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Cardiopulmonary Bypass Increases Permeability of the Blood Cerebrospinal Fluid Barrier

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Abstract

Background—Integrity of blood cerebrospinal fluid barrier (blood CSF barrier) during cardiopulmonary bypass (CPB) with hypothermic circulatory arrest (HCA) has not been systematically studied, especially in children. We tested the hypothesis that the blood CSF barrier is disrupted by CPB.

Methods—25 piglets (mean weight 11 kg) were randomly assigned to five groups (5 per group): anesthesia alone (control); CPB at 37°C with full-flow (FF); CPB at 25°C with very low flow (LF); HCA at 15°C and 25°C. pH-stat strategy was applied during CPB. An epidural catheter was inserted into cisterna magna for collection of CSF. CSF and blood samples were collected at 7 time points: after induction of anesthesia (baseline), 10, 50 and 115 min after start of CPB, just before end of CPB, 30 and 120 min after end of CPB. Albumin levels in CSF and plasma were measured to assess blood CSF barrier integrity and the albumin ratio (CSF/plasma) was calculated (Q_{alb}).

Results—In both HCA groups the Q_{alb} was significantly higher than control and 37°C FF groups (all $p < 0.05$), whereas Q_{alb} in 37 °C was not significantly different compared to control.

Conclusions—The blood CSF barrier is impaired by CPB with one hour of 15°C or 25°C HCA. Further investigations are needed to understand the behavior of the blood CSF barrier during CPB and its role in neuroprotection.

Keywords

Cerebrospinal fluid barrier; Cardiopulmonary bypass; Ischemia/reperfusion; Hypothermia/circulatory arrest

Introduction

Cognitive impairment has been consistently observed after cardiopulmonary bypass (CPB).¹ and in adults has been associated with the number of microemboli detected during coronary artery bypass grafting. However, there are many other potential sources of brain injury in children. In a study commissioned by the National Institutes of Health, the incidence of neurological complications in children undergoing cardiac surgery at various institutions ranged from 1 to 25%.²

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Disclosure statements

The brain is a unique tissue being protected from free exchange of blood by the blood–brain barrier, located at the tight junctions and cell walls of the cerebral endothelium. In addition, two fluid compartments are present: the brain interstitial fluid, surrounding the neurons and glia, and the cerebrospinal fluid (CSF). CSF fills the ventricles and the external surfaces of the brain, acting both as a fluid cushion and as a drainage route for the products of cerebral metabolism.³ The complex functions of the brain are critically dependent on the homeostasis of the above two fluids, and any variation in their ionic, amino acid, or peptide composition will markedly affect brain function. The homeostasis of the brain interstitial fluid depends on complex functions of the blood brain barrier and the blood CSF barriers, which are located at the choroid plexuses and the arachnoid membrane between the dura and subarachnoid fluid. 4-5 The endothelium, therefore, does not form a barrier to the movement of small molecules. Instead, the blood CSF barrier at the choroid plexus is formed by the epithelial cells and the tight junctions that link them. The other part of the blood CSF barrier is the arachnoid membrane, which envelops the brain. The cells of this membrane are linked by tight junctions.

Damage to the BBB and blood CSF barrier during CPB is one reason for neurological dysfunction after heart surgery.^{6, 7} Studies of BBB and blood CSF barrier during CPB in children are few and the affect of CPB on the BBB and BCSFB is controversial. The mechanisms underlying this susceptibility are not completely understood. Moreover, some studies have not demonstrated blood CSF barrier dysfunction and leakage of plasma proteins from CPB.^{8, 9}

The CSF/plasma ratio of albumin is used as an indicator of permeability of the BCSFB.¹⁰¹⁰ The calculated CSF/plasma concentration quotient, Q , e.g. for albumin: $CSF_{Alb} / plasma_{Alb} = Q_{Alb}$ has a higher sensitivity for barrier dysfunction than the absolute CSF concentrations. We developed a new model for collecting CSF samples intermittently during CPB. The purpose of this study was to test the hypothesis that the blood cerebrospinal fluid barrier is disrupted by CPB using albumin concentrations in CSF and plasma.

