Utility and Limitations of Near-Infrared Spectroscopy during Cardiopulmonary Bypass in a Piglet Model

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ABSTRACT

Near-infrared spectroscopy assessment of cytochrome oxygenation could be a valuable technique to monitor cerebral intraneuronal oxygen delivery during cardiopulmonary bypass. However, the validity of the cytochrome signal has been questioned as it could easily be overwhelmed by the Hb signal. Five- to six-week-old control piglets ($n = 5$) underwent cardiopulmonary bypass at 37°C. Study animals ($n = 10$) received 100 mg/kg of sodium cyanide to uncouple cytochrome from Hb. Hematocrit was then decreased in steps of 5% from 35 to 5% with crystalloid hemodilution. In study piglets, the initiation of cardiopulmonary bypass was associated with oxygenated Hb increasing from 0 to 62.9 ± 25.6 μM times the differential path-length factor, and oxidized cytochrome a,a3 increasing to 1.9 ± 1.8 μM times the differential path-length factor. Cyanide caused oxygenated Hb to increase further to 132.3 ± 48.9 μM times the differential path-length factor, and oxidized cytochrome c decreased to -7.0 ± 2.6 μM times the differential path-length factor as anticipated, confirming uncoupling of electron transport. However, hemodilution subsequently resulted in linear decreases in oxidized cytochrome a,a3 ($F = 8.57$, $p < 0.001$) suggesting important cross-talk between the Hb and cytochrome signals as cytochrome is only intracellular. In control piglets, tissue oxygenation index showed a positive correlation with pump flow ($r = 0.986$, $p = 0.013$). The cytochrome signal as presently measured by near-infrared spectroscopy is highly dependent on hematocrit. (*Pediatr Res* **49:** 770–776, 2001)

Abbreviations:
CPB, cardiopulmonary bypass
CytO$_2$, oxidized cytochrome a,a3
DPF, differential path-length factor
HbO$_2$, oxygenated Hb
HbT, total Hb
HHb, deoxygenated Hb
NIRS, near-infrared spectroscopy
TOI, tissue oxygenation index
CCD, charge-coupled device

The surgical outcome for congenital cardiac anomalies has improved in recent years (1). However, children undergoing surgical repair of congenital cardiac anomalies are at significant risk of brain injury (2, 3). Availability of a cerebral monitoring system for use during cardiac surgery, especially during CPB, could be useful in preventing postoperative cerebral impairment. NIRS has been used as a noninvasive tool for the assessment of cerebral oxygenation, especially in the fields of anesthesiology, neurology, and neonatology (4–6). Recently, NIRS has also been used in cardiac surgery, and its utility has been reported in both laboratory (7) and clinical applications (8, 9). NIRS assessment of cytochrome oxygenation could be a valuable technique to monitor cerebral intraneuronal oxygen delivery during CPB. This information is particularly important because of potentially limited oxygen unloading secondary to hypothermia and alkalosis during CPB, which may make Hb oxygenation uninformative. The interpretation of NIRS data, however, especially CytO$_2$ signal, remains controversial (8, 10, 11). The specific absorption spectra of HbO$_2$, HHb, and cytochrome oxidase overlap and are relatively featureless in the wavelength range that can penetrate several centimeters of tissue, and furthermore, the component of the tissue absorption coefficient caused by Hb is an order of magnitude greater than that from CytO$_2$ (12). For these reasons, the cytochrome signal is vulnerable to cross-talk with Hb, *i.e.* changes in the concentration or saturation of Hb in the head that cause artifactual changes in the cytochrome signal. Moreover, because NIRS is based on the modified Beer-Lambert law, a change of hematocrit might also affect the path length of

near-infrared light. Kurth and Uher (13) have reported a linear relationship between the intracerebral Hb level and path length using a time-resolved spectrophotometer. The situation is further complicated for children with congenital heart disease because the $CytO_2$ signal measured by current equipment detects only relative changes from baseline measurement. Children undergoing CPB have different levels of cerebral oxygenation at baseline before CPB because some are cyanotic whereas others are not cyanotic. It might be difficult to compare the relative $CytO_2$ signal of patients with very different hematocrits and oxygenation before cardiac repair (10). Furthermore, in cardiac surgery large changes of hematocrit occur at the initiation of CPB.

