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Interaction of temperature with hematocrit level and pH determines safe duration of hypothermic circulatory arrest

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Objective: Previous studies have demonstrated that both hematocrit level and pH influence the protection afforded by deep hypothermic circulatory arrest. The current study examines how temperature modulates the effect of hematocrit level and pH in determining a safe duration of circulatory arrest. The study also builds on previous work investigating the utility of near-infrared spectroscopy as a real-time monitor of cerebral protection during circulatory arrest.

Methods: Seventy-six piglets (9.3 ± 1.2 kg) underwent circulatory arrest under varying conditions with continuous monitoring by means of near-infrared spectroscopy (hematocrit level of 20% or 30%; pH-stat or alpha-stat strategy; temperature of 15°C or 25°C; arrest time of 60, 80, or 100 minutes). Neurologic recovery was evaluated daily by a veterinarian, and the brain was fixed in situ on postoperative day 4 to be examined on the basis of histologic score in a blinded fashion.

Results: Multivariable analysis of total histologic score revealed that higher temperature, lower hematocrit level, more alkaline pH, and longer hypothermic circulatory arrest duration were predictive of more severe damage to the brain ($P < .01$). Regression modeling revealed that higher temperature exacerbated the disadvantage of a lower hematocrit level and longer arrest times but not pH strategy. Normalized oxyhemoglobin nadir time, derived from near-infrared spectroscopy, was positively correlated with neurologic recovery on the fourth postoperative day and with total histologic injury score ($P < .0001$).

Conclusion: Hematocrit level and pH, as well as temperature, determine the safe duration of hypothermic circulatory arrest. Near-infrared spectroscopy is a useful real-time monitor of safe duration of circulatory arrest.

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Since the introduction of deep hypothermic circulatory arrest, clinicians and researchers have attempted to define a single safe duration.[1-8] There has also been an ongoing search to define a single optimal temperature.[9,10] It has been our hypothesis that the maximal safe duration of hypothermic circulatory arrest (HCA) is determined not only by the temperature but also by the conditions of the arrest, particularly hematocrit level and pH.[11-16] We have also hypothesized that near-infrared spectroscopy (NIRS) will be a useful method for real-time monitoring during circulatory arrest, thereby determining a safe duration.[15]

In 1998, we began a National Institutes of Health (NIH)–supported laboratory study that allowed us to study how the interaction of circulatory arrest variables affected cerebral outcome in a piglet model. Two temperatures (15°C and 25°C), 3

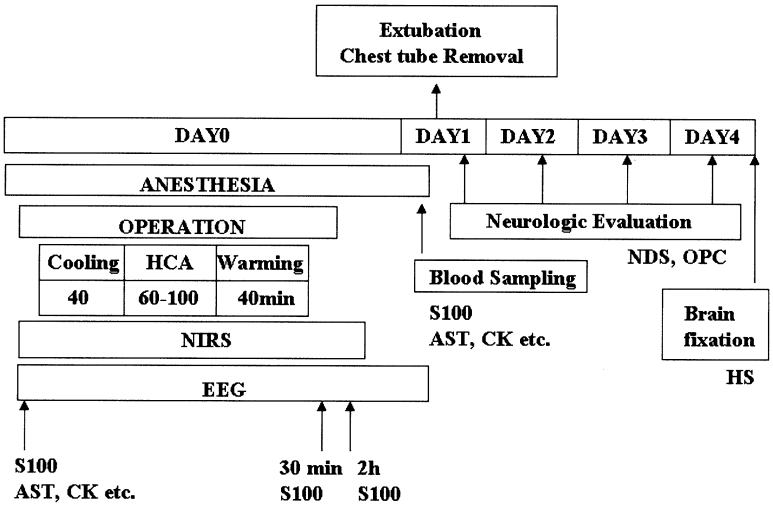

Figure 1. Study protocol. *HCA,* Hypothermic circulatory arrest; *NIRS,* near-infrared spectroscopy; *EEG,* electroencephalography; *S100,* S-100 protein; *AST,* aspartate aminotransferase; *CK,* creatinine kinase; *NDS,* neurologic deficit score; *OPC,* overall performance category; *HS,* histologic score.

durations (60, 80, and 100 minutes), 2 hematocrit values (20% and 30%), and 2 pH strategies (alpha-stat and pH-stat) were selected, thereby creating 24 scenarios. Each scenario was studied in 3 animals. Outcome variables included neurobehavioral outcome over 4 days of survival and histopathology after death on the fourth postoperative day (POD). This report describes the final results of this study conducted over 3 years.

Materials and Methods

Experimental Preparation

The study protocol is shown in Figure 1. Details of the survival piglet model have been described elsewhere.[15,16] Briefly, 76 Yorkshire piglets weighing 9.3 ± 1.2 kg underwent induction of anesthesia with intramuscular ketamine (20 mg/kg) and xylazine (4 mg/kg) and were intubated with a 5-mm cuffed endotracheal tube. Each animal was ventilated at a peak inspiratory pressure of 20 cm H_2O, an inspired oxygen fraction of 0.21, and a respiratory rate of 10 to 20 breaths/min controlled by means of a pressure-control ventilator (Healthdyne model 105; Healthdyne Technologies, Marietta, Ga) to achieve a normal pH and arterial carbon dioxide tension. After an intravenous bolus injection of fentanyl (50 μg/kg) and pancuronium (0.5 mg/kg), anesthesia was maintained by means of a continuous infusion of fentanyl (25 $\mu g \cdot kg^{-1} \cdot h^{-1}$), midazolam (0.2 $mg \cdot kg^{-1} \cdot h^{-1}$), and pancuronium (0.2 $mg \cdot kg^{-1} \cdot h^{-1}$) throughout the entire experiment, except during the period of circulatory arrest.

All surgical procedures were performed under sterile conditions. Arterial and venous lines were placed in the left superficial femoral artery and the right femoral vein for intraoperative monitoring and blood sampling, respectively. The right femoral artery was exposed for the cardiopulmonary bypass (CPB) arterial cannula, and a right anterolateral thoracotomy was performed in the third intercostal space to expose the right atrium for venous cannulation. After systemic heparinization (300 IU/kg), an 8F arterial cannula (Medtronic Bio-Medicus, Minneapolis, Minn) and a 28F venous cannula (Research Medical, Inc, Midvale, Utah) were inserted into the right femoral artery and right atrial appendage, respectively.