Methods

Surgical Procedure

Twenty-five experiments (each group: n=5) were performed on 5- to 6-week-old Yorkshire piglets (Archer Farms, Inc., Darlington, MD) with average body weight of 11.5 ± 1.3 kg. All animals received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health (NIH publication 85-23, revised 1985). Piglets were sedated with intramuscular ketamine (20 mg/kg) and xylazine (4 mg/kg). After endotracheal intubation (cuffed 5-mm tube) and a bolus of fentanyl (25 µg/kg IV) the piglets were ventilated with a pressure-controlled respirator using an inspiratory oxygen fraction of 21% pre-CPB and 100% post-CPB (rate 10–18 breaths per minute) to achieve arterial pCO₂ between 38–42 mmHg. Anesthesia was maintained with fentanyl (25 µg/kg/h), midazolam (0.2 mg/kg/h), and pancuronium (0.2 mg/kg/h) using an infusion pump. Animals were placed supine on a water-circulating heating mat to prevent hypothermia. Esophageal and rectal temperature probes were placed. The left femoral artery was cannulated and the catheter was advanced into the descending aorta for monitoring of blood pressure and blood gases. Blood pressure and body temperature were continuously monitored and recorded every 10 minutes. Blood gases were checked for pH, pO₂, pCO₂, sodium, potassium, calcium, glucose and lactate every 20 minutes during CPB in 0.5 mL samples using a blood-gas analyzer (Siemens, Rapidlab 1265, Erlangen, Germany). A catheter was placed through the femoral vein into vena cava for drug infusion.

The piglet was placed in the prone position for inserting an epidural catheter, which was used to draw the CSF intermittently from the cisterna magna. A small pillow was placed under the

shoulder to facilitate flexing the neck. After the midline of the occipital and cervical area was opened and the atlanto-occipital membrane was dissected, the dura mater of the cisterna magna was found. After placing the purse-string suture (6-0 prolene®) on the dura mater, an epidural catheter (Perifix® mini set, B. Braun medical company, PA) was gently inserted under that membrane without any blood contamination and was fixed to the dura mater (Figure 1).

After returning the animal to the supine position, the right femoral artery was exposed for arterial CPB cannulation and an anterolateral thoracotomy was performed in the third intercostal space. After administration of heparin (300 IU/kg), an arterial cannula (8Fr, Biomedicus) was advanced through the femoral artery into the abdominal aorta. A 28F venous cannula (Harvey, MA) was inserted into the right atrium. The heart was not opened during the procedure.

Bypass Management

A roller pump (Polystan, Vaerlose, Denmark) was used to generate nonpulsatile pump flow at 100 mL/kg body weight in all experiments. The oxygenator gas mixture consisted of 5% carbon dioxide and 95% oxygen in all CPB groups. pH-stat management strategy was used in all CPB groups. The cardiopulmonary bypass circuit consisted of 1000-mL filtered hard-shell venous reservoir (1361 Minimax, Medtronic, Minneapolis, MN), a membrane oxygenator (3381 Minimax Plus, Medtronic), a 40- μ m arterial filter (Terumo, Tokyo, Japan) and 1/4-inch tubing. Venous drainage was by gravity. No cardiotomy suction was used. The venous line was left open during circulatory arrest. A sterile circuit was used in each experiment. The CPB circuit was primed with 800 mL blood. The blood used for priming of the CPB circuit to achieve a hematocrit of 30% on CPB was drawn on the morning of the experiment from an adult donor piglet. Before the start of CPB and just before reperfusion methylprednisolone (30 mg/kg), 10 mL sodium bicarbonate 7.4%, and furosemide (0.25 mg/kg) were added to the prime. After 10 minutes of normothermic bypass, piglets in 15, 25°C HCA group and 25°C LF group underwent 40 minutes cooling to an esophageal temperature of 15°C and 25°C, respectively. After a 60-minute period of HCA at 15°C, 25°C and 10 mL/kg/min low-flow CPB at 25°C, animals were rewarmed on CPB to 37°C for 50 minutes. In the 37°C FF group, esophageal temperature was kept at 37°C during CPB. Esophageal temperature in the control group was maintained at 37°C without CPB. Following weaning from CPB, animals were observed for 120 minutes. Topical cooling was applied for hypothermic temperature groups.

Cerebrospinal Fluid and Blood Sample Collection

CSF and blood samples were collected from the epidural and arterial line at seven time points during the perioperative period: baseline (pre-CPB), 10, 50 and 115 after start of CPB, just before end of CPB (160 min after start of CPB), 30 (p-30) and 120 (p-120) min after the end of CPB. Plasma was prepared by centrifuging blood samples at $2000 \times g$ for 15 minutes at room temperature. CSF and plasma were reserved directly into polypropylene tubes and frozen at -20°C .