The purpose of the current study is to examine possible cross-talk between the cytochrome and Hb NIRS signals and to clarify the utility and limitations of NIRS during CPB.

METHODS

Experimental Preparation

Fifteen 5- to 6-wk-old Yorkshire piglets, weighing 8.79 ± 0.67 kg (Parson's Livestock, Hadley, MA, U.S.A.), were anesthetized with ketamine (20 mg/kg) and xylazine (4 mg/kg) intramuscularly and 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 rate of 12–15 breath/min, by means of a pressure control ventilator (Healthdyne model 105; Healthdyne Technologies, Marietta, GA, U.S.A.) to achieve a normal pH and arterial carbon dioxide tension. For the intraoperative monitoring and blood sampling, arterial and venous lines were placed in the left femoral artery and vein, respectively. After an i.v. bolus injection of fentanyl (25 μg/kg) and pancuronium (0.5 mg/kg), anesthesia was maintained by a continuous infusion of fentanyl (25 μg·kg^{-1}·h^{-1}) and midazolam (0.2 mg·kg^{-1}·h^{-1}) along with pancuronium (0.2 mg·kg^{-1}·h^{-1}) throughout the entire experiment. The right femoral artery was exposed for the CPB arterial cannula, and a median sternotomy was performed to expose the right atrium for venous cannulation. A venous line was placed in the jugular vein *via* the superior vena cava to measure the jugular venous saturation during the study. After systemic heparinization (300 IU/kg), an 8F arterial cannula (Medtronic Bio-Medicus, Minneapolis, MN, U.S.A.) and a 28F venous cannula (Research Medical, Inc., Midvale, UT, U.S.A.) 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 National Institutes of Health (NIH publication 86–23, revised 1985).

CPB Technique

The CPB circuit consisted of a roller-pump, membrane oxygenator (Minimax; Medtronic, Inc., Anaheim, CA, U.S.A.), and sterile tubing, with a 40-μm arterial filter (Olson Medical

Sales, Inc., Ashland, MA, U.S.A.). The pump circuit was primed with 800 mL of whole blood that was taken from a donor piglet on the same day. Methylprednisolone (30 mg/kg), furosemide (0.25 mg/kg), and 8.4% sodium bicarbonate (10 mL) were added to the priming solution, as is the clinical practice at our hospital. Full bypass flow was set at 100 mL·kg^{-1}·min^{-1}. CPB was started, and animals were perfused for 10 min at normothermia (37°C). Ventilation was stopped after the establishment of CPB. Esophageal temperature was maintained at 37°C.

Experimental Protocols

Experiment 1. After stabilization for 10 min, animals in study group (group CN, $n = 10$) received i.v. sodium cyanide (100 mg/kg) to uncouple cytochrome from Hb at an initial hematocrit of 35% (Fig. 1). The hematocrit was then stepwise decreased by 5% from 35% to a final hematocrit of 5% using crystalloid isovolemic hemodilution (Plasmalyte A, Baxter, Deerfield, IL, U.S.A.). NIRS data were recorded using an NIRO 300 device (Hamamatsu Photonics K.K., (Hamamatsu City, Japan), and the jugular venous saturation was measured. In control group (group C, $n = 5$), a venting cannula was inserted into the left ventricle, and the ascending aorta was clamped to prevent any residual forward blood flow from the heart. Hemodilution was undertaken without sodium cyanide. Pump flow was changed from 100 to 75, 50, and 25 mL·kg^{-1}·min^{-1} at each hematocrit value to alter cerebral blood flow and investigate the influence on NIRS data.

Experiment 2. The NIRO 300 device uses only four wavelengths, 775, 810, 850, and 910 nm, to measure and calculate the signals. This measurement system is simple and convenient to use clinically. In contrast, the CCD system (14) uses continuous multiple wavelengths. Thus, the results with the CCD system may be more accurate, although this instrument is less practical. The NIRO 300 device is based on the CCD system. The CCD system can also measure the path length that is dependent on wavelength simultaneously. For these reasons, one additional experiment was performed with the CCD system in place of the NIRO 300 in the same manner as group CN.
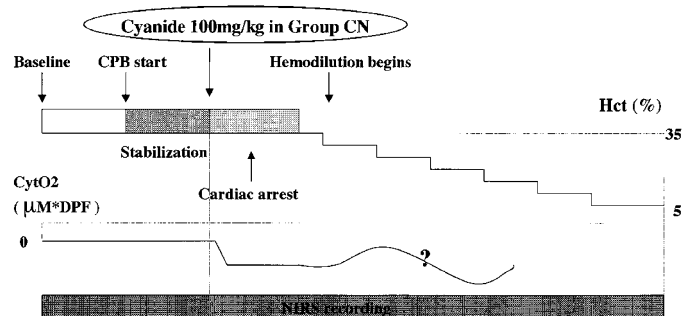