All animals received humane care in compliance 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 and published by the NIH (NIH publication no. 86-23, revised in 1985).

Experimental Groups

For hematocrit level during the cooling phase, a hematocrit level of either 20% or 30% was maintained. For pH strategy, either a pH-stat strategy or an alpha-stat strategy was used. For temperature during circulatory arrest, an esophageal temperature of either 15°C or 25°C was used. For duration of HCA, a circulatory arrest time of either 60, 80, or 100 minutes was used.

The experimental design included these 4 parameters with 2 or 3 possible levels, resulting in 24 ($2 \times 2 \times 2 \times 3$) experimental settings. Each setting was performed in 3 piglets (total $n = 72$). In the group with a hematocrit level of 20%, the CPB prime consisted of 400 mL of blood and 800 mL of crystalloid solution. The other group (hematocrit level of 30%) was prepared with 1200 mL of whole-blood prime.

CPB Technique

The circuit consisted of a roller-pump, a membrane oxygenator (Minimax; Medtronic, Inc, Anaheim, Calif), and sterile tubing with a 40-μm arterial filter (Olson Medical Sales, Inc, Ashland, Mass). The prime was determined by the experimental protocol. Methylprednisolone (30 mg/kg), furosemide (0.25 mg/kg), sodium bicarbonate (10 mL), cephazolin sodium (25 mg/kg), fentanyl (50

μg/kg), and pancuronium (0.5 mg/kg) were added to the prime. Full bypass flow was set at 100 mL · kg^{-1} · min^{-1}. Either pH-stat or alpha-stat strategy was selected according to the experimental protocol. Bypass was started, and animals were perfused for 10 minutes at normothermia (37°C). Animals were then cooled to an esophageal temperature of 13°C to 14°C or 23°C to 24°C over 40 minutes according to the experimental protocol. Ventilation was stopped after the establishment of bypass. Each group underwent 60, 80, or 100 minutes of HCA at 14°C to 15°C or 24°C to 25°C. Before reperfusion, methylprednisolone (30 mg/kg), furosemide (0.25 mg/kg), sodium bicarbonate (10 mL), and mannitol (0.5 g/kg) were administered into the pump. Reperfusion was begun at 100 mL · kg^{-1} · min^{-1}, and animals were warmed to 37°C. The heart was defibrillated as necessary at an esophageal temperature of 30°C. Fresh heparinized whole blood from a donor pig, drawn on the operative day, was transfused into the prime as required to increase the hematocrit level to at least 25% in all groups during rewarming. Ventilation (100% oxygen) was started 10 minutes before weaning from bypass. After 40 minutes of rewarming, animals were weaned from bypass, and the arterial and atrial cannulae were removed. Protamine (5 mg/kg) was administered intravenously after the animal was hemodynamically stable. The wound was closed in a sterile fashion.

Postoperative Management

Animals remained sedated and paralyzed and were mechanically ventilated and monitored continuously for 12 hours after the operation, at which time chest tubes were removed, and the animals were weaned from ventilation and extubated. Neurologic and behavioral evaluations were performed at 24-hour intervals beginning on POD 1. Neurologic scoring data were adapted from the neurologic deficit score (NDS) and overall performance category (OPC). On POD 4, the brain was fixed by means of perfusion with 4 L of 4% formaldehyde solution, and the histologic assessment was done.

Data Collection

Blood gas analyses. Arterial blood gas values, including electrolyte, glucose, and lactate concentrations, were measured at baseline, every 10 minutes during cooling and rewarming, and after the procedure, as needed (NOVA 900; Nova Biomedical, Waltham, Mass).

NIRS. A pair of fiberoptic optodes was attached to the head of the animal with a probe holder after induction of anesthesia. The optode spacing was 4.0 cm in a coronal plane. These 2 optodes, one a transmitter and one a receiver of laser light of near-infrared wavelength, were connected to an NIRS device (NIRO300; Hamamatsu Photonics KK, Hamamatsu City, Japan). This device calculated the relative concentration changes in oxygenated hemoglobin (HbO_2), deoxygenated hemoglobin (HHb), total hemoglobin (HbT), and oxidized cytochrome a,a3 ($CytO_2$), as well as the tissue oxygenation index (TOI), which is a calculated ratio of HbO_2 to HbT. Data were recorded every 10 seconds after the induction of anesthesia through 3 hours after weaning from bypass.

Electroencephalography. Electroencephalography (EEG) and spectral analysis were performed at 3 time points during the study: (1) after anesthesia and before thoracotomy (baseline); (2) 6 hours after circulatory arrest; and (3) 18 hours after circulatory arrest. After scrubbing with alcohol, platinum needle electrodes were placed in the frontal, central, temporal, and occipital regions by using the nasion, inion, and external auditory meatus as anatomic landmarks. Electrodes placed directly behind the external auditory meatus were used as reference electrodes. Electrodes were fixed to the scalp with collodion, and resistances were maintained at less than 10,000. A minimum of 20 minutes of EEG was recorded at each of the 3 time points. Recordings were obtained by using a digital EEG with cut-off frequencies of 1.0 Hz and 70 Hz. The EEG was blindly interrupted for ictal events, operationally defined as rhythmic epileptiform activity lasting greater than 10 seconds, by an investigator blinded to treatment group.

In addition to examination of the EEG, spectral analysis of the EEG was performed offline after the recording of the EEG. The fast Fourier transformation was calculated for 2-second epochs (256 samples) by using quantitative EEG software from Nicolet Biomedical. These parameters provided a frequency resolution of 0.5 Hz within each epoch. Ten epochs of 2 seconds duration of awake and artifact-free recording were averaged for the final analysis. Total power (μV2/Hz) was calculated across all bandwidths (1-16 Hz) and within the frequency bands of δ (0-4 Hz), θ (>4-9 Hz), and α (>9-13 Hz) for each channel. Three different analyses were made for each recording, with the aim to reduce any subjective interpretation. In addition to absolute power in the bandwidth, ratios of δ, θ, and α to total power were also calculated.

Brain marker (S-100 protein). Concentrations of the brain ischemic marker S-100 protein were assessed at baseline, 30 minutes, and 2 hours after weaning from bypass and on POD 1. The blood samples were immediately centrifuged, and the serum was stored at −70°C for later measurement by means of luminescence immunoassay (Sangtec LIA 100; AB Sangtec Medical, Bromma, Sweden).[17]

Neurologic and behavioral evaluations. Details of neurologic and behavioral evaluations have been described elsewhere.[18] NDS (500, brain death; 0, normal) and OPC (5, brain death; 1, normal) were used for neurologic and behavioral evaluations and were carried out by one veterinarian who was blinded to the experimental protocol.

