Effects of pH Management During Deep Hypothermic Bypass on Cerebral Microcirculation: Alpha-Stat Versus pH-Stat

Lennart F. Duebener, MD; Ikuo Hagino, MD; Takahiko Sakamoto, MD; Lotfi Ben Mime, MD; Christof Stamm, MD; David Zurakowski, PhD; Hans-Joachim Schäfers, MD; Richard A. Jonas, MD

Objective—There is controversy regarding the optimal pH strategy during deep hypothermic bypass in children. We directly visualized the effects of the pH-stat and alpha-stat strategy on cerebral microcirculation (including leukocyte/endothelial cell interactions) in a piglet model using intravital fluorescence microscopy.

Methods—Two groups of 5 piglets (mean weight 9.6±1.3 kg) with a cranial window over parietal cerebral cortex underwent 10-minute normothermic bypass, 40-minute cooling on cardiopulmonary bypass ([CPB] Hct 30%, 100 mL/kg/min), 60-minute circulatory arrest at 15°C, and 40-minute rewarming with alpha-stat (group alpha) or pH-stat (group pH). Plasma was labeled with fluorescein-ITC-dextran for assessment of microvascular diameter. Circulating leukocytes were labeled and observed in postcapillary venules for adhesion before and up to 120 minutes after CPB. Cerebral tissue oxygenation was evaluated by quantification of NADH autofluorescence, which increases during ischemia.

Results—At the end of normothermic bypass diameter of cerebrocortical microvessels increased to 116±9% (alpha) versus 119±10% (pH) of pre-CPB baseline values. During cooling microvascular diameter decreased in group alpha and significantly increased in group pH (89±11% (alpha) versus 132±13% (pH) at the end of cooling; $P<0.001$). During the first 10 minutes of rewarming, the cerebral microvascular diameter was significantly larger when the pH stat strategy was used. Tissue oxygenation at the end of cooling was significantly greater in the pH-stat group ($P=0.008$). On reperfusion, the pH-stat strategy resulted in significantly more rapid return of tissue oxygenation toward baseline although at the end of rewarming the metabolic recovery was complete in both groups. The whole body lactate during early rewarming was significantly less with the pH stat strategy. There was no significant difference between the groups regarding the number of adherent leukocytes throughout the time course of the experiment.

Conclusions—pH-stat management increases tissue oxygenation during deep hypothermic bypass and after circulatory arrest. Leukocyte/endothelial cell interactions during hypothermic bypass are mild with both alpha-stat and pH-stat.

Key Words: cardiopulmonary bypass • brain • cerebral ischemia • surgery
There are theoretical advantages and disadvantages for both pH strategies. Alpha-stat has been said to better preserve intracellular pH and enzyme activity.\textsuperscript{2} It has been argued that it better preserves autoregulation of cerebral vasculature and cerebral flow-metabolism coupling.\textsuperscript{3} Disadvantages of alpha-stat include less efficient and less homogeneous cooling and less reduction of oxygen consumption.

Advantages of the pH-stat strategy include increased cerebral blood flow (CBF), more homogeneous cooling, greater reduction of oxygen consumption and increased tissue oxygen availability because of a shift in the oxyhemoglobin dissociation curve. On the other hand pH-stat might result in loss of autoregulation and luxuriant cerebral blood flow that has the potential to increase the risk of microembolism to the brain.\textsuperscript{4}

Studies using microspheres have consistently demonstrated greater cerebral blood flow during hypothermia with pH-stat when compared with alpha-stat.\textsuperscript{5} However, information about the impact of pH management during deep hypothermic bypass on the cerebral microcirculation is very limited. Little is known about the effects of pH strategy on cerebral blood flow at microvascular and capillary level. We used intravitral microscopy to directly study the effects of pH strategy on cerebral microcirculation and tissue oxygenation in a piglet model. In addition, the effects of pH on leukocyte/endothelial cell interactions were studied.

