Prolonged mild hypothermia after experimental hypothermic circulatory arrest in a chronic porcine model

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See related editorial on page 621.

Objectives: We sought to evaluate the potential efficacy of prolonged mild hypothermia after hypothermic circulatory arrest.

Methods: Twenty pigs, after a 75-minute period of hypothermic circulatory arrest, were randomly assigned to be rewarmed to 37°C (normothermia group) or to 32°C and kept at that temperature for 14 hours from the start of rewarming (hypothermia group).

Results: The 7-day survival was 30% in the hypothermia group and 70% in the normothermia group (P = .08). The hypothermia group had poorer postoperative behavioral scores than the normothermia group. Prolonged hypothermia was associated with lower oxygen extraction and consumption rates and higher mixed venous oxygen saturation levels during the first hours after hypothermic circulatory arrest. Decreased cardiac index, lower pH, and higher partial pressure of carbon dioxide were observed in the hypothermia group. There was a trend for beneficial effect of prolonged hypothermia in terms of lower brain lactate levels until the 4-hour interval and of intracranial pressure until the 10-hour interval. Postoperatively, total leukocyte and neutrophil counts were lower, and creatine kinase BB was significantly increased in the hypothermia group. At extubation, the hypothermia group had higher oxygen extraction rates and lower brain tissue oxygen tension.

Conclusions: A 14-hour period of mild hypothermia after 75-minute hypothermic circulatory arrest seems to be associated with poor outcome. However, the results of this study suggest that mild hypothermia may preserve its efficacy when it is used for no longer than 4 hours, but the potentials of a shorter period of postoperative mild hypothermia still require further investigation.

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The metabolic benefits achieved by the ischemic brain under hypothermic conditions have brought interest in hypothermia as a treatment method, even in fields other than cardiac surgery. Despite perceived detrimental effects, as reported by several studies in the 1970s,[1] the use of prolonged mild hypothermia in the management of stroke,[2,3] brain injury,[4-7] and neonatal hypoxic-ischemic encephalopathy[8] has regained interest, and it is currently under extensive experimental and clinical evaluation. The encouraging results achieved with the use of

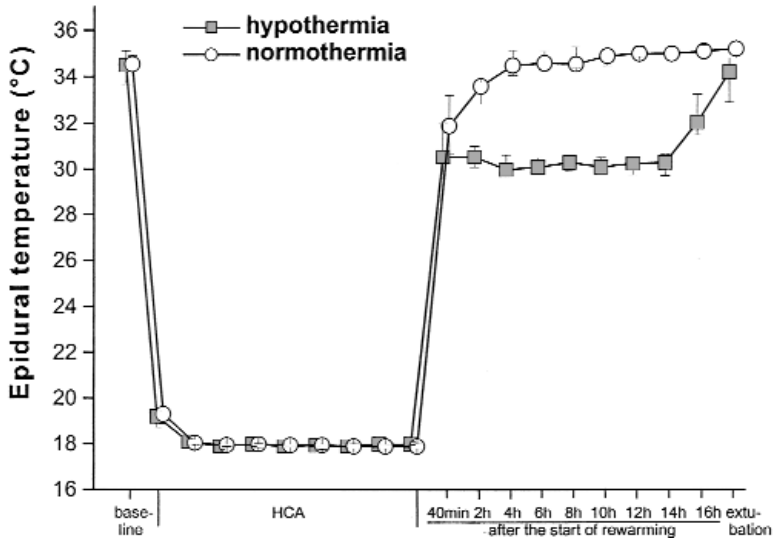

Figure 1. Epidural temperatures in pigs during the experimental protocol. Values are expressed as medians and interquartile ranges (25th-75th percentile).

intraischemic and postischemic prolonged mild hypothermia and the need for improved cerebral protection during surgical repair of the aortic arch under hypothermic circulatory arrest (HCA)[9] led us to evaluate the potential efficacy of a 14-hour period of mild hypothermia after 75 minutes of HCA in a chronic porcine model.

Material and Methods

Twenty female juvenile pigs (age, 8-10 weeks) of a native stock, after a 75-minute period of HCA at a core temperature of 20°C, were randomly assigned to be rewarmed to 37°C during 60 minutes of reperfusion (normothermia group) or to undergo a 5-minute period of reperfusion at a core temperature of 20°C and then to be rewarmed to 32°C during 60 minutes of reperfusion and kept at that temperature for 14 hours from the start of rewarming (hypothermia group).

Preoperative Management

All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. The study was approved by the Research Animal Care and Use Committee of the University of Oulu.

Anesthesia and Hemodynamic Monitoring

Anesthesia was induced with medetomidine hydrochloride (1 mg administered intramuscularly) and midazolam (30 mg administered intramuscularly). A peripheral venous catheter was inserted into the right ear for administration of drugs and to maintain fluid balance with Ringer acetate. Anesthesia was deepened with thiopental sodium (125-250 mg administered intravenously), and pancuronium bromide (4 mg administered intravenously) was given for muscular paralysis. Cefuroxime, 1.5 g, was given as antibiotic prophylaxis at anesthesia induction, 8 hours after the start of rewarming, and before extubation.

After endotracheal intubation, the animals were maintained on positive-pressure ventilation with 40% oxygen, and anesthesia was maintained with isoflurane (1.2%-1.3%). Electrocardiographic monitoring was started. An arterial catheter was positioned in the left femoral artery for arterial pressure monitoring and blood sampling. A thermodilution catheter (CritiCath, 7F; Ohmeda GmbH & Co, Erlangen, Germany) was placed through the left femoral vein to allow blood sampling and pressure monitoring in the pulmonary artery and for recording the blood temperature and cardiac output. A 10F catheter was placed in the urinary bladder for monitoring urine output. Temperatures were monitored from blood, the rectum, and the esophagus and from the epidural and intracerebral spaces.

Brain Microdialysis and Intracerebral Monitoring

A temperature probe was placed into the epidural space through a cranial hole made in the left side of the coronal suture. Two catheters for the measurement of intracerebral tissue oxygen partial pressure (Revodoxe Brain Oxygen Catheter-Micro-Probe, REF CC1.SB; GMS, Mielkendorf, Germany) and temperature (Thermocouple Temperature Catheter-Micro-Probe, REF. C8.B, GMS) were inserted through a hole located at the right side anteriorly to the coronal suture. These parameters were monitored with the Licox CMP Monitor (GMS). An intracerebral microdialysis catheter and a pressure-monitoring catheter (Codman Micro-Sensor ICP Transducer, Codman ICP Express Monitor; Codman & Shurtleff, Inc, Raynham, Mass) were placed through a hole located at the right side posteriorly to the coronal suture.