Analysis of pH, partial pressure of oxygen and carbon dioxide and Lactate in CSF and plasma

Data regarding pH, partial oxygen pressure (PO_2), partial carbon dioxide pressure (PCO_2) and lactate were measured using the blood gas machine (Rapid lab 1246; Bayer, Germany) immediately to avoid mixing with room air, after drawing samples of CSF and blood.

Analysis of albumin concentrations in CSF and plasma samples

The piglet albumin concentration in CSF was determined using a Piglet Albumin ELISA (Enzyme-Linked Immunosorbent Assay) Quantitation Kit (Bethyl laboratories Inc., TX) according to the manufacturer's protocol. In short, 96-well plates were coated with 100 μ L of

capture antibody (10µg/mL) for porcine albumin for 1h at room temperature and post-coated with 200µl of 1% BSA-tris-buffered saline for 30min. The 100µL of samples and standard solutions were incubated for 1h, and detective reaction by 100µL of antibody/enzyme conjugate was performed for 1h. Then 100µL of tetramethyl benzidine was reacted for 10 minutes. The reaction was stopped by 100µL of 0.5M sulfuric acid, and absorbance was read at 450nm using platereader. Mean values and standard deviations were calculated from three independent experiments determined in duplicate. Albumin concentrations in plasma samples were measured using BCP assay (BioAssay Systems, CA).

Statistical Analysis

Continuous variables are expressed as mean $\pm$ standard deviation (SD). Repeated-measures analysis of variance (ANOVA) was used to compare variables between the experimental groups and to assess differences over the time course (within group). Slopes were compared by the group-by-time two-way interaction F-test. Statistical analysis was performed by SPSS version 16.0 (SPSS Inc, Chicago, IL). Two-tailed values of $p < 0.05$ were considered statistically significant. Power analysis indicated that the sample sizes of 5 piglets per group (measured at each of 7 time points) would provide 80% power to detect 30% mean differences in pH and lactate (plasma and CSF levels) between the different CPB experimental conditions and control group using repeated-measures ANOVA and assuming a pooled standard deviation of 20% (nQuery Advisor, Statistical Solutions, Saugus, MA). The Q_{Alb} ; the albumin ratio (CSF/plasma), was compared between the five experimental groups at the 7 different time points (baseline through 280 min) using repeated-measures analysis of variance (ANOVA) with the Bonferroni method for multiple group comparisons.

Results

Baseline data for age, weight, pH, arterial PO_2 , arterial PCO_2 , hematocrit level, mean arterial pressure (MAP), and saturation of right atrium: pre-operative variables were compared between the five experimental groups and presented for all five experimental groups reflecting no differences by the F-tests in one-way ANOVA (all $p \geq 0.10$). (Table 1) The pH, partial pressure of oxygen and carbon dioxide in CSF (Figure 2a, b, c): The pH in CSF was significantly lowered towards acidosis levels in both HCA groups during hypothermic circulatory arrest relative to the control group. In particular, pH at 115 minutes and just before the end of CPB in both HCA groups was significantly lower than the other three groups. Partial pressure of oxygen (PO_2) of both HCA and 25°C LF was significantly higher at 50 min than other groups. The values of both HCA groups were higher at 115 min than others. Partial carbon dioxide pressure (PCO_2) in the continuous bypass groups was significantly higher at 115 min than control. The values in both HCA groups were significantly higher at 115 min than the continuous bypass groups.

Lactate concentrations in plasma and CSF (Figure 3a, b): Plasma lactate levels at 25°C with LF and in both HCA groups were significantly increased compared with control. Lactate levels in both HCA groups at 115, 160, p-30 and p-120 min were significantly increased compared with others in CSF ($p < 0.05$). Control and 37°C with FF groups were not increased during the experimental time course.