**Methods**

**Surgical Procedure**

Ten experiments (each experimental group: n = 5) were performed on 5- to 6-week-old Yorkshire pigs (Parsons Farms, Hadley, MA) with an average body weight of 9.6 ± 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).

The piglets were premedicated intramuscularly with Ketamine (20 mg/kg) and Xylazine (4 mg/kg). After endotracheal intubation (cuffed 5-mm tube) and a bolus of Fentanyl (25 mg/kg) and Xylazine (4 mg/kg). After endotracheal intubation (NIH publication 85-23, revised 1985).

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The piglets were premedicated intramuscularly with Ketamine (20 mg/kg) and Xylazine (4 mg/kg). After endotracheal intubation (cuffed 5-mm tube) and a bolus of Fentanyl (25 mg/kg IV) the piglets were artificially ventilated with a pressure-controlled respirator using an inspiratory oxygen fraction of 50% pre-CPB and 100% post-CPB at a rate between 12 to 18 breaths per minute to achieve an arterial pCO\textsubscript{2} between 35 and 40 mm Hg. 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. The animals were placed supine on a water-circulated heating mat to prevent hypothermia. A nasopharyngeal and rectal temperature probe 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. The blood pressure and body temperature were continuously monitored and recorded every 10 minutes. Blood gases were checked for pH, pCO\textsubscript{2}, potassium and lactate every 10 minutes during CPB in 1-mL samples using a blood-gas analyzer (Nova, Stat Profile Plus9, Waltham, MA). A catheter was placed through the left femoral vein into the inferior vena cava for infusion of drugs and fluorescent dyes. The right femoral artery was exposed because of the FITC fluorescence of blood plasma. The functional capillary density (FCD), defined as total number of red blood cells/m vessel length.

**Arteriolar Diameter, Functional Capillary Density**

To visualize microvascular diameters and microvascular perfusion, plasma was labeled with 5% fluorescein-isothiocyanate (FITC)-Dextran (Fluka, Buchs, Switzerland, molecular weight 150,000) by intravenous injection of 2 mL FITC-dextran for baseline recordings and 0.5 mL FITC-Dextran before each subsequent measurement (intravenous injection before and after CPB, intra-arterial injection during CPB). FITC fluorescence was excited using 450- to 490-nm light and the emitted light (>515 nm) (blue filter set, Leica) was transferred from the microscope to a video camera. Thus, the plasma was highlighted and the red blood cells appeared dark (negative contrast). To minimize heating of the cover slip and the underlying tissue the window was regularly washed with physiological saline solution.

The diameter of arterial and venous cerebrocortical microvessels (range 40 to 130 μm) was measured from video still images by use of an image analysis program after calibration of the software with a video caliper. On average, 4 to 5 arterioles and 5 to 6 venules per observation area were measured.

After focusing below the level of pial vessels the cortical capillaries could be visualized because of the FITC fluorescence of blood plasma. The functional capillary density (FCD), defined as total length of red blood cell-perfused capillaries per observation area [cm\textsuperscript{-1}] was determined.

**LE Interactions**

For observation of leukocyte-endothelial cell interactions, the circulating leukocytes were labeled by intravenous injection of 2 mL Rhodamine-6G solution (Sigma, St. Louis, MO) before each measurement. A green filter set (excitation wavelength 536 to 556 nm, emission wavelength >590 nm, Leica) was used to excite Rhodamine fluorescence. In identical areas, leukocyte adherence in post-capillary venules was recorded for 60 seconds at defined time points. Adherent leukocytes were defined as white blood cells (WBC) that attached to the endothelium and did not detach within a 20-second observation period. Numbers of adherent leukocytes were expressed per 100-μm vessel length.
**Tissue Oxygenation**

NADH is a natural intracellular fluorophore and its concentration increases during ischemia. Therefore, it can be used for tissue oxygenation.6 NADH fluorescence was excited using 340- to 380-nm light (UV-Filter set: emission wavelength >420 nm, Leica). During these measurements the remote control of the video camera for brightness and contrast was disabled. The optical densities (black=0, white=255) of the recorded still images were determined by densitometry using the NIH image program.