Histologic assessment. Details of histologic assessment have been described elsewhere.[11,19] Histologic evaluation (5, necrosis; 0, normal) was performed by an experienced neuropathologist in a blinded fashion. Twenty-four areas, including the neocortex, dentate gyrus, caudate nucleus, and hippocampus, were scored and summed to determine the total histologic score (total HS; range, 0-20 points).

Statistical Analysis

The experimental design included 4 perfusion variables (pH strategy, hematocrit, temperature, and duration of HCA). To simplify the duration of HCA, we treated the 80- and 100-minute levels together, so that 16 settings were used for statistical analysis. Neurologic outcome measures consisted of NDS, OPC, EEG power, and HSs, with the total HS used as the gold standard. Univariable analysis was conducted to assess differences in neurologic outcomes between levels of each perfusion variable by using the nonparametric Mann-Whitney *U* test of medians. Continuous variables were compared by using the 2-sample Student *t* test after checking for normality (Gaussian-shaped distribution). Multiple stepwise linear regression was used to identify the per-

fusion variables that were independent predictors of outcome and to develop an algorithm for predicting total HS on the basis of the combination of these predictors. Regression equations were derived to model the interaction of temperature with pH management, hematocrit value, and duration of HCA. NIRS parameters, including HbO_2 signal, HHb, HbT, $CytO_2$, and TOI, were correlated with neurologic measures by using the Pearson correlation *(r)*. In addition, the relationship between normalized HbO_2 nadir time (adjusted to account for different metabolic rates at different temperatures and pH)[10,20-22] and total HS was determined by using regression through the origin (no-intercept model) and 95% prediction intervals, with the coefficient of determination (R^2) used to measure the proportion of variability in total HS explained by normalized HbO_2 nadir time. The Pearson product-moment correlation *(r)* was used for normally distributed variables, and the Spearman rho (ρ) correlation was used for discrete categoric data. Statistical analysis was performed with the PROC GLM and REG procedures in the SAS software package (version 8.1; SAS Institute, Cary, NC). *P* values are all 2-tailed.

Results

Target Hematocrit Level

At the end of the cooling phase, the hematocrit levels were 33.14% ± 2.19% for the hematocrit 30% groups and 20.94 ± 1.64% for the hematocrit 20% groups.

Operative Results

Two animals that underwent HCA of 100 minutes at 25°C did not survive because of sudden ventricular fibrillation after weaning from CPB. Two other animals did not survive as a result of lung problems (tension pneumothorax and severe pneumonia). The data from these 4 animals were excluded from analysis. The results from the remaining 72 animals were used for analysis. Among these 72 animals, 8 animals did not survive for 4 days because of severe brain damage with seizures after extubation (6 on POD 1, 1 on POD 2, and 1 on POD 4). Data from these animals were included for analyses, although some data were not obtained. No animal experienced a systemic reaction in response to transfusion with fresh heparinized blood.

NIRS Data

NIRS data are shown in Figure 2. The HbO_2 signal increased significantly during the cooling phase in all animals. In the animals with hematocrit levels of 30%, HbO_2 signal continued to increase during the entire cooling phase, whereas HbO_2 signal reached a plateau level after about 20 minutes in the animals with hematocrit levels of 20%. HbT showed almost the same pattern as HbO_2 during the cooling phase. On the other hand, TOI demonstrated an increase without plateau in all animals. From the onset of HCA, there was a decrease in HbO_2, $CytO_2$, and TOI, whereas HHb showed a reciprocal increase. HbO_2 signal decreased to a plateau (nadir) during circulatory arrest. Time to nadir was significantly shorter with lower hematocrit levels, alpha-stat strategy, and higher temperature ($P < .001$). As we reported previously, the duration from reaching nadir until reperfusion was calculated in each case and was termed HbO_2 nadir time. Then HbO_2 nadir time was normalized according to the perfusion conditions.[15,16]

EEG

No EEG events were recorded in any animals at any of the time points.

S-100 Protein

S-100 protein levels showed significant changes after bypass with HCA. However, almost all animals had a normal value of less than 0.5 μg/dL, even when they had abnormal NDS, OPC, or HS results. Also, there was no significant relationship between S-100 protein data and evidence of brain damage (data not shown).

Neurologic Recovery (NDS and OPC) and HS

Regarding NDS and OPC, most animals recovered and showed normal performance without neurologic deficit by PODs 3 or 4. Histologic damage was found predominantly in the caudate nucleus. All 3 animals with the higher hematocrit level (30%), pH-stat strategy, lower temperature (15°C), and shortest HCA (60 minutes) showed no evidence of brain damage, either functionally or structurally.

Effect of Perfusion Variables on Neurologic Outcome

EEG on POD 1. Linear regression was performed to determine the effect of hematocrit level, pH strategy, temperature, and HCA duration on EEG power. The only significant univariable predictor was temperature, such that a lower temperature (15°C) was predictive of a higher EEG power ($P = .01$). Hematocrit level, pH strategy, and HCA duration were not associated with EEG power by means of univariable analysis (all $P > .2$). Among the 45 animals measured, the mean EEG power was 370 ± 342 μV2/Hz for the 18 animals at 15°C and 176 ± 170 μV2/Hz for the 27 animals at 25°C ($P = .01$, Student *t* test). Multiple stepwise linear regression revealed that temperature was the only perfusion variable independently associated with EEG power ($P = .01$).