The microdialysis catheter (CMA 70; CMA/Microdialysis, Stockholm, Sweden) was placed into the brain cortex to a depth of

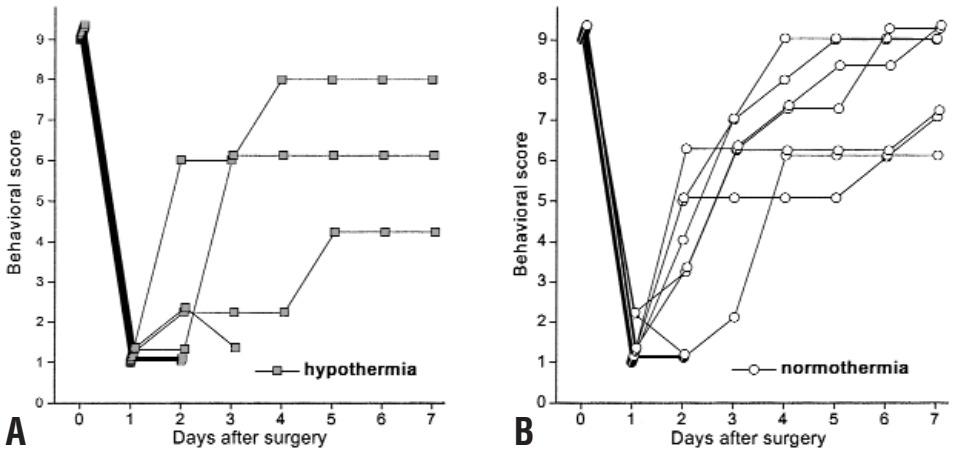

Figure 2. Daily score indicating behavioral recovery after 75 minutes of HCA. A score of 8 or 9 indicates essentially complete recovery.

15 mm below the dura mater. The catheter was connected to a 2.5-mL syringe placed into a microinfusion pump (CMA 106, CMA/Microdialysis) and perfused with Ringer solution (Perfusion Fluid CNS, CMA/Microdialysis). Samples were collected at different time points. The concentrations of cerebral tissue glucose, lactate, glutamate, and glycerol were measured immediately after collection with a microdialysis analyzer (CMA 600, CMA/Microdialysis) by using ordinary enzymatic methods.

Electroencephalography Monitoring

Cortical electrical activity was registered by 4 stainless-steel screw electrodes of 5 mm in diameter implanted in the skull over the parietal and frontal areas of the cortex by using a digital electroencephalography (EEG) recorder (Nervus, Reykjavik, Iceland) and an amplifier (Magnus EEG 32/8, Reykjavik, Iceland). Sampling frequency was 1024 Hz, and bandwidth was 0.03 to 256 Hz. All EEG recordings were referenced to a frontal screw electrode, which, together with a ground screw electrode, was implanted over the frontal sinuses.

The isoflurane level was adjusted so that EEG showed a steady burst-suppression pattern. Then isoflurane end-tidal concentration was kept at this steady level until the end of monitoring. EEG was recorded for 10 minutes to get a baseline recording of steady burst-suppression activity before the cooling period. After HCA, EEG recording was restarted and continued until extubation. The duration of bursts was measured from 5-minute EEG samples at 1-hour intervals. Artifact periods were excluded from each 5-minute sample, and after that, the sum of bursts was counted as a percentage of the sum of artifact-free bursts and suppressions. This percentage was used as a measure of EEG activity in the analysis.

Cardiopulmonary Bypass

Through a right thoracotomy in the fourth intercostal space, the right thoracic vessels were ligated, the pericardium was opened, and the heart and great vessels were exposed. A membrane oxygenator (Midiflow D 705; Dideco, Mirandola, Italy) was primed with 1 L of Ringer acetate and heparin (5000 IU). After systemic heparinization (500 IU/kg), the ascending aorta was cannulated with a 16F arterial cannula, and the right atrial appendage was cannulated with a single 24F atrial cannula. Nonpulsatile cardiopulmonary bypass (CPB) was initiated at a flow rate of 100 mL $\cdot kg^{-1} \cdot min^{-1}$, and the flow was adjusted to maintain a perfusion pressure of 50 mm Hg. A 12F intracardiac sump cannula was positioned in the left ventricle through the apex of the heart for decompression of the left side of the heart during CPB. A heat exchanger was used for core cooling**.** The pH was maintained with alpha-stat principles at 7.40 ± 0.05, with an arterial carbon dioxide tension of 4.0 to 5.0 kPa uncorrected for temperature.

Experimental Protocol

A cooling period of 60 minutes was carried out to attain a rectal temperature of 20°C. Then a 75-minute period of HCA was started. The ascending aorta was crossclamped just distal to the aortic cannula, and cardiac arrest was induced by injecting potassium chloride (3 g) through the aortic cannula. Topical cardiac cooling with ice slush was begun and maintained throughout the HCA period. During HCA, the epidural and intracerebral temperatures were maintained at a level of 18°C with ice packs placed over the head. After 75 minutes of HCA, rewarming was started. Animals in the hypothermia group underwent a 5-minute period of reperfusion at a rectal temperature of 20°C, and then they were rewarmed to a rectal temperature of 32°C during 60 minutes of reperfusion and kept at that temperature for 14 hours from the start of rewarming, whereas animals in the normothermia group were rewarmed to a rectal temperature of 37°C during 60 minutes of reperfusion. In both groups the temperatures were regulated by heat-exchanger mattress, heating lamps, and ice packs. Both groups were extubated 18 hours after the start of rewarming, when the rectal temperature approximated 37°C. We decided that the experiment should have been driven by the rectal temperature because it is the one most clinically relevant in this model closely resembling HCA during cardiac surgery, and it is likely not affected by the presence of ice slush into the pericardium during HCA.

