, Zhang N, et al. Effect of hypothermia on
cerebral blood flow and metabolism in the pig. Ann Thorac Surg
2002;73:191–7. https://doi.org/10.1016/S0003-4975(01)03273-8.

46. Neumar RW, Nolan JP, Adrie C, et al. Post-cardiac arrest syndrome.
Circulation 2008;118:2452–83. https://doi.org/10.1161/
CIRCULATIONAHA.108.190652.

47. Cho JW, Lee CH, Jang JS, Kwon OC, Roh WS, Kim JE. Moderate
and deep hypothermia produces hyporesposiveness to
phenylephrine in isolated rat aorta. Korean Journal of Thoracic and
Cardiovascular Surgery 2013;46:402–12. https://doi.org/10.5090/
KJTCS.2013.46.6.402.

48. Ruggeri L, Franco A, Alba AC, et al. Coagulation derangements in
patients with refractory cardiac arrest treated with extracorporeal
cardiopulmonary resuscitation. J Cardiothorac Vasc Anesth
2019;33:1877–82. https://doi.org/10.1053/j.jvca.2018.11.014.

49. Tsuchida T, Wada T, Gando S. Coagulopathy induced by veno-
arterial extracorporeal membrane oxygenation is associated with a
poor outcome in patients with out-of-hospital cardiac arrest. Front
Med (Lausanne) 2021;8. https://doi.org/10.3389/
FMED.2021.651832.