Three tables (Table 2–4) present the exact data in terms of means and standard deviations as well as specific results from two-way repeated-measures ANOVA showing the p -values for within group (7 time points: baseline through 120 minutes post bypass), between group (5 groups), and the group-by-time interaction (ie, slope test). Significant interactions are followed by post-hoc Bonferroni comparisons to assess where differences lie. Table 2 presents pH, pO_2 , and pCO_2 data, Table 3 summarizes the lactate data, and Table 4 the albumin ratio (CSF/plasma). There are a total of six variables in Table 2–Table 4 and therefore a within group,

between group, and interaction p -value (3 columns) for each of these six variables. Interestingly, all six group-by-time interaction tests are highly significant ($p < 0.001$), indicating that for each variable there are significant differences between experimental groups although only at certain time points. Typically, highly significant two-way interactions suggest that differences between groups are conditional on specific time points and not across the board. A significant group $\times$ time interaction test in this context can also indicate that certain groups – but not all groups – demonstrate changes in a variable over the time course (ie, different time-related slopes or profiles). Again, the concept is that the two variables (group condition and time) are not independent but rather conditional or dependent. For example, the analysis of the albumin concentration data shown in Table 4 indicates significant differences between the groups but only at 115 min and 160 min after onset of CPB, and at 30 minutes post-bypass (p-30). The interaction test also confirmed, by virtue of Bonferroni comparisons, significant changes in the albumin (CSF/plasma) ratio, Q_{ALB} , but only for the first three experimental groups, 15°C HCA, 25°C HCA, and 25°C low flow (LF). Specifically, repeated-measures ANOVA revealed no significant group differences in Q_{ALB} at baseline ($p = 0.88$), 10 min ($p = 0.81$), or 50 min ($p = 0.86$), however the albumin ratio was significantly higher in the 15°C HCA group compared to 37°C FF and 37°C OFF (control) at 115 min, 160 min, and p-30 (all $p < 0.05$). In addition, Q_{ALB} in the 25°C HCA group was significantly higher compared to 25°C LF at 115 min ($p = 0.03$), 37°C FF at 115, 160, and 190 min (all $p < 0.01$), and compared to 37°C OFF (control) at 115 min ($p < 0.01$), 160 min ($p < 0.01$), and 190 min ($p = 0.03$). At p-120 minutes, none of the five experimental groups showed any differences with respect to the albumin ratio ($p = 0.31$). Changes in the Q_{alb} were in themselves indicative of disruption of the blood-CSF barrier. Both HCA groups demonstrated a significant increase in blood CSF barrier permeability after reperfusion ($p < 0.01$). The leakage of albumin was still maintained even after the end of bypass. There were no changes in Q_{alb} at 37°C, FF. Anesthesia alone did not affect the permeability of BCSFB over 6 hours. (Figure 4)

Discussion

We are aware of only a small number of investigations regarding the blood CSF barrier during CPB.^{11, 12, 13} There are no systematic research studies evaluating the effects of CPB on the blood CSF barrier in children. In the current study we have examined the integrity of the BCSFB during various CPB techniques using a new experimental model.

The values of PO_2 and PCO_2 in CSF in contrast to the pH remained within normal range at the end of CPB under this protocol. It is generally reported that CO_2 in blood diffuses easily to CSF through the blood CSF barrier. On the other hand, pH in CSF tends to acidosis after reperfusion, and time is required to return to the normal range. Some investigators have reported that the decline toward acidosis in CSF results in brain impairment.^{14, 15} A bypass condition such as a deep hypothermic circulatory arrest of 15°C did not eliminate acidosis in CSF. However, in this study, ultra low flow bypass reduced acidosis in CSF. Specifically, LF bypass at 25°C was effective and adequate for maintaining pH.

This experiment has also demonstrated that changes in CSF and blood lactate levels vary according to bypass condition. Lactate levels in CSF in the 25°C HCA group, which is the experimental setting that poses the greatest ischemic impact, were highest among all groups.¹⁶ Although CSF pH levels were stable with continuous LF at 25°C plasma lactate levels increased at 25°C within the LF group. These data suggest that ultra low flow bypass at 25°C is adequate for maintaining oxygen supply to the brain, not but to other organs and that LF bypass is preferable to deep hypothermic circulatory arrest at 15°C for oxygen supply.¹⁷ Khaladj N. et al.¹⁸ concluded that lactate levels in CSF appear to be sensitive in terms of the time course of events in characterizing an oxygen supply shortage to the central nervous system and that lactate in CSF potentially may mark anaerobic metabolism without necessarily being

associated with any permanent cellular damage. The mechanism or mechanisms underlying these changes remain unclear, however may be due in part to a failure of the blood brain barrier or of altered metabolism during ischemia. Our data suggest that 25°C LF with pH-stat strategy during CPB was adequate for oxygen supply to the brain.