**Measurements: Intravital Microscopy**

Microvascular and capillary perfusion, NADH fluorescence, and LE interactions were recorded every 10 to 15 minutes during CPB. In addition, NADH fluorescence was recorded every 15 minutes during circulatory arrest. The duration of epi-illumination was limited to 1 minute to avoid thermal injury of tissue. The epi-illumination of brain tissue was always shut off between video recordings.

**Bypass Management**

After baseline recordings of the cerebral microcirculation, the venous and the arterial cannulas were connected to the venous and arterial lines of the cardiopulmonary bypass circuit, respectively. A roller pump (Cardiovascular Instrument Corp., Wakefield, MA) was used to generate a nonpulsatile pump flow of 100 mL/kg bodyweight in all experiments. The oxygenator gas mixture consisted of 5% carbon dioxide and 95% oxygen in the pH-stat group. Pure oxygen was used as oxygenator gas in the alpha-stat group. The gas flow was adjusted to achieve an arterial pCO2 of 40 to 45 mm Hg (pH-stat corrected to nasopharyngeal temperature, alpha-stat not temperature corrected). The cardiopulmonary bypass circuit in all experiments consisted of a 1000-mL filtered hard-shell venous reservoir (1361 Minimax, Medtronic, Minneapolis, MN), a membrane oxygenator (3381 Minimax plus, Medtronic), a 40-μm arterial filter (Terumo), 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 pig. Before the start of CPB and on reperfusion methylprednisolone (25 mg/kg), cephalizin (25 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 underwent 40 minutes of cooling to a nasopharyngeal temperature of 15°C. After 60 minutes of deep hypothermic arrest, animals were rewarmed on CPB to 37°C. Following weaning from CPB, animals were observed for 120 minutes. The room temperature in the animal operating room was thermostatically controlled (range, 15 to 30°C) to aid cooling and rewarming of animals. No topical cooling was applied.

**Statistics**

Continuous variables are expressed as means±SD. Variables were tested for normality using the Kolmogorov-Smirnov goodness-of-fit test and no significant skewness was detected. Therefore, repeated-measures ANOVA was performed to evaluate changes over time and to compare rates of change between the groups. The conservative Greenhouse-Geisser procedure was used to account for the small sample sizes.7 For time point comparisons within an experimental group, paired t tests were used. To evaluate group differences at fixed time points, one-way factorial ANOVA with the post-hoc Bonferroni method was utilized. Data analysis was conducted using the SPSS software package (release 11.0, SPSS Inc., Chicago, IL).