Figure 1. Experimental protocol. CPB was established with right femoral artery and right atrial cannulations at 37°C. Full bypass flow was set at 100 mL·kg^{-1}·min^{-1}n. Hematocrit was decreased 5% in steps from 35 to 5% with crystalloid hemodilution. At each hematocrit value in control piglets, pump flow was changed from 100 to 75, 50, and 25 mL·kg^{-1}·min^{-1}. The question mark indicates that the interpretation of the cytochrome signal is controversial.

Data Collection

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 two optodes, one a transmitter and one a receiver of laser light of near-infrared wavelength, were connected to the NIRO 300 device. This device calculates the relative concentration changes in HbO_2, HHb, and $CytO_2$, and also calculates TOI. TOI is an index of average tissue Hb saturation and is calculated independently from the change in HbO_2 and HHb. Whereas HbO_2, HHb, and $CytO_2$ are shown as the changes in concentrations from an arbitrary point, TOI is an absolute measure of tissue oxygen saturation independent of Hb. Data collection was begun immediately after intubation, and baseline values were 0 $\mu M \times DPF$ for HbO_2, HHb, HbT, and $CytO_2$ signals. Data were recorded every 5 s throughout the experiment. Measurement was expressed as micromoles per liter times DPF.

Blood gas analyses. Arterial and venous (jugular vein) blood gas values, including electrolyte, glucose, and lactate concentrations, were measured at baseline, 10 min after beginning of CPB (before injection of sodium cyanide), 10 min after injection of cyanide, and 5 min after the completion of each hemodilution (NOVA 900; Nova Biomedical, Waltham, MA, U.S.A.).

Statistical Analysis

All results were expressed as mean ± SD of the mean. In control piglets in experiment 1, the average values of hematocrit and NIRS data at each hemodilution point were plotted, and their relationships were determined by comparing linear and nonlinear regression models at each CPB flow. The optimal model, according to correlation coefficients, to describe the relation between hematocrit and $CytO_2$ was a nonlinear logarithmic equation. Relationships between TOI, CPB flow, and jugular vein oxygen saturation were analyzed in the same manner, and a simple linear regression model fits well. A two-tailed $p < 0.05$ was considered statistically significant. In study piglets in experiment 1, the relationships between hematocrit and NIRS data were determined by a mixed model ANOVA to account for the repeated measurements within the same piglets. A significant F test was used to indicate a relationship between changes of hematocrit and changes of NIRS data. A two-tailed $p < 0.05$ was considered statistically significant. In experiment 2, the Pearson correlation coefficient

was used to evaluate the strength of association between $CytO_2$, HbT, and path-length data. All data were analyzed by a statistical analysis software package (SPSS version 10.0, SPSS Inc., Chicago, IL, U.S.A.).

RESULTS

Experiment 1

Control piglets. Change of hematocrit and hemodynamic state. The hematocrit of animals before CPB was 30.0 ± 3.0%. It increased to 33.6 ± 3.6% after establishment of CPB. Hematocrit was then decreased 5% in steps to 7.2 ± 0.8% by crystalloid hemodilution. Animals showed mean arterial pressure of 79.4 ± 10.1 mm Hg before CPB and 91.4 ± 9.1 mm Hg at a pump flow of 100 $mL \cdot kg^{-1} \cdot min^{-1}$ after the beginning of CPB. There was a decrease of mean arterial pressure during hemodilution such that perfusion pressure was 28.8 ± 2.6 mm Hg at hematocrit of 7.2 ± 0.8%.