The piglet model we have developed confirms that the blood CSF barrier is indeed disrupted by CPB. The CSF/plasma ratio of albumin is used as an indicator of permeability of the BCSFB. 10^{10} The calculated CSF/plasma concentration quotient, Q , e.g. for albumin: $CSF_{Alb} / plasma_{Alb} = Q_{Alb}$ has a higher sensitivity for barrier dysfunction than the absolute CSF concentrations. In particular, if CSF and plasma are analyzed in the same analytical run, the precision of quotients is higher and values are independent of method. In this study, the Q_{Alb} was showed the increase of permeability of the blood- CSF barrier in both HCA group., the increase of Q_{Alb} demonstrated a correlation with ischemic impact. In short, the disruption and dysfunction of blood CSF barrier was considerable in the both HCA groups. It suggests that there is cerebral ischemia during CPB and this is related to lactate levels in CSF and plasma. It should be noted that ultra low flow bypass reduced the albumin leakage from the blood CSF barrier. However, Q_{Alb} was not improved during experiments, even after the end of bypass.

Our data suggest that ischemic impact and reperfusion injury of CPB and post- CPB inflammation affect the barrier function. There were no significant changes in albumin levels for the control and 37°C with FF conditions. Anesthesia alone and 37°C with FF did not affect the blood CSF barrier. Ischemia was one cause for breaching of the blood CSF barrier. However the lactate levels in plasma and increased in 25°C with LF in spite of unchanging lactate levels in CSF. The blood-CSF barrier was maintained in 25°C with LF. Even ultra low flow bypass might be useful for barrier protection. Further studies are needed to understand the restoration of a barrier function.

In summary, we have shown increased permeability of the Blood Cerebrospinal Fluid Barrier during cardiopulmonary bypass using a new experimental piglet model. The bypass condition of 25°C with LF was effective in maintaining cerebral oxygen metabolism however inadequate for supplying oxygen to other body organs. This new piglet model has numerous possibilities for investigating and understanding the behavior of the blood CSF barrier function during CPB. Future studies should assess the optimal combination of bypass conditions including temperature, bypass flow and possible drug treatment for maintaining the barrier function.

Acknowledgments

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Figure 1.

Introduction of catheter into subdural space. The piglet was placed in the prone position for inserting an epidural catheter, which was used to draw the CSF intermittently from the cisterna magna. A small pillow was placed under the shoulder to facilitate flexing the neck. After the midline of the occipital and cervical area was opened and the atlanto-occipital membrane was dissected, the dura mater of the cisterna magna was found. After placing the purse-string suture (6-0 prolene®) on the dura mater, an epidural catheter (Perifix® mini set, B. Braun medical company, PA) was gently inserted under that membrane without any blood contamination and was fixed to the dura mater.

Figure 2-a

Figure 2-b

Figure 2-c

Figure 2.

Figure 2-a: Changes of pH in CSF for all groups. Both 15 and 25°C HCA were significantly lower than control group. The pH of both HCA groups at 115, 160 min was lower than other three groups. (* $p < 0.05$, repeated-measures ANOVA), p-30; 30 min after the end of CPB, p-120; 120 min after the end of CPB.

Figure 2-b: Changes of partial oxygen pressure (PO_2) in CSF for all groups. PO_2 of Both HCA and 25°C, LF was significantly higher at 50 min than others. The values of both HCA were higher at 115 min than others (* $p < 0.05$, one-way factorial ANOVA).

Figure 2-c: Changes of partial carbon dioxide pressure (PCO_2) in CSF for all groups. PCO_2 in bypass groups were significantly higher at 115 min than control. The values in both HCA were significantly higher at 115 min than other bypass groups (*, † $p < 0.05$, one-way factorial ANOVA).

Figure 3-a

Figure 3-b

Figure 3.

Figure 3-a: Changes of lactate levels in plasma for all groups. Lactate levels in 25°C with LF and both HCA groups were significantly increased compared with control. (* $p < 0.05$, repeated-measures ANOVA).

Figure 3-b: Changes of lactate levels in CSF for all groups. Lactate levels in both HCA groups at 115, 160, p-30 and p-120 min were significantly increased compared with others. (* $p < 0.05$, one-way factorial ANOVA).