NDS, OPC, and HS. Differences of temperature and HCA duration demonstrated pronounced effects, less so for pH strategy and hematocrit level, by means of univariable analysis (Tables 1-4). As in our previous reports, in which there were several potentially important predictors, there was often confounding or multicolinearity among variables, and therefore multivariable analysis was essential to determine the independent effects. Results of multivariable analysis indicated that all 4 perfusion variables contributed to a significant independent effect on total HS. An algorithm was constructed by using the regression coefficients from the multivariable model to predict total HS on the basis of

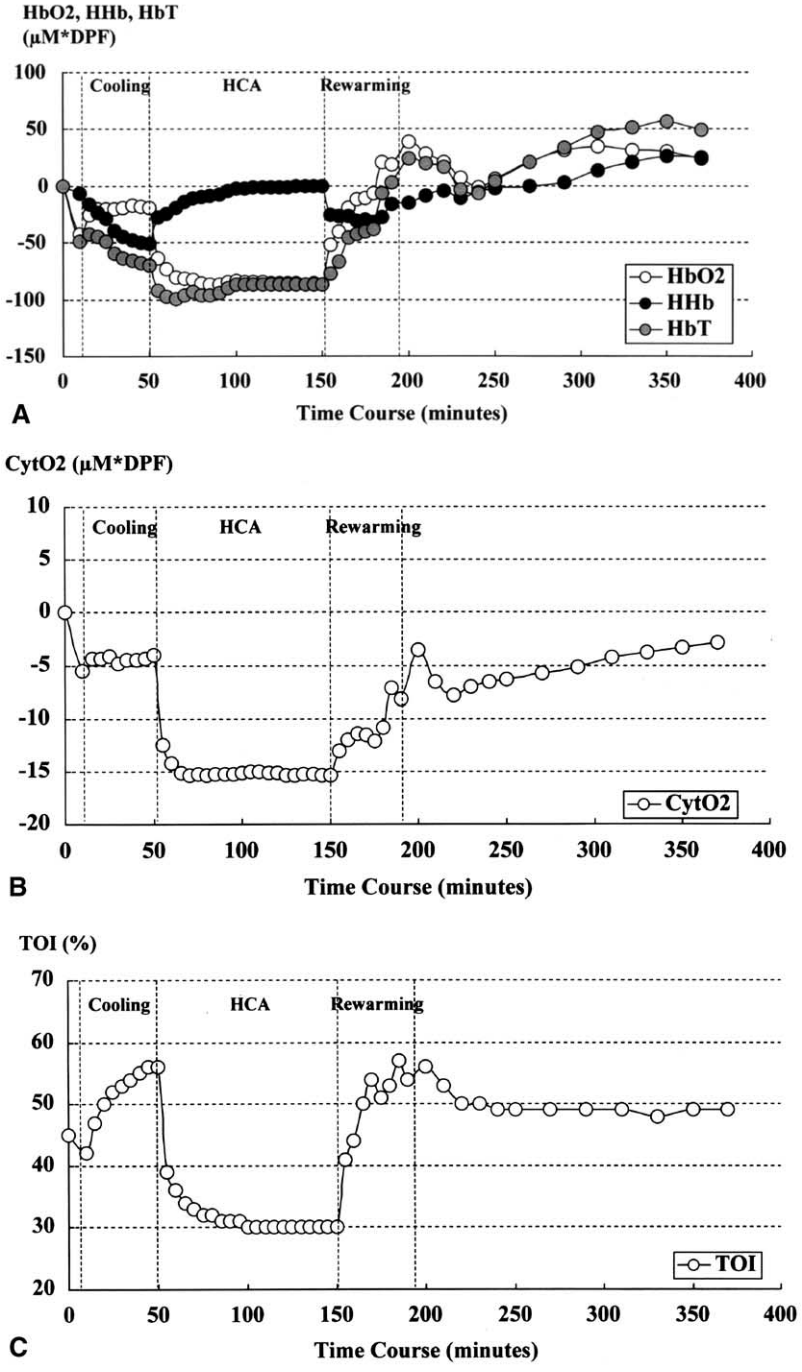

Figure 2. Changes in NIRS data for a representative case showing HbO_2, HHb, and HbT (A); $CytO_2$ (B); and TOI (C). This animal underwent the following respective conditions: hematocrit level of 20%, alpha-stat strategy, temperature of 15°C, and HCA duration of 100 minutes.

the 16 (24) possible combinations of the 4 multivariable predictors (Table 5). For example, animals undergoing circulatory arrest with a temperature of 15°C, pH-stat strategy, hematocrit level of 30%, and HCA of 60 minutes are expected to have a total HS of 0.0. On the other hand, a combination of a temperature of 25°C, alpha-stat strategy, hematocrit level of 20%, and HCA of 80 to 100 minutes is associated with a predicted total HS of 8.0.

Because temperature was found to be such a powerful determinant of neurologic and histologic outcomes, a com-

TABLE 1. Histologic and neurologic outcomes according to esophageal temperature

	15° C			25° C			
Variable	**Median**	**IQR**	**Min-Max**	**Median**	**IQR**	**Min-Max**	***P* value**
Total HS	2	1-3	0-6	4	3-7	2-16	<.001
Neocortex	0	0-0	0-4	1	0-2	0-5	<.001
Dentate gyrus	0	0-0	0-2	1	0-2	0-4	<.001
Hippocampus	0	0-0	0-1	0	0-2	0-4	<.001
Caudate	2	1-2	0-4	2	2-3	1-4	.02
NDS							
1 POD	115	70-175	30-255	182	155-230	100-265	<.001
2 POD	0	0-32	0-190	120	40-150	0-210	<.001
3 POD	0	0-5	0-185	105	20-135	0-205	<.001
4 POD	0	0-0	0-130	65	0-120	0-215	<.001
OPC							
1 POD	3	2-3	1-4	3	3-4	2-4	<.001
2 POD	1	1-2	1-3	2	2-3	1-4	<.001
3 POD	1	1-1	1-3	2	1-3	1-4	<.001
4 POD	1	1-1	1-2	2	1-2	1-4	<.001

All *P* values were determined by using the nonparametric Mann-Whitney *U* test.
IQR, Interquartile range (25^{th}-75^{th} percentile); *HS,* histologic score; *NDS,* neurologic deficit score; *POD,* postoperative day; *OPC,* overall performance category.