Change of HbO_2 and $CytO_2$. HbO_2 increased from 0 to 43.1 ± 35.9 $\mu M \times DPF$, and $CytO_2$ increased to 3.4 ± 3.3 $\mu M \times DPF$ after the beginning of CPB (Table 1, Fig. 2). Hemodilution subsequently resulted in a linear decrease in HbO_2 ($R^2 = 0.993$, $p < 0.0001$) and a logarithmic decrease of $CytO_2$ ($R^2 = 0.951$, $p < 0.0001$) at a pump flow of 100 $mL \cdot kg^{-1} \cdot min^{-1}$. The logarithmic correlation was affected by the pump flow.

Change of HHb. HHb changed little at the initiation of CPB. There was significant correlation between hematocrit and HHb during hemodilution ($R^2 = 0.923$, $p < 0.0001$).

Change of TOI. Animals showed a mean TOI of 48.4 ± 3.2% before CPB. TOI increased to 56.8 ± 4.1% after the establishment of CPB. TOI was not much influenced by hematocrit, but showed a positive correlation with pump flow ($R^2 = 0.976$, $p = 0.013$).

Study piglets. Change of hematocrit and hemodynamic state. The hematocrit of animals before CPB was 28.4 ± 2.3% and increased to 34.7 ± 2.7% after establishment of CPB. The injection of sodium cyanide caused some change of hematocrit to 32.5 ± 2.7% because 50 mL of saline was used to dissolve the sodium cyanide. Hemodilution decreased the hematocrit stepwise by 5% to 6.7 ± 0.8%. Animals showed mean arterial pressure of 72.8 ± 6.4 mm Hg before CPB and 80.4 ± 15.2 mm Hg after the beginning of CPB. Sodium cyanide resulted in a decrease to 31.6 ± 4.6 mm Hg. There was a mild decrease of mean arterial pressure during hemodilution to 24.5 ± 17.0 mm Hg at the end of the experiment.

Table 1. *Change of NIRS signals before hemodilution in both groups*

Group	HbO_2 ($\mu M \times DPF$)	HHb ($\mu M \times DPF$)	HbT ($\mu M \times DPF$)	$CytO_2$ ($\mu M \times DPF$)	TOI (%)*
Group CN					
Before CPB	0	0	0	0	46.4 ± 1.7
During CPB before cyanide	62.9 ± 25.6	−4.6 ± 7.8	58.4 ± 27.0	1.9 ± 1.8	55.4 ± 4.0
After cyanide	132.3 ± 48.9	−70.8 ± 22.1	61.5 ± 41.0	−7.0 ± 2.6	73.7 ± 3.9
Group C					
Before CPB	0	0	0	0	48.4 ± 3.2
During CPB	43.1 ± 35.9	−0.8 ± 27.4	42.3 ± 44.0	3.4 ± 3.3	56.8 ± 4.1