**Results**

**Experimental Conditions**

There were no significant differences between the alpha-stat and pH-stat groups with respect to nasopharyngeal temperature, hematocrit level, or arterial oxygen tension (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Pre CPB</th>
<th>10' NT</th>
<th>End Cool</th>
<th>10' Rewarm</th>
<th>End Rewarm</th>
<th>120' off CPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial pH</td>
<td>alpha-stat</td>
<td>7.46±0.09</td>
<td>7.52±0.05</td>
<td>7.77±0.03</td>
<td>7.62±0.09</td>
<td>7.51±0.07</td>
<td>7.45±0.03</td>
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<td>pH-stat</td>
<td>7.49±0.07</td>
<td>7.41±0.11</td>
<td>7.39±0.09</td>
<td>7.39±0.07</td>
<td>7.37±0.04</td>
<td>7.47±0.06</td>
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<tr>
<td>Arterial pCO2</td>
<td>alpha-stat</td>
<td>36.6±5.3</td>
<td>29.0±6.1</td>
<td>13.1±2.5</td>
<td>20.5±2.0</td>
<td>33.3±5.5</td>
<td>42.1±4.9</td>
</tr>
<tr>
<td></td>
<td>pH-stat</td>
<td>38.1±5.1</td>
<td>43.2±4.2</td>
<td>44.3±4.8</td>
<td>41.6±7.2</td>
<td>46.5±6.0</td>
<td>40.9±5.2</td>
</tr>
<tr>
<td>Arterial pO2</td>
<td>alpha-stat</td>
<td>355±45</td>
<td>544±45</td>
<td>719±82</td>
<td>583±107</td>
<td>551±39</td>
<td>418±92</td>
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<tr>
<td></td>
<td>pH-stat</td>
<td>326±32</td>
<td>422±51</td>
<td>651±72</td>
<td>491±89</td>
<td>505±76</td>
<td>360±97</td>
</tr>
<tr>
<td>Arterial hematocrit</td>
<td>alpha-stat</td>
<td>28.9±3.5</td>
<td>32.3±2.4</td>
<td>30.0±2.5</td>
<td>29.9±3.3</td>
<td>32.7±3.3</td>
<td>29.8±1.2</td>
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<td></td>
<td>pH-stat</td>
<td>27.8±2.2</td>
<td>32.0±2.3</td>
<td>32.0±2.3</td>
<td>31.1±3.6</td>
<td>32.2±3.8</td>
<td>31.3±4.0</td>
</tr>
<tr>
<td>Nasopharyngeal, temperature</td>
<td>alpha-stat</td>
<td>37.4±0.6</td>
<td>37.1±0.4</td>
<td>13.4±0.6</td>
<td>28.7±1.3</td>
<td>37.3±0.5</td>
<td>34.5±0.7</td>
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<td>pH-stat</td>
<td>37.0±0.9</td>
<td>36.7±0.6</td>
<td>13.3±0.7</td>
<td>27.1±1.6</td>
<td>36.7±0.8</td>
<td>34.3±0.6</td>
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<tr>
<td>Mean arterial pressure</td>
<td>alpha-stat</td>
<td>73±14</td>
<td>84±15</td>
<td>87±12</td>
<td>77±17</td>
<td>95±17</td>
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<td>pH-stat</td>
<td>70±11</td>
<td>86±16</td>
<td>57±13*</td>
<td>57±8</td>
<td>86±14</td>
<td>90±14</td>
</tr>
</tbody>
</table>

Values for arterial pH, pCO2, and pO2 are corrected to nasopharyngeal temperature. pre CPB indicates baseline values before cardiopulmonary bypass; 10' NT, after 10 minute normothermic bypass; End Cool, end of cooling; 10' Rewarm, after 10 minutes of rewarming; 120' off CPB, 120 minutes after weaning from CPB.

*P<0.05.

**Microvascular Diameter of Pial Vessels**

During 10 minutes of normothermic CPB, the arteriolar diameter increased significantly relative to baseline (pre bypass) values in both groups (alpha 116±9%, pH 119±10%) (Figure 1). After 10 minutes of cooling, the microvascular diameter increased further (133±13%) in the pH-stat group, but declined to 104±11% in the alpha-stat group (P=0.02). At the end of cooling, the arteriolar diameter reached 132±13% with pH-stat and 89±11% with alpha-stat (P<0.001). At the end of DHCA, the diameter of pial arterioles was about 25% of baseline values in both groups.
(The venous line to the reservoir was left open during circulatory arrest for exsanguination.) During early reperfusion, the arteriolar diameter was significantly larger with pH-stat relative to alpha-stat (5-minute reperfusion 82% vs 67%; \( P = 0.007 \); 10-minute reperfusion, \( P = 0.03 \)). However, the microvascular diameter did not significantly differ between the groups after 20 minutes of rewarming (nasopharyngeal temperature 32.5°C). The arteriolar diameter was not significantly different from baseline values in any group after weaning from CPB.