TABLE 2. Histologic and neurologic outcomes according to pH management strategy

	pH-stat			Alpha-stat			
Variable	**Median**	**IQR**	**Min-Max**	**Median**	**IQR**	**Min-Max**	***P* value**
Total HS	2	1-4	0-14	2	2-5	1-16	.02
Neocortex	0	0-0	0-4	2	0-1	0-5	.05
Dentate gyrus	0	0-1	0-4	2	0-1	0-3	.55
Hippocampus	0	0-0	0-4	2	0-0	0-3	.78
Caudate	2	1-3	0-4	2	2-3	1-4	.19
NDS							
1 POD	138	101-175	30-265	190	135-240	60-255	.01
2 POD	20	0-120	0-175	40	0-135	0-210	.12
3 POD	0	0-108	0-175	30	0-120	0-205	.36
4 POD	0	0-78	0-175	10	0-65	0-215	.34
OPC							
1 POD	3	2-3	1-4	3	3-4	2-4	.02
2 POD	1	1-2	1-3	2	1-3	1-4	.05
3 POD	1	1-2	1-3	2	1-2	1-4	.27
4 POD	1	1-2	1-3	1	1-2	1-4	.22

All *P* values were determined by using the nonparametric Mann-Whitney *U* test.
IQR, Interquartile range (25^{th}-75^{th} percentile); *HS,* histologic score; *NDS,* neurologic deficit score; *POD,* postoperative day; *OPC,* overall performance category.

parison was made to show categories of total HS according to temperature level (Figure 3). On the basis of the Pearson χ^2 test, the distribution of total HS on the basis of 3 chosen score intervals was significantly different between the 15°C and 25°C groups ($P < .0001$).

Relationship Between the NIRS Data and Neurologic Outcome

Normalized HbO_2 nadir time was positively correlated with NDS on POD 4 (Spearman $\rho = 0.82$, $P < .001$, n = 65), OPC on POD 4 (Spearman $\rho = 0.77$, $P < .001$, n = 65), and total HS (Pearson $r = 0.81$, $P < .001$, n = 68). We tested other NIRS-derived parameters and found significant inverse correlations between HbO_2 before HCA and NDS, OPC, and total HS ($P < .01$ in each case). In addition, TOI was inversely correlated with NDS, OPC, and total HS at 5 and 10 minutes after the start of HCA ($P < .01$ in each case). No significant correlations were detected between HHb, HbT, or $CytO_2$ and the neurologic outcomes. HbO_2 nadir time and total HS were tested for normality and were found to have normal (Gaussian-shaped) distributions. Linear regression analysis was performed to illustrate the rela-

TABLE 3. Histologic and neurologic outcomes according to level of hematocrit

Variable	Hematocrit 30%			Hematocrit 20%			*P* value
	Median	IQR	Min-Max	Median	IQR	Min-Max	
Total HS	3	2-4	0-8	3	2-5	0-16	.16
Neocortex	0	0-1	0-3	0	0-1	0-5	.21
Dentate gyrus	0	0-1	0-3	0	0-1	0-4	.77
Hippocampus	0	0-0	0-2	0	0-1	0-4	.04
Caudate	2	1-3	0-4	2	2-3	0-4	.13
NDS							
1 POD	155	100-205	30-255	165	120-220	40-265	.38
2 POD	20	0-82	0-165	45	20-142	0-210	.05
3 POD	0	0-50	0-145	30	0-120	0-205	.17
4 POD	0	0-40	0-135	0	0-98	0-215	.23
OPC							
1 POD	3	2-3	1-4	3	2-3	2-4	.47
2 POD	2	1-2	1-3	2	1-3	1-4	.12
3 POD	1	1-1	1-3	2	1-3	1-4	.06
4 POD	1	1-1	1-3	2	1-2	1-4	.07

All *P* values were determined by using the nonparametric Mann-Whitney *U* test.
IQR, Interquartile range (25^{th}-75^{th} percentile); *HS,* histologic score; *NDS,* neurologic deficit score; *POD,* postoperative day; *OPC,* overall performance category.

TABLE 4. Histologic and neurologic outcomes according to duration of HCA

Variable	HCA (60 min)			HCA (80 or 100 min)			*P* value
	Median	IQR	Min-Max	Median	IQR	Min-Max	
Total HS	2	1-3	0-4	4	2-6	0-16	<.01
Neocortex	0	0-0	0-2	0	0-1	0-5	.03
Dentate gyrus	0	0-0	0-1	0	0-2	0-4	<.01
Hippocampus	0	0-0	0-2	0	0-1	0-4	<.02
Caudate	2	1-2	0-4	2	2-3	0-4	.08
NDS							
1 POD	135	80-175	40-255	175	120-220	30-265	.03
2 POD	0	0-40	0-160	40	20-145	0-210	<.01
3 POD	0	0-5	0-120	40	0-120	0-205	<.01
4 POD	0	0-0	0-60	30	0-105	0-215	<.01
OPC							
1 POD	3	2-3	2-4	3	3-4	1-4	.02
2 POD	1	1-2	1-3	2	1-3	1-4	<.01
3 POD	1	1-1	1-3	2	1-2	1-4	<.01
4 POD	1	1-1	1-2	2	1-2	1-4	<.01

All *P* values were determined by using the nonparametric Mann-Whitney *U* test.
HCA, Hypothermic circulatory arrest; *IQR,* interquartile range (25^{th}-75^{th} percentile). *HS,* histologic score; *NDS,* neurologic deficit score; *POD,* postoperative day; *OPC,* overall performance category.

tionship between shorter HbO_2 nadir times and less histologic damage (Figure 4). Regression through the origin (no-intercept model) was applied because the y-intercept based on least squares was nonsignificant and negligible. On the basis of the coefficient of determination, the model indicated that 86% of the variability in total HS is explained by the normalized HbO_2 nadir time ($R^2 = 0.86$). The fitted regression line can be described by the following equation: $y = 0.042x$. For example, the model predicts that HbO_2 nadir times of 50 and 150 minutes are expected to have total HSs of 2 and 6 points, respectively. In fact, the total HSs for all 23 animals that had normalized HbO_2 nadir times of 50 minutes or less were between 0 and 3 points (7 were free of any histologic evidence of brain injury from the neocortex, dentate gyrus, hippocampus, or caudate nucleus).