Figure 4.

The Q_{Alb} (CSF/plasma) for experimental groups at each time point. Error bars denote standard deviations. Asterisks indicate significantly higher mean Q_{Alb} (reflecting greater permeability) compared to 37°C FF and 37°C control (* $p < 0.05$, ANOVA with Bonferroni correction).

Table 1

Baseline Characteristics of the Five Experimental Groups (n = 5 animals per group)

Characteristic	15°C, HCA	25°C, HCA	25°C, LF	37°C, FF	37°C, OFF	p Value ^a
Age, d	26.0 ± 2.0	24.8 ± 1.5	23.0 ± 2.3	24.6 ± 1.5	23.6 ± 1.2	0.10
Weight, kg	11.8 ± 1.4	11.4 ± 1.7	11.8 ± 1.6	11.7 ± 1.2	11.1 ± 0.7	0.90
pH	7.50 ± 0.03	7.50 ± 0.07	7.49 ± 0.04	7.50 ± 0.05	7.50 ± 0.04	0.98
pO ₂ , mm Hg	83.4 ± 11.7	80.2 ± 7.8	84.6 ± 4.6	88.0 ± 1.2	81.0 ± 6.9	0.48
pCO ₂ , mm Hg	39.9 ± 2.1	40.7 ± 1.6	40.5 ± 0.8	40.6 ± 2.2	40.3 ± 1.9	0.96
Hematocrit, %	27.8 ± 2.1	26.8 ± 3.1	25.9 ± 2.5	26.4 ± 3.4	27.8 ± 1.9	0.75
MAP, mm Hg	90.2 ± 17.1	79.2 ± 8.6	96.6 ± 14.0	77.6 ± 10.1	84.8 ± 10.4	0.13
SVO ₂ , %	66.9 ± 11.2	63.0 ± 7.2	67.2 ± 10.4	63.8 ± 7.3	67.5 ± 3.4	0.86

The values are mean ± SD.

HCA = hypothermic circulatory arrest; FF = full flow; LF = low flow; pO₂ = partial pressure of oxygen; pCO₂ = partial pressure of carbon dioxide; MAP = mean arterial blood pressure; SVO₂ = mixed venous oxygen saturation.

^aF-test in one-way ANOVA indicated no significant group differences for any of the variables.

Table 2

pH, pO₂ and pCO₂ in the CSF for Each Group From Baseline Through 120 Minutes Post CPB (n = 5 animals per group)

Parameter	Baseline	10 min	50 min	115 min	160 min	p-30 min	p-120 min	Within group p value	Between group p value	Interaction group × time p value
pH								0.027	<0.001	<0.001
15°C HCA	7.47±0.07	7.39±0.04	7.27±0.02	7.09±0.07	7.30±0.04*	7.44±0.03	7.46±0.05			
25°C HCA	7.47±0.05	7.39±0.04	7.35±0.05	7.04±0.07	7.20±0.10*	7.38±0.12	7.46±0.07			
25°C LF	7.46±0.09	7.36±0.04	7.30±0.02	7.29±0.03	7.35±0.09	7.43±0.05	7.49±0.07			
37°C FF	7.44±0.05	7.37±0.06	7.31±0.05	7.32±0.08	7.34±0.07	7.42±0.07	7.43±0.07			
37°C OFF	7.40±0.03	7.39±0.02	7.41±0.03	7.43±0.02	7.44±0.04	7.44±0.02	7.44±0.02			
pO ₂ , mm Hg								<0.001	<0.001	<0.001
15°C HCA	83±24	110±17	259±63	157±27	116±13	117±12	110±8			
25°C HCA	100±12	105±12	160±31	139±22	120±15	108±12	106±24			
25°C LF	92±20	101±17	152±15	115±9	127±27	120±40	125±36			
37°C FF	88±15	103±17	102±9	102±19	96±10	105±17	104±13			
37°C OFF	78±10	85±5	88±10	79±5	92±14	84±7	83±8			
pCO ₂ , mm Hg								0.020	<0.001	<0.001
15°C HCA	37.1±3.3	44.4±4.1	54.6±5.7	81.7±12.0	46.0±8.7	38.4±4.8	41.2±5.1			
25°C HCA	36.5±4.2	46.2±3.9	46.3±8.1	86.1±21.7	50.6±7.4	41.7±7.2	39.9±6.1			
25°C LF	39.8±8.0	46.6±3.9	54.2±4.3	62.9±4.4	53.8±5.5	43.8±4.5	40.4±2.9			
37°C FF	42.8±4.3	47.0±4.8	57.8±2.4	61.8±9.5	58.0±6.1	46.5±5.2	48.7±6.4			
37°C OFF	44.1±4.4	43.8±3.0	42.5±2.7	44.2±1.7	40.2±3.4	42.6±1.6	41.9±2.8			