### Functional Capillary Density

There was a slight decline of functional capillary density (FCD) during cooling in both groups relative to baseline (Figure 2). At the end of cooling, the FCD was 93±4% in the pH-stat and 81±6% in the alpha-stat group (\( P = 0.13 \)). During early reperfusion, there was a tendency to higher FCD values with pH-stat, although the differences did not reach statistical significance (5 minute rewarming: 56±7% (pH) versus 45±5% (alpha) \( P = 0.07 \); 10-minute rewarming 93±5% vs 82±6% \( P = 0.1 \)). At the end of rewarming and 120 minutes after weaning from CPB, the FCD was not significantly different from baseline values in either group. There were no significant differences between the groups at these time points.

### Tissue Oxygenation

The NADH autofluorescence of the parietal cortex decreased to 93±2% (alpha) versus 94±3% (pH) during normothermic bypass relative to baseline, indicating better cerebral tissue oxygenation in both groups (Figure 3). During cooling the tissue oxygenation declined below baseline levels in the alpha-stat group. At the end of cooling, the NADH fluorescence reached 98±2% and 103±3% in the pH and alpha-stat group, respectively (\( P = 0.008 \)), indicating significantly poorer tissue oxygenation in the alpha-stat group. During deep hypothermic circulatory arrest there was a tendency for more severe de-oxygenation in the alpha-stat group. In the early rewarming phase, the tissue oxygenation was significantly higher with the pH-stat strategy (5-minute rewarming: 116±4% (pH) versus 106±4% (alpha) \( P = 0.001 \); 10-minute rewarming: 116±4% (pH) versus 106±4% (alpha) \( P = 0.01 \)). Furthermore, the recovery time to reach baseline values again was significantly shorter in the pH-stat group (10 minutes versus 35 minutes; \( P = 0.02 \)). However, at the end of reperfusion and after weaning from CPB the metabolic recovery was complete in both groups. There were no significant differences between the groups at final recovery.

### Systemic Lactate Level

There were no statistical differences between the groups with respect to systemic lactate levels during cooling. However, during early reperfusion the whole body lactate levels were significantly higher in the alpha-stat group (10-minute re-
warming 10.6±1.9 mmol/L (alpha) versus 7.3±1.4 mmol/L (pH) \( P = 0.04 \).

**White Blood Counts**

At baseline, the number of circulating leukocytes as measured by white blood counts was 14.4±5.0 versus 15.0±6.1 \times 1000/\mu L for alpha and pH-stat, respectively. The white blood count decreased to 8.7±1.8 (alpha) versus 6.6±2.7 (pH) \times 1000/\mu L at the end of 40-minute cooling. At the end of the observation period (ie, 2 hours after weaning), the number of circulating leukocytes reached 12.5±2.3 (alpha) versus 14.1±4.2 (pH). None of these differences between the groups was statistically significant.

**LE Interactions**

The number of adherent leukocytes to cerebral venules (length 100 \mu m) was 4.8±1.6 versus 5.0±2.1 in the alpha and pH-stat group, respectively. Leukocyte adherence decreased to 2.9±0.6 (alpha) versus 2.2±0.9 (pH) at the end of cooling. Two hours after weaning, the number of adherent leukocytes was 4.5±1.1 in the alpha-stat group and 4.7±1.4 in the pH-stat group. At no time point were the differences between the experimental groups statistically significant.

**Discussion**

This intravital microscopy study focused on the effects of pH management during hypothermic bypass on cerebral microcirculation and tissue oxygenation.

In both groups, the microvascular diameter increased relative to baseline during normothermic bypass. During hypothermic bypass we found an increase of arteriolar diameter with the pH-stat strategy. This is not surprising because the pH-stat strategy involves addition of carbon dioxide, a well known cerebral vasodilator. When the alpha-stat strategy was employed during cooling a progressive decrease of diameter of cerebrocortical microvessels could be demonstrated.

There was a tendency for higher functional capillary density values during hypothermic bypass and early reperfusion in the pH-stat group relative to alpha-stat, however these differences in capillary perfusion did not reach statistical significance. Functional capillary density is defined as total length of capillaries perfused with red blood cells per observation area. The actual capillary blood flow (ml/min) cannot be reliably evaluated by intravital microscopy. Thus, there might have been differences in capillary blood flow that were not detectable by this method.