The duration of time from which the HbO_2 signal reaches its nadir until the end of the circulatory arrest period (ie, HbO_2 nadir time) is significantly shorter for higher hematocrit levels, lower body temperatures, and less alkaline pH. Specifically, mean normalized HbO_2 nadir times for 30% and 20% hematocrit levels were 75 ± 50 and 108 ± 55 minutes, respectively ($P < .01$). For temperatures of 15°C

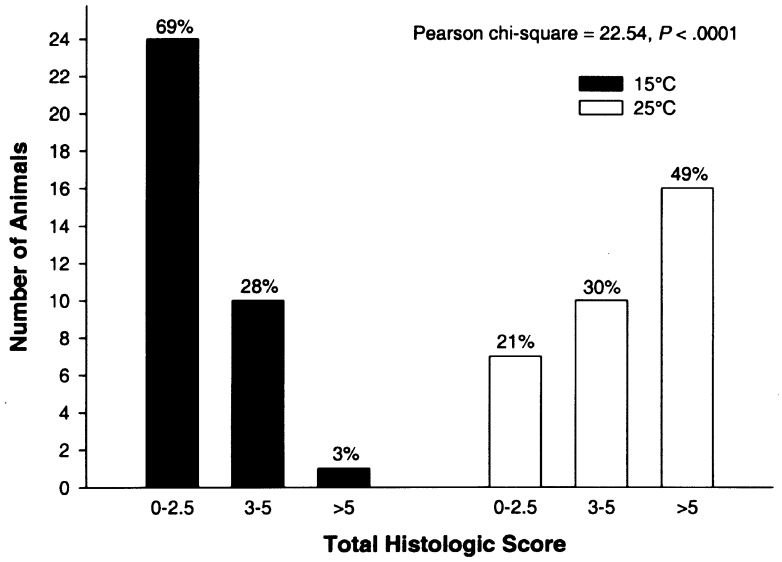

Figure 3. Frequency histogram showing the empiric data for the number and percentage of animals by histologic score interval according to the 2 different temperature conditions. The score distributions reveal significantly higher scores among animals in the 25°C temperature condition ($P < .0001$).

TABLE 5. Algorithm for predicting total histologic score on the basis of possible conditions

Multivariable predictor*				
Temperature (°C)	pH Strategy	Hematocrit level (%)	HCA (min)	Predicted total histologic score
15	pH-stat	30	60	0.0
15	pH-stat	20	60	0.5
15	Alpha-stat	30	60	1.0
15	pH-stat	30	80 or 100	1.5
15	Alpha-stat	20	60	2.0
25	pH-stat	30	60	2.5
15	Alpha-stat	30	80 or 100	3.0
15	pH-stat	20	80 or 100	3.0
25	pH-stat	20	60	3.5
25	Alpha-stat	30	60	4.0
15	Alpha-stat	20	80 or 100	4.0
25	pH-stat	30	80 or 100	4.5
25	Alpha-stat	20	60	5.0
25	pH-stat	20	80 or 100	6.0
25	Alpha-stat	30	80 or 100	7.0
25	Alpha-stat	20	80 or 100	8.0

HCA, Hypothermic circulatory arrest.
*Multiple stepwise linear regression analysis revealed that temperature ($P < .001$), pH strategy ($P = .005$), hematocrit level ($P = .01$), and duration of HCA ($P < .001$) were all multivariable predictors of total histologic score.

and 25°C, nadir times were 50 ± 30 and 130 ± 48 minutes, respectively ($P < .001$). Mean normalized HbO_2 nadir times were 77 ± 54 minutes for pH-stat and 106 ± 55 minutes for alpha-stat ($P = .02$). Therefore normalized HbO_2 nadir time might provide a useful predictive index of cerebral protection during HCA.

Temperature and pH Strategy

Figure 5 illustrates the interaction of temperature and pH strategy in determining total HS. It can be seen that the advantage of the pH-stat strategy relative to the alpha-stat strategy increases at lower temperatures. This is predictable in that the 2 strategies become more disparate at lower

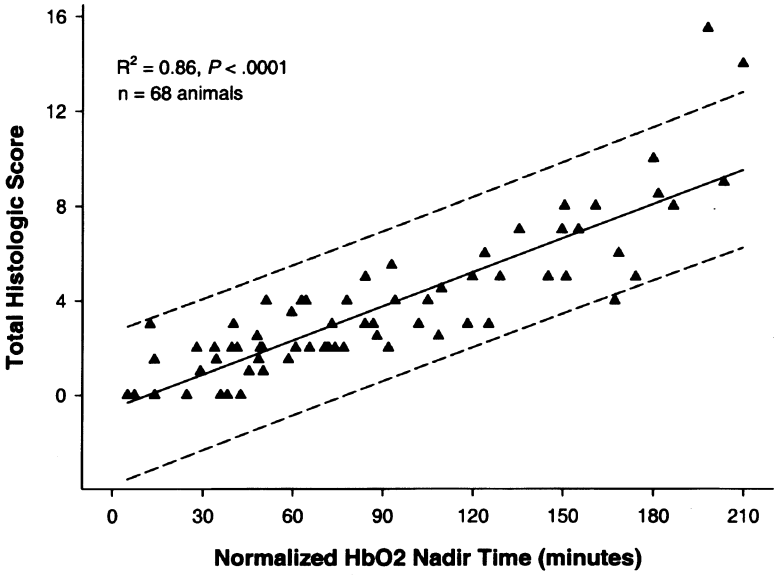

Figure 4. Least-squares regression analysis depicting the relationship between normalized HbO_2 nadir time in minutes and total histologic score. Lower scores were associated with shorter nadir times. The fitted theoretic regression model has the following linear form: *y = 0.042x (solid line).* The 95% prediction intervals *(dashed lines)* are shown to provide an estimate of precision in estimating histologic score from nadir time.

temperature. This is the probable explanation as to why studies of pH strategy at mild and moderate hypothermia have often failed to demonstrate differences, particularly when continuous bypass has been studied rather than HCA.

Temperature and Hematocrit Level

Figure 6 illustrates the interaction of temperature and hematocrit level in determining total HS. In contrast to the result with pH strategy, the disadvantage of using a low hematocrit level (20%) relative to a higher hematocrit level (30%) is amplified by use of a higher temperature for the arrest.

Temperature and Duration of Circulatory Arrest

Figure 7 illustrates the interaction of temperature and duration of circulatory arrest in determining total HS. Not surprisingly, use of a higher arrest temperature exacerbates the disadvantage of arresting for a longer duration. This can also be expressed by stating that the safe duration of circulatory arrest is shorter if one arrests at a higher temperature.

Table 6 presents an overview of the interaction of temperature with pH-strategy, hematocrit level, and duration of HCA with respect to the modifying effect of temperature on predicted total HS and how the temperature effect changes according to hematocrit level, pH strategy, and duration of circulatory arrest.

Discussion

This study has confirmed the hypothesis that temperature, duration of circulatory arrest, hematocrit level, and pH strategy all influence neurologic outcome after HCA. The study also defines the relative effect of these variables in determining outcome, as determined on the basis of neurobehavioral outcome and histopathology.