During rewarming, the left ventricular sump cannula was removed, and furosemide (40 mg), mannitol (15 g), methylprednisolone (80 mg), lidocaine (40-150 mg), and calcium chloride (1375 mg) were administered. After weaning from CPB, cardiac support was provided with dopamine. The animals were kept in

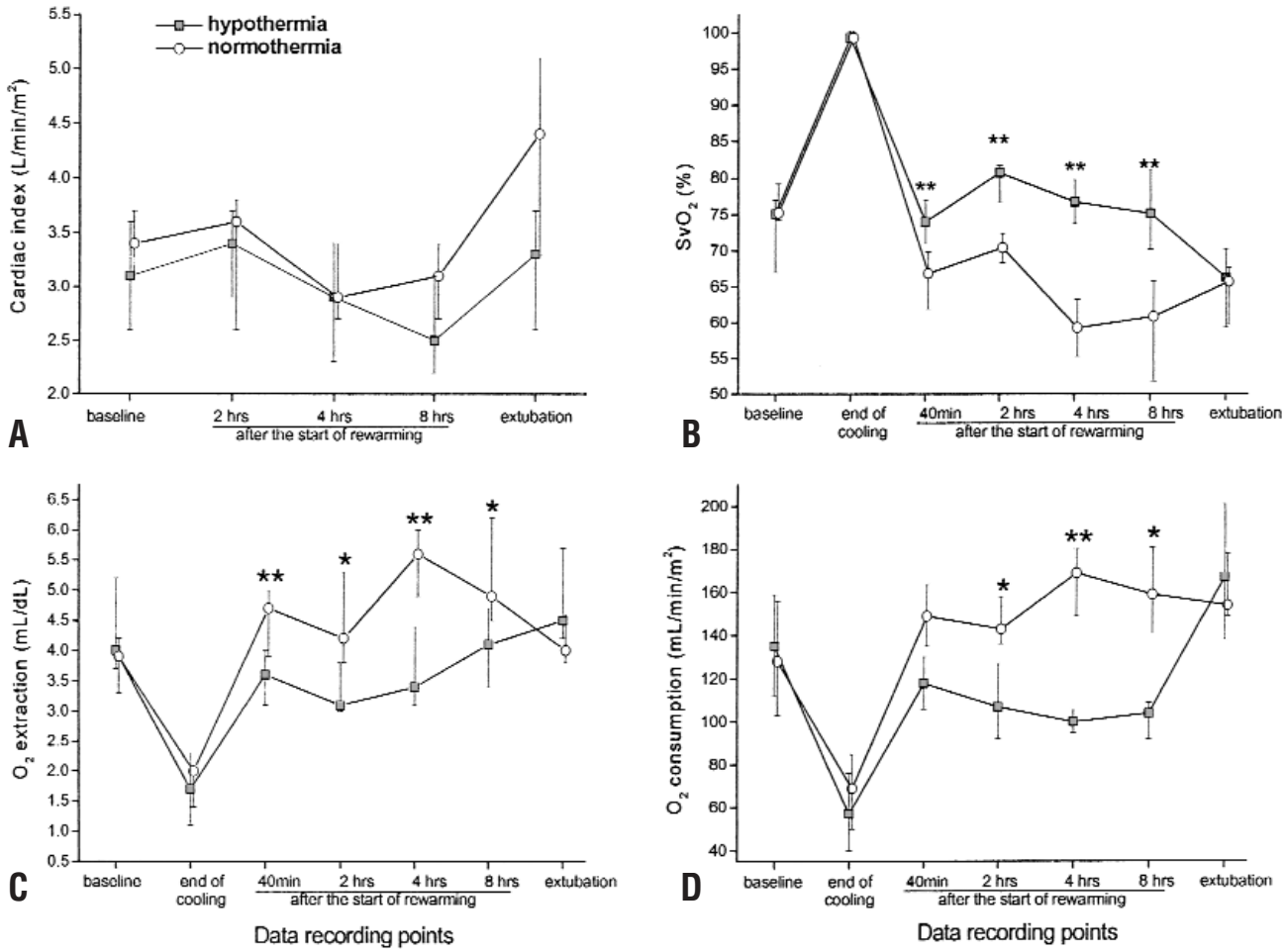

Figure 3. Cardiac index and oxygen kinetics parameters. Values are expressed as medians with interquartile ranges (25th-75th percentile). *$P < .05$, interaction between time versus baseline and other intervals in the study groups; **$P < .01$, interaction between time versus baseline and other intervals in the study groups. Svo_2, Mixed venous oxygen saturation.

isoflurane anesthesia until the following morning, and then they were extubated and moved to a recovery room.

During the experiment, hemodynamic and metabolic measurements (pulse rate, systemic and pulmonary arterial pressures, central venous pressure, pulmonary capillary wedge pressure, cardiac output, intracranial pressure, intracerebral tissue oxygen partial pressure, temperatures, arterial and venous pH, oxygen and carbon dioxide partial pressure, oxygen saturation, oxygen concentration, hematocrit, hemoglobin, sodium, potassium, and glucose [Ciba-Corning 288 Blood Gas System; Ciba-Corning Diagnostic Corp, Medfield, Mass]; lactate [YSI 1500 analyzer; Yellow Springs Instrument Co, Yellow Springs, Ohio]; leukocyte differential count [Cell-Dyn analyzer; Abbot, Santa Clara, Calif]; and creatine kinase [CK] and its isoenzymes [CK-MM, CK-MB, CK-BB; Hydrasys LC-electrophoresis, Hyrys-densitometry, Sebia, France]) were recorded continuously or at baseline; at the end of cooling (at 20°C immediately before institution of HCA); 40 minutes, 2 hours, 4 hours, and 8 hours after the start of rewarming; and before extubation.

Postoperative Evaluation

Postoperatively, all the animals were evaluated daily by an experienced observer who was blinded to the study group using a species-specific quantitative behavioral score, as reported earlier.[10] The assessment quantified mental status (0 = comatose, 1 = stuporous, 2 = depressed, and 3 = normal), appetite (0 = refuses liquids, 1 = refuses solids, 2 = decreased, and 3 = normal), and motor function (0 = unable to stand, 1 = unable to walk, 2 = unsteady gait, and 3 = normal). Numeric summing of these functions provides a final score, with the maximum (score of 9) reflecting apparently normal neurologic function, and lower values indicating substantial brain damage.