Cerebral tissue oxygenation was significantly higher in the pH-stat group at the end of cooling and during early reperfusion after deep hypothermic circulatory arrest. This finding might be related to the effect of carbon dioxide in counteracting the leftward shift of the oxyhemoglobin dissociation curve during hypothermia. The rightward shift with the pH-stat strategy should increase oxygen availability for tissues. The finding of a higher lactate level with the alpha-stat strategy supports the concept that there is improved oxygen availability with the pH-stat strategy. This is consistent with a previous study from our laboratory where we compared hyperoxic with normoxic management and found an improved histological outcome with hyperoxia, ie, in the setting of deep hypothermic circulatory arrest and cardiopulmonary bypass increased tissue oxygenation is associated with an improved outcome.8

Other studies have demonstrated that the rate of depletion of brain oxygen during deep hypothermia and circulatory arrest is significantly slower with pH-stat strategy in comparison to alpha-stat strategy.9 As a possible explanation it has been argued that the more acidic intracellular pH causes a depression of metabolism that should further decrease the oxygen consumption during deep hypothermic circulatory arrest.10 We found that the cerebral tissue oxygenation was lower at all times during circulatory arrest with alpha-stat relative to pH-stat. However, in our intravital microscopic study the rate of depletion of brain oxygen was not significantly different between the groups.

The finding of increased tissue oxygenation with pH-stat is in keeping with our previous laboratory studies of piglets undergoing microsphere studies of cerebral blood flow as well as magnetic resonance studies of cerebral high energy phosphates and intracellular pH.5 When the pH-stat technique for acid base management was chosen, the return of cerebral ATP and phosphocreatinine after reperfusion was significantly more rapid. Near infrared spectroscopy studies have shown an improved redox state of cytochrome aa3 with pH-stat relative to alpha-stat.11

In another experimental study using a piglet survival model of deep hypothermic arrest, it was shown that pH-stat cardiopulmonary bypass management improves neurologic outcome compared with alpha-stat bypass.12

A clinical randomized study from our institution demonstrated that infants managed with the pH-stat strategy had better outcomes than patients in the alpha-stat group.13 In the subset of patients with transposition of the great arteries, pH-stat patients had a higher cardiac index and a shorter duration of mechanical ventilation and intensive care unit stay. The data suggest that pH-stat management improves cerebral and systemic protection in infants undergoing deep hypothermic bypass.

The inflammatory response as measured by leukocyte/endothelial cell interactions to hypothermic cardiopulmonary bypass including circulatory arrest was mild with both alpha-stat and pH-stat strategy. In fact there was a 50% reduction in circulating and adherent leukocytes in both groups. Our experimental model involved administration of steroids, slow cooling over 40 minutes, deep hypothermic circulatory arrest at 15°C for only 60 minutes, and slow rewarming over 40 minutes. These “mild” experimental conditions might account for the weak inflammatory response observed.

**Limitations**

One of the limitations of this intravital microscopy study of cerebral microcirculation is the fact that only superficial cerebrocortical vessels can be assessed. Deeper subcortical microvessels can not be studied with this direct method in a nondisruptive manner. However, hypoperfusion of deeper brain regions is most likely important in the pathogenesis of neurological injury after cardiopulmonary bypass. The study by Aoki et al14 from our institution showed that regional blood
flow in the brain was significantly higher in the pH-stat group relative to the alpha-stat group in the basal ganglia, midbrain, and cerebellum during cooling.

Summary
Using intravital fluorescence microscopy we were able to directly visualize and measure the microcirculatory effects of pH management during deep hypothermic bypass on the brain. The pH-stat strategy resulted in greater tissue oxygenation at the end of cooling and at the beginning of reperfusion after deep hypothermic arrest. This adds further evidence to support the use of pH-stat strategy during deep hypothermic bypass with and without circulatory arrest. We conclude that the pH-stat strategy is preferable in patients undergoing deep hypothermia because it improves cerebral tissue oxygenation.

References
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