Optimal Hematocrit Level for Circulatory Arrest

This study has demonstrated that a higher hematocrit level of 30% is associated with a significantly improved histologic outcome relative to a lower hematocrit level of 20%, irrespective of the other perfusion conditions; that is, it is an advantage to arrest with a higher hematocrit level. This is an important new finding of this study because histology is the gold standard primary outcome of this survival study. In a previous smaller pilot study examining hematocrit level in a univariate fashion, we were unable to demonstrate a significant advantage of a hematocrit level of 30% relative to a level of 20%, although there was a significant disadvantage with a hematocrit level of 10%.[11] In a previous report describing an earlier phase of the current study, hematocrit level had a significant effect on NIRS-derived indices, such as time to HbO_2 nadir, but was not associated with worse histologic outcome per se.[15] The greater power of the current report, in which a total of 72 animals were analyzed, has confirmed the importance of a higher hematocrit level.

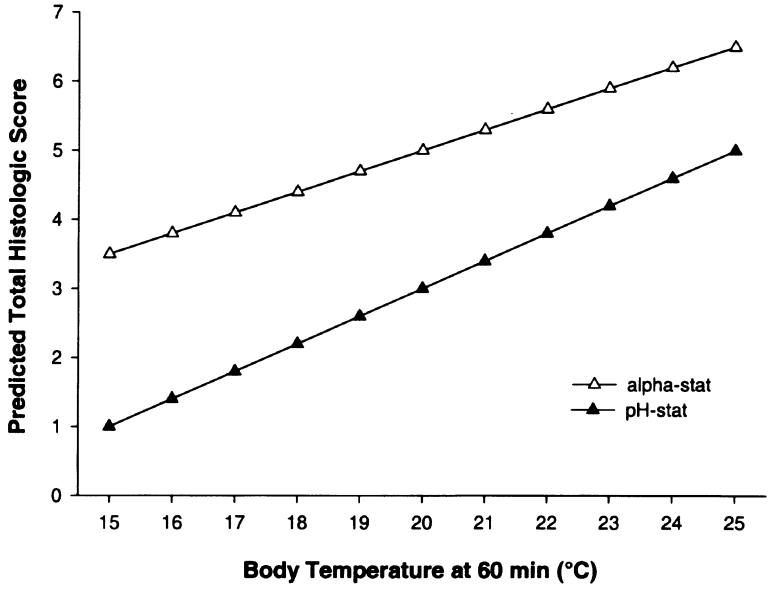

Figure 5. Predicted total histologic score as a function of temperature for the alpha-stat and pH-stat conditions. Throughout the temperature range, pH-stat demonstrated a highly significant advantage over alpha-stat ($P < .001$). Although the 2 lines are roughly parallel, the protective effect of the pH-stat strategy compared with that of the alpha-stat strategy is most evident at lower temperatures.

TABLE 6. Interaction of esophageal temperature on predicted total histologic score*

	pH Strategy		Hematocrit level		Duration of HCA	
Temperature	pH-stat	Alpha-stat	30%	20%	60 min	80/100 min
15°C	1.0	3.5	1.25	2.0	1.25	2.0
20°C	3.0	5.0	2.5	4.25	2.0	4.0
25°C	5.0	6.5	3.75	6.5	2.75	6.0

HCA, Hypothermic circulatory arrest.

*Predicted total histologic scores above were determined according to the appropriate regression equation for each perfusion variable as follows. pH-stat: score = 0.40 × temperature − 5.0. alpha-stat: score = 0.30 × temperature − 1.0. Hematocrit 30%: score = 0.25 × temperature − 2.5. Hematocrit 20%: score = 0.45 × temperature − 4.75. Duration of HCA 60 minutes: score = 0.15 × temperature − 1.0. Duration of HCA 80 or 100 minutes: score = 0.40 × temperature − 4.0.

This finding is consistent with ongoing studies in our laboratory examining the cerebral microcirculation during circulatory arrest. These studies have demonstrated that use of a hematocrit level of 30% is associated with improved reperfusion (functional capillary density) relative to a hematocrit level of 10%. There is no evidence of capillary plugging caused by increased viscosity or white cell activation with use of a higher hematocrit level. In fact, severe hemodilution is associated with a greater number of rolling leukocytes than mild hemodilution.[23]

The normal hematocrit level of the pig is approximately 30%. Therefore it is not realistic to evaluate with this model whether a hematocrit level of greater than 30% is more protective. It remains unknown whether a normal hematocrit level of 40% in human subjects would be preferable for circulatory arrest relative to 30%, although it seems unlikely that a hematocrit level of 20% is ideal, despite its common use.

Optimal pH Strategy for Circulatory Arrest

This study has confirmed the results of 2 previous univariate studies of pH strategy from our laboratory suggesting that the pH-stat strategy protects the brain more effectively than the alpha-stat strategy during HCA.[13,14] The 2 previous studies were acute experiments using magnetic resonance spectroscopy in the first and a combination of NIRS and magnetic resonance spectroscopy in the second. In a previous report describing an earlier phase of the current study, when 36 animals had been studied, the pH-stat strategy had already been proved to be important in reducing histologic

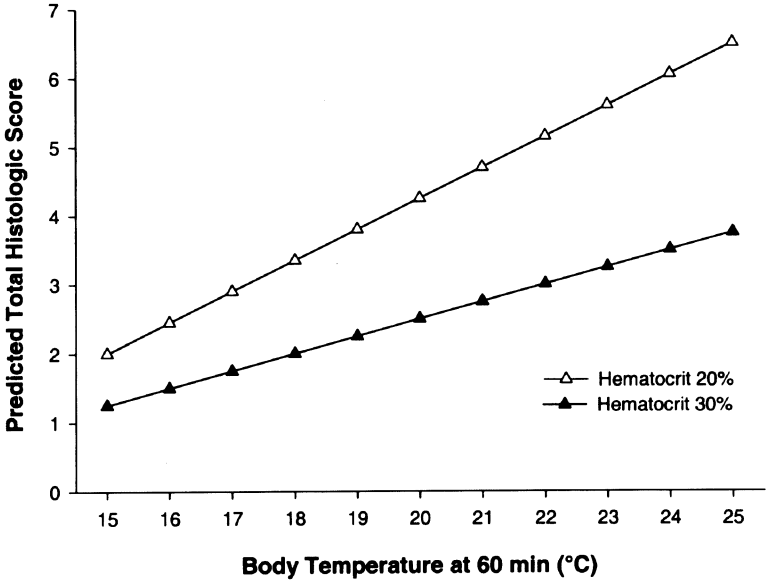

Figure 6. Predicted total histologic score as a function of temperature for the 20% and 30% hematocrit conditions. The F test in analysis of covariance indicated a highly significant interaction between temperature and hematocrit level ($P < .01$), suggesting that the advantage of the 30% hematocrit level is amplified at higher temperatures.

injury.[16] After study of 72 animals, the effect of the pH strategy is even more convincing, as shown in Table 5.