Perfusion Fixation

Each surviving animal was electively killed on the seventh postoperative day. Immediately after intravenous injection of pentobarbital (60 mg/kg) and heparin (500 IU/kg), the thoracic cavity was opened, and the descending thoracic aorta was clamped.

TABLE 1. Experimental data

	Baseline	End of cooling	After the start of rewarming				Extubation	*P* value between groups
			40 min	2 h	4 h	8 h		
Mean arterial pressure (mm Hg)								
Hypothermia	90 (78-95)	56 (52-60)	67 (59-76)	70 (64-77)	64 (58-74)	70 (69-72)	55 (53-61)	.39
Normothermia	90 (81-105)	54 (52-56)	74 (69-79)	72 (63-80)	70 (58-77)	63 (61-65)	60 (58-64)	
Heart rate (1/min)								
Hypothermia	111 (94-122)	—	120 (116-129)	119 (106-140)	106 (104-117)	114 (108-127)	128 (115-154)	.11
Normothermia	115 (105-123)	—	138 (128-165)	131 (116-142)	130 (114-147)	128 (121-141)	129 (117-136)	
Dopamine administered (mg/h)								
Hypothermia	—	—	—	8.5 (6.1-10.2)	8.5 (7.2-10.2)‡	10.8 (6.1-14.5)‡	15.5 (13.3-18.0)	—
Normothermia	—	—	—	7.5 (4.0-11.8)	5.3 (3.7-6.4)‡	6.1 (6.0-8.3)‡	12.0 (9.9-17.5)	
Hemoglobin (g/L)								
Hypothermia	102 (99-104)	67 (61-69)	80 (75-86)	96 (94-102)	99 (94-108)*	101 (98-104)†	88 (86-95)†	.003
Normothermia	98 (94-105)	60 (57-70)	83 (78-89)	92 (87101)	86 (82-96)*	81 (75-87)†	67 (60-76)†	
Hematocrit (%)								
Hypothermia	29.5 (29.0-30.5)	19.5 (18.1-20.5)	23.4 (22.0-25.2)	28.5 (27.5-30.0)	29.2 (27.6-31.7)*	29.8 (28.8-30.6)†	25.8 (25.2-27.9)†	.003
Normothermia	28.6 (27.7-31.3)	17.4 (16.7-20.5)	24.2 (22.7-25.9)	27.0 (25.5-29.5)	25.2 (23.9-28.1)*	24.3 (22.1-25.6)†	20.0 (17.7-22.2)†	
Total leukocyte count (1×10^9/L)								
Hypothermia	15.2 (13.7-19.2)	2.5 (2.0-3.3)	6.7 (5.2-10.6)	14.7 (12.4-25.9)†	17.7 (14.2-27.3)†	—	—	.18
Normothermia	14.9 (11.7-17.8)	2.2 (1.6-2.8)	10.8 (6.8-11.7)	24.0 (20.5-27.4)†	30.2 (26.0-34.1)†	—	—	
Neutrophil count (1×10^9/L)								
Hypothermia	6.2 (4.7-7.5)	1.2 (0.7-1.5)	2.7 (1.9-3.8)	9.1 (6.6-16.6)*	12.5 (9.2-21.1)†	—	—	.07
Normothermia	5.1 (3.4-6.9)	0.8 (0.6-1.4)	3.7 (2.0-5.8)	17.2 (15.0-20.3)*	23.2 (20.4-26.6)†	—	—	
Rectal temperature (°C)								
Hypothermia	37.4 (36.4-37.7)	20.1 (19.5-20.4)	29.0 (27.9-31.0)	32.1 (31.7-32.2)	32.2 (31.9-32.3)	32.0 (31.8-32.4)	35.2 (34.9-36.8)	—
Normothermia	37.5 (36.8-37.7)	20.2 (20.0-20.4)	32.4 (31.4-32.9)	35.2 (35.0-35.6)	37.2 (37.0-37.4)	37.0 (36.8-37.2)	37.1 (36.7-37.2)	
Blood temperature (°C)								
Hypothermia	37.4 (36.4-37.7)	19.3 (18.7-19.7)	35.0 (34.8-35.6)	32.3 (31.9-32.6)	32.1 (32.0-32.3)	32.0 (31.9-32.4)	35.7 (35.3-37.0)	—
Normothermia	37.5 (36.8-37.8)	18.7 (18.2-19.4)	37.3 (36.3-38.0)	35.3 (34.8-35.7)	37.3 (37.1-37.4)	37.1 (37.0-37.3)	37.1 (36.7-37.3)	
Esophageal temperature (°C)								
Hypothermia	37.5 (36.3-37.6)	19.8 (18.6-20.2)	32.5 (31.3-33.6)	32.5 (31.8-32.6)	32.2 (32.0-32.4)	32.1 (31.9-32.3)	35.3 (34.9-36.8)	—
Normothermia	37.4 (36.8-37.6)	19.2 (18.5-19.7)	34.2 (33.5-35.5)	35.4 (35.2-35.5)	37.2 (37.1-37.4)	37.1 (37.0-37.2)	37.1 (36.7-37.2)	
Epidural temperature (°C)								
Hypothermia	34.5 (33.7-35.1)	19.2 (18.7-19.5)	30.6 (29.6-32.0)	30.6 (30.1-31.0)	30.0 (29.8-30.6)	30.4 (30.0-30.5)	34.3 (32.9-34.8)	—
Normothermia	34.6 (34.2-34.9)	19.3 (18.9-19.7)	31.9 (30.7-33.2)	33.6 (32.9-33.8)	34.5 (34.1-35.2)	34.6 (34.4-35.3)	35.2 (34.8-35.6)	
Intracerebral temperature (°C)								
Hypothermia	36.0 (35.9-36.5)	19.0 (18.6-19.4)	30.6 (29.5-33.7)	31.1 (31.0-31.8)	30.4 (30.3-31.5)	30.9 (30.5-31.5)	35.4 (33.9-36.8)	—
Normothermia	35.9 (35.4-36.4)	18.3 (18.3-18.8)	34.7 (32.3-35.2)	34.5 (33.6-35.5)	36.3 (35.4-36.5)	36.2 (35.6-36.7)	36.6 (35.5-36.9)	

Values are shown as medians and interquartile ranges (25th-75th percentile). *P* values between groups represent tests of between-subject effects.
*$P < .05$, interaction between time versus baseline and other intervals in the study groups.
†$P < .01$, interaction between time versus baseline and other intervals in the study groups.
‡$P < .05$, normothermia versus hypothermia.