Data are mean ± SD. All p-values are based on repeated-measures ANOVA with the significant interaction for each parameter indicating group differences although only at specific time points (see Figure 2 for more details).

Table 3

Lactate Measured in Plasma and CSF for Each Group Baseline Through 120 Minutes Post CPB (n = 5 animals per group)

Parameter	Baseline	10 min	50 min	115 min	160 min	p-30 min	p-120 min	Within group p value	Between group p value	Interaction group × time p value
Lactate in Plasma, mmol/L										
15°C HCA	1.04±0.25	2.20±0.58	2.47±0.67	5.12±0.94	4.51±1.27	3.52±1.06	1.90±0.70	<0.001	<0.001	<0.001
25°C HCA	1.12±0.23	2.34±0.38	2.41±0.18	7.54±1.45	6.79±1.06	5.89±1.74	5.25±2.09			
25°C LF	1.20±0.29	2.73±0.65	3.00±0.62	9.17±1.39	6.56±1.28	4.83±1.32	2.85±0.83			
37°C FF	0.99±0.25	2.41±0.76	1.42±0.35	1.46±0.46	1.87±0.68	2.26±0.78	1.73±0.41			
37°C OFF	0.96±0.32	0.93±0.57	0.84±0.44	0.80±0.32	0.92±0.29	0.90±0.17	0.80±0.10			
Lactate in CSF, mmol/L										
15°C HCA	2.21±0.24	1.84±0.30	1.43±0.34	3.24±0.59	4.79±1.65	3.79±1.07	2.89±0.45	<0.001	<0.001	<0.001
25°C HCA	1.86±0.20	1.77±0.18	1.34±0.11	4.76±1.36	5.83±1.40	4.81±1.01	3.61±0.90			
25°C LF	2.16±0.42	1.95±0.30	1.59±0.24	1.80±0.27	2.00±0.59	2.31±0.37	2.69±0.45			
37°C FF	2.23±0.11	2.01±0.16	1.51±0.15	1.41±0.19	1.48±0.36	1.89±0.34	2.17±0.39			
37°C OFF	2.04±0.38	1.78±0.23	1.67±0.29	1.63±0.27	1.43±0.27	1.53±0.24	1.67±0.18			

Data are mean ± SD. All p-values are based on repeated-measures ANOVA with a significant interaction test for each parameter indicating group differences but only at specific time points (see Figure 3 for more details).

Table 4

Albumin Ratio (CSF/Plasma) for Each Group From Baseline Through 120 Minutes Post CPB (n = 5 animals per group)

QALB	Baseline	10 min	50 min	115 min	160 min	p-30 min	p-120 min	Within group p value	Between group p value	Interaction group × time p value
Group								<0.001	0.450	<0.001
15°C HCA	4.9±1.7	7.2±5.3	10.9±5.8	19.7±9.7	15.7±5.5	14.8±6.5	15.6±7.8			
25°C HCA	3.1±1.4	4.0±3.3	6.5±3.3	23.9±12.3	21.2±10.3	17.1±8.3	16.9±9.8			
25°C LF	6.3±3.5	6.1±2.6	8.9±5.2	14.3±7.6	14.8±6.2	12.4±7.2	12.6±7.3			
37°C FF	7.5±4.8	9.3±8.7	7.3±7.2	8.4±6.7	9.5±8.1	7.0±4.2	8.5±6.9			
37°C OFF	6.0±3.8	6.8±8.5	7.5±7.7	7.6±7.2	6.8±5.4	7.5±5.2	9.0±5.5			

Data are mean ± SD. All p-values are based on repeated-measures ANOVA with the significant interaction indicating group differences, but only at 115, 160, and p-30 time points. In addition, significant changes were detected in QALB across time, although only for 15°C HCA, 25°C HCA, and 25°C LF groups (see Figure 4 for more details).