Probable Mechanisms Causing the Interactions Observed

Completion of this study, particularly with analysis of the NIRS findings, has strengthened our understanding of the probable mechanisms responsible for the interactions observed between temperature, duration of HCA, pH strategy, and hematocrit level. Lower temperature reduces oxygen consumption. A more acidotic milieu, such as that provided by the pH-stat strategy, also reduces cerebral oxygen consumption. The pH-stat strategy also increases oxygen availability by increasing cerebral blood flow and by shifting the HbO_2 curve to the right, thereby counteracting the leftward shift induced by hypothermia. A higher hematocrit level increases oxygen availability because it increases the oxygen carrying capacity of blood. This is particularly important when cooling is achieved with CPB because the flow rate usually used is less than a normal cardiac output and does not increase to compensate for the decreased oxygen carrying capacity in the same way that cardiac output increases to compensate for acute anemia in an intact animal. A higher hematocrit level is also advantageous at the onset of circulatory arrest because there is an increased reservoir of oxygen from which the brain can draw throughout the early phase of the arrest period. When there is no more oxygen available for the reduced but nevertheless ongoing aerobic metabolism, there is a flattening of the HbO_2 signal detected by NIRS. The subsequent duration, which we have termed the HbO_2 nadir time, is predictive of cerebral injury.[15]

Neuromonitoring

Completion of all 72 animals in this study has confirmed the potential utility of NIRS to monitor safe duration of circulatory arrest for specific conditions of pH, hematocrit level, and temperature. The normalized HbO_2 nadir time (normalized to account for the different metabolic rates associated with different temperatures and with alpha-stat vs pH-stat strategy) was directly correlated with NDS on the fourth POD, OPC on the fourth POD, and total HS (all $P < .001$). The study has also confirmed what others have described previously, namely that other methods of neuromonitoring are unreliable or of little help.[24] EEG in the current study (specifically EEG power on the first POD) was only able to distinguish temperature as an important predictor of outcome. Postoperative levels of S-100 protein had no relationship with functional or structural evidence of brain injury.[25]

Application of Study Findings to Adults

Cerebral embolization of atherosclerotic debris is an important mechanism of cerebral injury in adults undergoing CPB.[26,27] Thus there have been appropriate concerns in adults that CPB techniques that increase cerebral blood flow to luxuriant levels might increase the risk of microembolic injury. However, we believe the results of the current study are applicable to adults for the following reason. Cerebral blood flow is the mechanism whereby the brain is cooled before circulatory arrest for both adults and children. (In

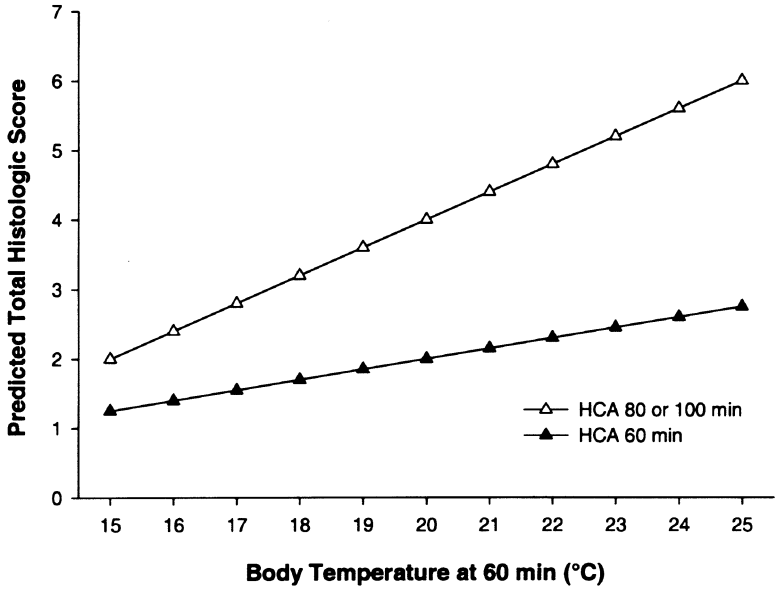

Figure 7. Predicted total histologic score as a function of temperature for the 2 different circulatory arrest conditions. Results of the F test in the analysis of covariance indicated a highly significant interaction between temperature and duration ($P < .01$), suggesting that the advantage of 60 minutes compared with 80 to 100 minutes of HCA is amplified at higher temperatures.

fact, core cooling is more important in adults than children because of the more rapid surface cooling of children caused by their greater surface area to volume ratio.) Total flow to the brain during the cooling phase is determined by the cooling function rather than the oxygen requirement: for example, if the alpha-stat strategy is used, a longer duration of cooling will be needed to compensate for the lesser flow rate relative to the pH-stat strategy. Thus the total embolic load will be determined by the weight of the brain, its specific heat, the starting temperature, and the temperature at arrest rather than by any bypass factors.

Application of Study Findings to Continuous CPB

Although circulatory arrest is being used more frequently today for adults undergoing aortic surgery, it is being used less frequently for repair of congenital anomalies, in large part in response to clinical trials, such as the Boston Circulatory Arrest Study.[28] It is important to remember that the latter study was conducted more than 10 years ago and used the alpha-stat strategy, a hematocrit level of 20%, no arterial filter, and outmoded hardware with a large priming volume. Furthermore, at 4 and 8 years of age, there is no detectable difference in IQ between patients from that trial randomized to circulatory arrest versus patients who underwent continuous low-flow bypass.[29] However, both groups now score less than would be anticipated. Similar developmental findings have been reported by other groups using different (higher) flow rates but similar pH and hematocrit conditions.[30] We have speculated that the conditions of bypass are responsible for the suboptimal outcome, possibly involving inadequate oxygen delivery. In future studies, we plan to study the interaction of temperature, flow rate, and hematocrit level in determining the adequacy of oxygen delivery during continuous bypass. We will also assess the usefulness of NIRS in monitoring the adequacy of oxygen delivery to the brain under varying conditions of continuous CPB.

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