CSP

Ringer solution (1 L) was infused through the ascending thoracic aorta through the upper body, and blood was suctioned from the superior vena cava until the perfusate was clear of blood. Then 10% formalin solution (1 L/15 min) was infused through the brain in the same manner to accomplish a perfusion fixation. Immediately thereafter, the entire brain was harvested, weighed, and immersed in 10% neutral formalin. The same method of fixation procedure was carried out in those animals that died before the seventh postoperative day.

Histopathologic Analysis

The brain was allowed to fix for 1 week en bloc. Thereafter, 3-mm thick coronal samples were sliced from the left frontal lobe, thalamus (including the adjacent cortex), and hippocampus (including the adjacent brainstem and temporal cortex), and sagittal samples from the posterior brainstem (medulla oblongata and pons) and cerebellum were obtained. The specimens were fixed in fresh formalin for another week. After fixation, the samples were processed as follows: rinsing in water for 20 minutes and immersion in 70%

TABLE 2. Metabolic data

	Baseline	End of cooling	After the start of rewarming				Extubation	*P* value between groups
			40 min	2 h	4 h	8 h		
Arterial pH								
Hypothermia	7.53 (7.50-7.55)	7.45 (7.35-7.46)	7.39 (7.36-7.41)	7.39 (7.37-7.43)*	7.47 (7.47-7.49)†	7.50 (7.47-7.52)	7.53 (7.50-7.60)	.002
Normothermia	7.54 (7.51-7.57)	7.42 (7.38-7.45)	7.42 (7.40-7.44)	7.48 (7.46-7.49)*	7.55 (7.52-7.58)†	7.57 (7.56-7.58)	7.55 (7.54-7.57)	
Venous pH								
Hypothermia	7.48 (7.46-7.49)	7.40 (7.33-7.42)	7.33 (7.28-7.34)	7.35 (7.34-7.40)	7.44 (7.42-7.46)	7.46 (7.42-7.47)	7.47 (7.44-7.54)	.032
Normothermia	7.50 (7.47-7.52)	7.37 (7.34-7.41)	7.35 (7.32-7.37)	7.43 (7.41-7.44)	7.49 (7.46-7.52)	7.50 (7.49-7.51)	7.50 (7.47-7.52)	
$Paco_2$ (kPa)								
Hypothermia	4.8 (4.6-4.8)	5.4 (4.7-6.0)	4.5 (4.3-4.8)	5.7 (5.3-6.2)*	5.6 (5.5-6.0)*	5.5 (5.1-5.9)	4.9 (4.6-5.6)	<.001
Normothermia	4.4 (4.2-4.9)	5.2 (4.9-6.1)	4.0 (3.8-4.3)	4.7 (4.5-4.9)*	4.6 (4.6-5.1)*	4.8 (4.5-4.9)	4.6 (4.3-4.7)	
$Pvco_2$ (kPa)								
Hypothermia	5.8 (5.4-6.1)	6.0 (5.6-6.8)	5.6 (5.4-6.0)	6.6 (6.1-7.0)	6.5 (6.2-6.8)	6.4 (6.1-7.1)	6.1 (5.6-6.8)	.005
Normothermia	5.5 (5.1-5.7)	5.9 (5.6-6.7)	5.4 (5.0-5.5)	5.7 (5.6-6.1)	5.9 (5.8-6.2)	6.0 (5.9-6.2)	5.4 (5.3-5.5)	
Venous lactate (mmol/L)								
Hypothermia	1.2 (1.0-1.5)	2.2 (1.7-2.6)	5.6 (4.9-5.9)	4.3 (3.6-4.6)	1.9 (1.4-2.4)	—	—	.36
Normothermia	1.1 (0.8-1.2)	2.1 (1.8-2.2)	4.9 (4.3-5.7)	3.8 (3.2-4.9)	1.8 (1.2-1.8)	—	—	
Venous CK-BB (U/L)								
Hypothermia	208 (160-323)	162 (116-203)	257 (203-352)	624 (508-732)	614 (500-680)	783 (692-905)†	684 (617-1007)*	<.001
Normothermia	166 (149-290)	125 (110-150)	216 (197-291)	389 (359-506)	347 (276-505)	249 (134-350)†	373 (257-590)*	

Values are shown as medians and interquartile ranges (25th-75th percentile). *P* value between groups represents tests of between-subject effects. $Paco_2$, Arterial partial pressure of carbon dioxide; $Pvco_2$, venous partial pressure of carbon dioxide; *CK-BB,* creatine kinase BB isoenzyme.
*$P < .05$, interaction between time versus baseline and other intervals in the study groups.
†$P < .01$, interaction between time versus baseline and other intervals in the study groups. $Pvco_2$, mixed venous partial pressure of carbon dioxide.

ethanol for 2 hours, 94% ethanol for 4 hours, and absolute ethanol for 9 hours. Then the specimens were kept for 1 hour in absolute ethanol-xylene mixture and 4 hours in xylene and embedded in warm paraffin for 6 hours. The specimens were sectioned at 6 μm and stained with hematoxylin and eosin. The sections of the brain specimens of each animal were screened by an experienced senior pathologist (J.H.) unaware of the experimental design and the identity and fate of individual animals. Each section was carefully examined for the presence or absence of any ischemic or other kinds of tissue damage.

The signs of injury were scored as follows: 1 (slight edema, dark or eosinophilic neurons or cerebellar Purkinje cells); 2 (moderate edema, at least 2 hemorrhagic foci in the section); 3 (severe edema, several hemorrhagic and infarct foci [local necrosis]). The total regional score was the sum of the scores in each specific brain area (cortex, thalamus, hippocampus, posterior brainstem, and cerebellum). A total histopathologic score was calculated by summing all the regional scores to allow semiquantitative comparisons between the animals.

Statistical Analysis

Values are expressed as the median with interquartile ranges (25th-75th percentile). Statistical analysis was performed with SPSS software (SPSS version 10.0; SPSS Inc, Chicago, Ill). The Kaplan-Meier curve with the log-rank test was used to evaluate survival. Differences between these 2 groups were determined by the *t* test or the Mann-Whitney test. Variance analysis of repeated measurements was used, and tests of between-subject effects and interactions between baseline and other interval measurements in these 2 groups were reported.

Results

Comparability of the Groups

The median weight of pigs was 29.8 kg (interquartile range, 27.5-31.5 kg) in the hypothermia group and 29.6 kg (interquartile range, 28.2-32.6 kg) in the normothermia group ($P = .96$). The median CPB cooling time was 61.0 minutes (interquartile range, 61.0-62.5 minutes) in the hypothermia group and 62.0 minutes (interquartile range, 61.0-64.5 minutes) in the normothermia group ($P = .24$). The median CPB rewarming time was 64.0 minutes (interquartile range, 62.7-68.5 minutes) in the hypothermia group and 64.5 minutes (interquartile range, 62.7-68.5 minutes) in the normothermia group ($P = .95$). The median total CPB time was 127.0 minutes (interquartile range, 124.0-129.7 minutes) in the hypothermia group and 128.5 minutes (interquartile range, 125.5-132.5 minutes) in the normothermia group ($P = .34$). During HCA, the temperatures did not differ between the groups (Figure 1).

Mortality and Morbidity

Survival figures on the second and seventh postoperative days were 70% and 30% in the hypothermia group and 90% and 70% in the normothermia group, respectively (*P*

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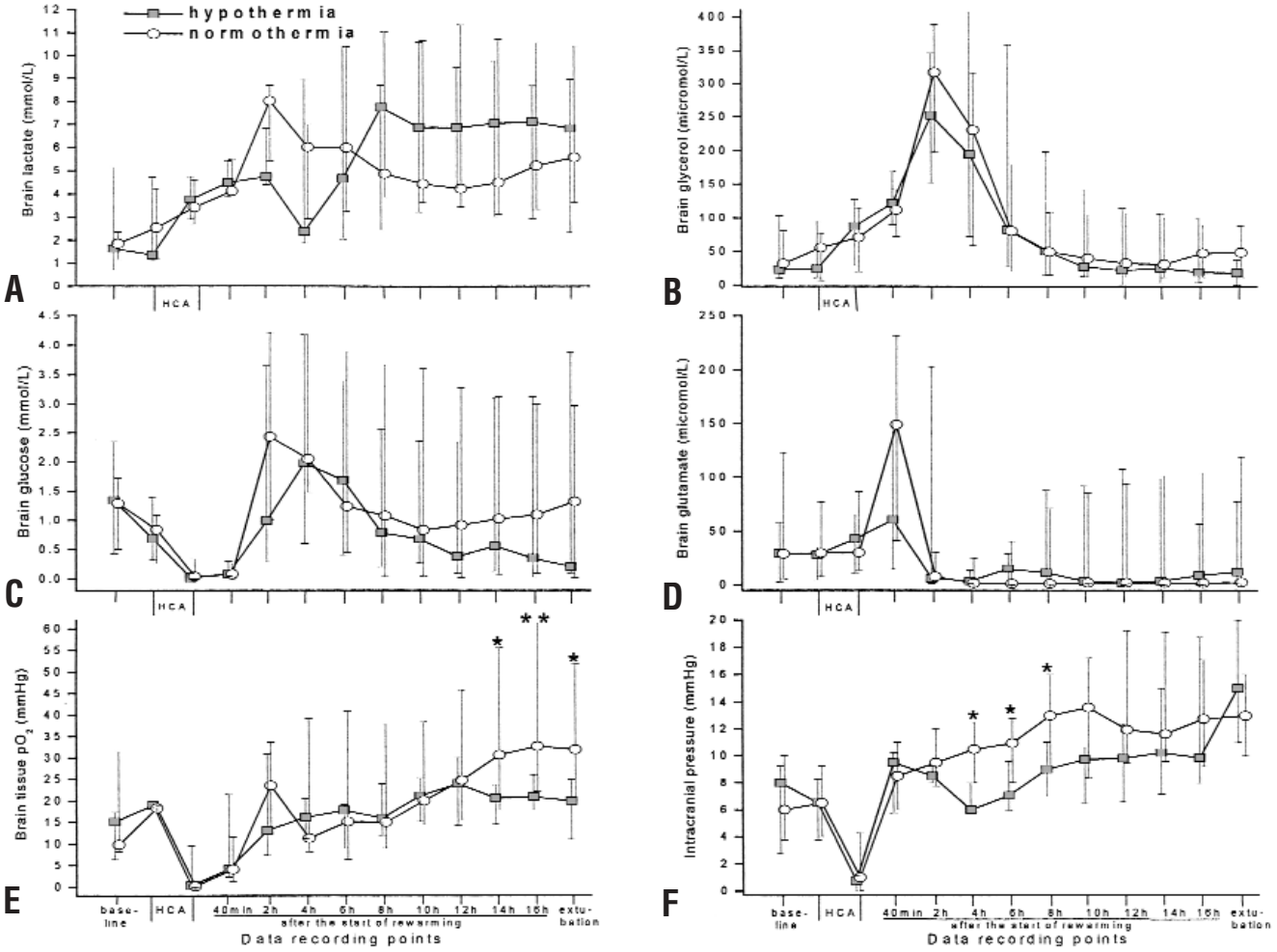

Figure 4. Results of brain microdialysis, intracranial pressure, and brain tissue oxygen partial pressure measurements. Values are expressed as medians with interquartile ranges (25th-75th percentile). *$P < .05$, interaction between time versus baseline and other intervals in the study groups; **$P < .01$, interaction between time versus baseline and other intervals in the study groups.

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= .08). Animals that underwent prolonged mild hypothermia after HCA had lower behavioral scores than those of the normothermia group throughout the postoperative surveillance (Figure 2). Such differences were not statistically significant, probably because only a few animals of the hypothermia group survived. However, the survivors of the hypothermia group failed to reach complete recovery on the seventh postoperative day, as otherwise observed in 4 of 7 survivors of the normothermia group (P = .09, Figure 2).

Hemodynamic and Metabolic Data

Experimental and metabolic data are presented in Tables 1 and 2 and in Figure 3. After 75 minutes of HCA, pigs that underwent prolonged hypothermia had lower oxygen extraction and consumption rates and higher mixed venous oxygen saturation levels during the first hours after HCA (Figure 3, *B-D*). At extubation, such a condition was almost reversed, with animals of the normothermia group having lower oxygen extraction and consumption rates than those of the hypothermia group (Figure 3, *C*). Median oxygen extraction rates at extubation tended to be higher in those animals that died postoperatively (4.5 vs 3.9 mL/dL, P = .3).

The apparent advantage of better oxygen kinetic parameters was not coupled by the cardiac index because, even if without statistical significance, it was lower in the hypothermia group at the 8-hour interval and at extubation (Figure 3, *A*). It is worth noting that animals of the hypothermia group required significantly higher doses of dopamine during the postoperative period (Table 1).

Furthermore, lower pH values associated with higher arterial and venous carbon dioxide partial pressure values

TABLE 3. Histopathologic scores

Protocol	Pig No.	Survival	Cortex score	Thalamus score	Hippocampus score	Posterior brainstem score	Cerebellum score	Total score
Hypothermia	1	7	4	1	1	1	1	8
	2	2	3	4	2	3	2	14
	3	2	4	4	3	5	5	21
	4	7	3	2	3	4	0	12
	5	1	5	5	2	4	2	18
	6	1	2	4	1	4	2	13
	7	7	1	0	1	1	1	4
	8	1	4	4	3	5	2	18
	9	2	4	3	2	2	2	13
	10	3	2	2	1	1	1	7
Mean			3.2	2.9*	1.9	3	1.8	12.8
Normothermia	1	7	2	2	3	2	2	11
	2	7	5	1	5	2	2	15
	3	7	2	2	2	1	1	8
	4	7	6	1	2	2	3	14
	5	2	4	2	3	3	2	14
	6	2	2	1	1	2	2	8
	7	7	4	1	2	2	1	10
	8	7	4	1	2	0	1	8
	9	1	3	3	2	3	2	13
	10	7	5	2	2	2	2	13
Mean			3.7	1.6*	2.4	1.9	1.8	11.4

Signs of injury were scored as follows: 1 (slight edema, dark or eosinophilic neurons or cerebellar Purkinje cells); 2 (moderate edema, at least 2 hemorrhagic foci in the section); 3 (severe edema, several hemorrhagic and infarct foci [local necrosis]). The total score is the sum of scores in each specific brain area.

*P = .05, hypothermia versus normothermia.

were observed among animals of the hypothermia group (Table 2). This observation suggest that metabolic acidosis without respiratory compensation occurred under prolonged mild hypothermia after HCA, as also indicated by slightly higher venous lactate concentrations in this group of animals (Table 2).

Measurements of serum concentrations of CK-BB showed that this parameter was significantly higher in pigs that underwent prolonged mild hypothermia ($P < .001$, Table 2). Serum concentrations of total CK and of the other isoenzymes (CK-MM and CK-MB) did not differ between the study groups.

Total leukocyte and neutrophil counts were found to be significantly decreased in the hypothermia group after the procedure compared with those in the normothermia group (Table 1).

Intracranial Measurements

Intracranial measurement data are presented in Figure 4. Intracranial microdialysis measurements failed to show any statistically significant differences between these 2 groups. However, there was a trend for a beneficial effect of prolonged hypothermia in terms of lower brain lactate levels and intracranial pressure values until the 4-hour interval (Figure 4, *A* and *F*). Worsening of these 2 parameters coincided with a second slight increase in glutamate levels (Figure 4, *D*). The normothermia group tended to have a quicker increase in intracerebral concentration of glucose during the first hours after rewarming, and its concentration was higher at extubation as well ($P = .92$; Figure 4, *C*). Also, the brain tissue oxygen partial pressures during the last intervals became significantly higher in the normothermia group compared with those in the hypothermia group (Figure 4, *E*).

Histopathologic Data

No statistically significant differences were observed in the overall histopathologic scores between these 2 groups (12.8 in the hypothermia group vs 11.4 in the normothermia group, $P = .47$), probably because of too few surviving animals in the hypothermia group (Table 3). However, the histopathologic score of the thalamus was significantly higher in the hypothermia group ($P = .05$), and the score of the posterior brainstem tended to also be higher in this group of animals (Table 3).

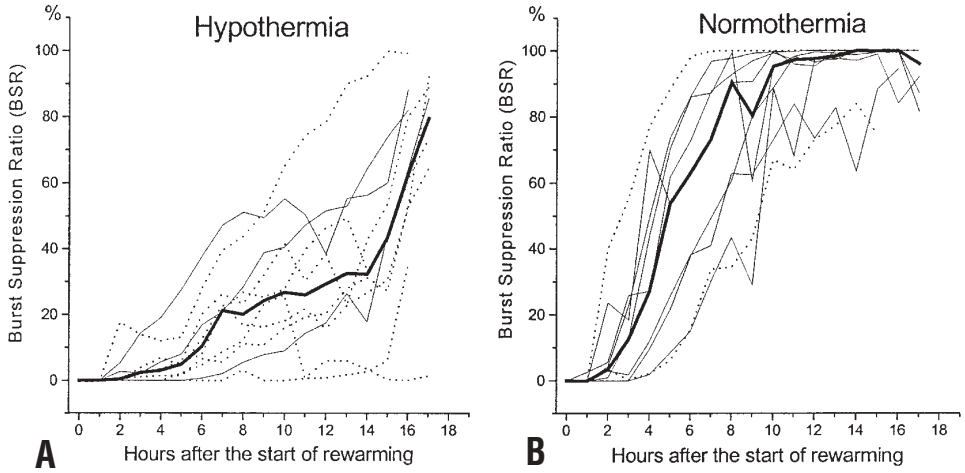

Figure 5. EEG burst recovery in pigs after 75 minutes of HCA. Burst-suppression ratio = [EEG burst time/(EEG burst time + EEG suppression time)] × 100. *Heavy black lines,* Median; *light solid lines,* alive at the seventh postoperative day; *dashed lines,* dead before the seventh postoperative day.

EEG Findings

The baseline EEG findings were similar between the study groups. The median rate of EEG burst recovery was significantly lower in the hypothermia group from the 4-hour interval after the start of rewarming ($P = .02$) throughout all the subsequent intervals in the postoperative period ($P < .01$, Figure 5). Also, before extubation, the EEG burst recovery tended to be lower in the hypothermia group ($P = .05$).

Discussion

Several studies have recently shown that mild or moderate (30°C-34°C) hypothermia can provide substantial neuroprotection after global and focal cerebral ischemia and that, to some extent, this method may be effective even in the setting of traumatic brain injury.[11] These experimental observations have led to some clinical studies in patients with stroke,[3,12] neonatal asphyxia,[13] and head injury.[7]

The mechanisms by which postischemic hypothermia is potentially neuroprotective are not well established. There is evidence that hypothermia can inhibit apoptosis simply by reducing cell activity.[14] It is probably such a reduction in metabolism that prevents cell injury. Decreased depletion of high-energy phosphates may therefore be the main mechanism underlying the delayed neuronal depolarization and the reduction of release of excitotoxins along with stabilization of the cell membrane and blood-brain barrier.[15-17]

Chatzipanteli and colleagues[5] observed a reduction of myeloperoxidase activity with the use of hypothermia after experimental traumatic brain injury both in injured and uninjured cerebral areas. This observation suggests that hypothermia may effectively reduce the accumulation of leukocytes within brain tissues after injury. Ishikawa and coworkers[6] have also shown a reduction of adhering leukocytes in arterioles and venules after reperfusion during moderate hypothermia.

Chello and associates[18] reported decreased complement and neutrophil activation during hypothermic CPB compared with during normothermic CPB.

The optimal timing, duration, and depth of mild hypothermia for neuroprotection, however, is not yet well established. Hypothermia has been evaluated in a preischemic, intraischemic, and postischemic fashion with encouraging results, even several hours after the ischemic insult.[19,20] In most studies hypothermia has been safely used for 1 to 3 hours after the ischemic event.[20,21] However, some studies indicated that hypothermia must be prolonged to 24 to 48 hours to provide its benefits,[19,22] whereas mild hypothermia (33°C-34°C) can be tolerated in human patients for 48 to 72 hours.[3]

A recent experimental study by Ehrlich and colleagues[23] in an acute porcine model of 90-minute HCA showed that reperfusion at 20°C for 20 minutes followed by rewarming significantly reduced intracranial pressure compared with that in animals that have been rewarmed immediately after HCA. These results suggest the efficacy of a hypothermic regimen after HCA, even when used in a short-term fashion, but its safety still should be assessed in a chronic experimental model.

There are several studies that reported detrimental effects of hypothermia as well.[6,24-28] Both moderate and mild hypothermia during CPB have been shown to be associated with endogenous endotoxemia, acidosis, prolonged partial thromboplastin and prothrombin times, and impairment of cardiovascular and hepatic function.[24-28]

Furthermore, clinical experience showed that the use of mild hypothermia can be associated with hemodynamic

instability, cardiac arrythmias, increase in serum lipase and amylase levels, thrombocytopenia, and prolonged reduction of total leukocyte, lymphocyte, and neutrophil counts, with an increased incidence in bacteremia and more severe pneumonia.[3,6]

The results of a large series of patients who underwent coronary artery bypass surgery with CPB showed that patients with a bladder core temperature of less than 36°C had significantly higher postoperative mortality, prolonged mechanical ventilation, and longer intensive care unit and hospital length of stay and required transfusion of more blood units.[29]

The present study provides the first experimental results on prolonged mild hypothermia after HCA in a chronic porcine model, a condition somewhat different from the others previously studied. It confirmed that prolonged mild hypothermia is effective in reducing the intracranial pressure, and this makes this method particularly interesting because increased intracranial pressure after a period of brain ischemia may result in severely impaired recovery.

This positive effect on intracranial pressure was associated with somewhat lower concentrations of brain lactate during the first 6 hours after the start of rewarming compared with that seen in the animals of the normothermia group. The significantly lower EEG burst-suppression rates observed in the hypothermia group can be viewed as the result of decreased cerebral metabolic activity, which may further positively affect the outcome after HCA. However, before extubation, when the temperatures of the groups approximated each other, the EEG burst recovery was still significantly lower in the hypothermia group.

The possible beneficial effects of postoperative hypothermia are probably time limited because most of the benefits seems to vanish after a few postoperative hours. In fact, apart from the increased postoperative mortality that occurred in the hypothermia group, severe brain metabolic derangements seemed to occur, as suggested by increased intracranial pressure, increased concentration of brain lactate, decreased concentration of brain glucose, and significantly decreased brain tissue oxygen partial pressure observed at extubation and during the last intervals preceding extubation. Furthermore, the increased serum levels of CK-BB among the animals that underwent postoperative prolonged hypothermia seem to confirm that this method, at least with this duration and temperature, may have detrimental effects on the brain. Despite the limitations as a result of a limited number of animals surviving in the hypothermia group, which prevents any conclusive statement, the histopathologic findings of the thalamus showed a significantly higher score in the hypothermia group, and findings in the posterior brainstem also tended to be worse in this group.

Initial beneficial metabolic effects of prolonged mild hypothermia after HCA were observed also in terms of increased mixed venous oxygen saturation and decreased oxygen extraction and consumption rates. However, these parameters were subsequently negatively reversed, with the normothermia group having better oxyhemodynamic parameters at extubation. A decrease of arterial and venous pH and increased mixed venous partial pressure of carbon dioxide suggests an underlying metabolic derangement in the overall body that might have had a negative effect on brain metabolism as well.

A reduced postoperative cardiac index and an increased need for dopamine confirm the negative effect of mild hypothermia on cardiac function and provide further evidence of the detrimental effect of this method, which resulted in an increased, but not statistically significant, postoperative mortality rate.

Prolonged postoperative hypothermia was associated with lower total leukocyte and neutrophil counts, which is supposed to lessen the reperfusion injury. In this study total leukocyte and neutrophil counts were measured only until the fourth hour after the start of rewarming, thus preventing any conclusion on the role of these parameters on the outcome. However, an eventual prolonged decrease of total leukocyte and neutrophil counts should be viewed with suspicion in surgical patients because it may be associated with severe postoperative septic complications, as observed in clinical studies.[6]

In conclusion, a 14-hour period of mild hypothermia after 75 minutes of HCA seems to be associated with poor outcome. However, the results of this study suggest that this method may preserve its efficacy when it is used for no longer than 4 hours after the start of rewarming. Further studies are required to elucidate the efficacy of short-term mild hypothermia after HCA.

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