* TOI is the ratio of tissue oxygenated and total hemoglobin, as follows: TOI = (HbO_2 vol/HbT vol) × 100%.
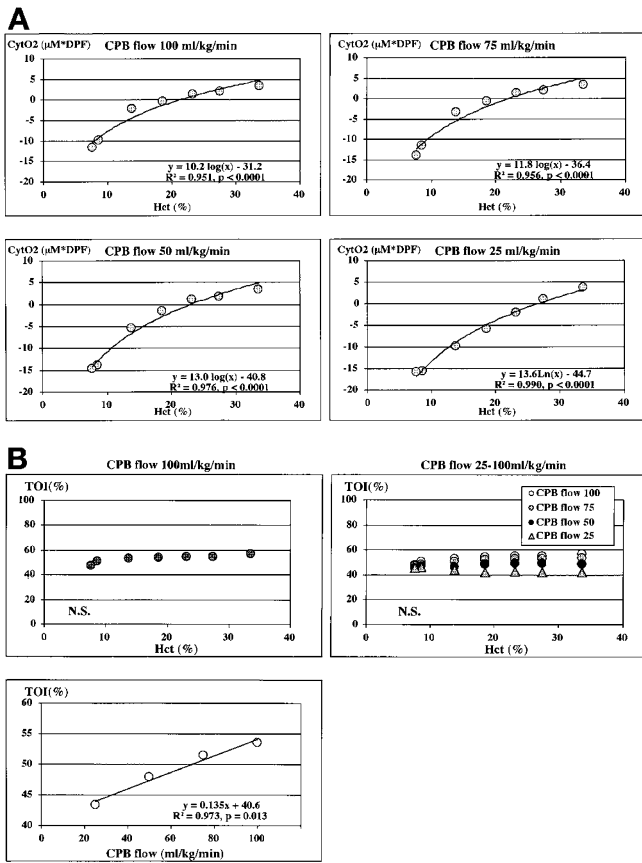
Figure 2. Change of CytO$_2$ (*A*) and TOI (*B*) during hemodilution in group C. CytO$_2$ signal decreased logarithmically during hemodilution ($R^2 = 0.951, p < 0.0001$) and was affected by the pump flow. TOI was not much influenced by hematocrit value, but showed a positive correlation with pump flow ($R^2 = 0.973, p = 0.013$).
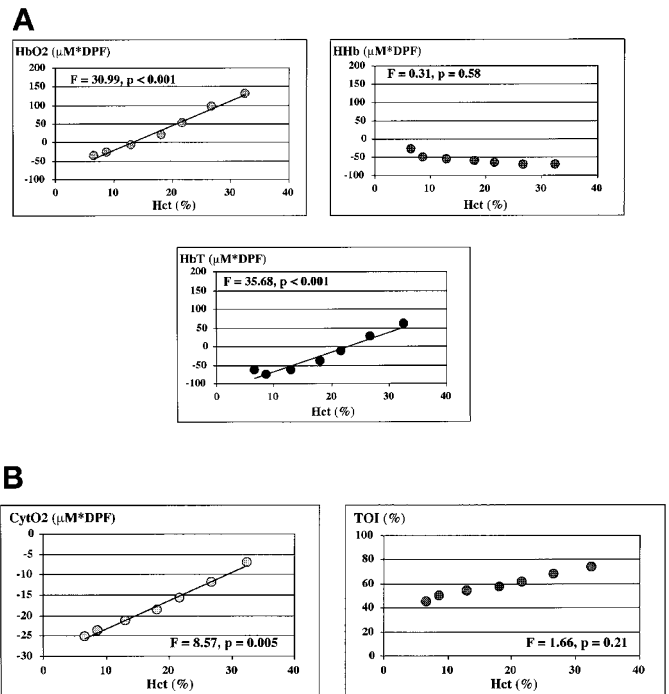

Figure 3. *A, B,* Change of NIRS signals during hemodilution in group CN. Hemodilution resulted in linear decreases in CytO$_2$ ($F = 30.99, p < 0.001$), suggesting important cross-talk between the Hb and cytochrome signals inasmuch as cytochrome is only intracellular.

Blood gas analyses. The results are shown in Table 2. The administration of sodium cyanide caused large increases in potassium, lactate, and jugular venous saturation.

Change of HbO$_2$ and CytO$_2$. HbO$_2$ increased 62.9 ± 25.6 μM×DPF units, and CytO$_2$ increased 1.9 ± 1.8 μM×DPF units after the beginning of CPB (Table 1, Fig. 3). Sodium cyanide caused HbO$_2$ to increase 132.3 ± 48.9 μM×DPF, and CytO$_2$ decreased by 7.0 ± 2.6 μM×DPF units as anticipated, confirming uncoupling of electron transport. Hemodilution subsequently resulted in linear decreases of HbO$_2$ ($F = 30.99, p < 0.001$) and CytO$_2$ ($F = 8.57, p < 0.001$).

Change of HHb. HHb did not change importantly at the initiation of CPB. After the bolus injection of sodium cyanide, HHb decreased −70.8 ± 22.1 μM×DPF. There was no significant relationship between hematocrit and HHb during hemodilution ($F = 0.31, p = 0.58$).

Relationship between HbT and CytO$_2$. Changes in CytO$_2$ were highly correlated with changes in HbT during hemodilution. If blood flow to the brain is constant during hemodilution, changes in HbT indicate the intracerebral Hb concentration.

Change of TOI. Animals showed a mean TOI of 46.4 ± 1.7% before CPB. TOI increased to 55.4 ± 4.0% after the establishment of CPB and showed further increase to 73.7 ± 3.9% after the injection of sodium cyanide. There was no significant relationship between hematocrit and TOI during hemodilution ($F = 1.66, p = 0.21$).

Experiment 2

The data derived with the CCD device are shown in Figure 4. During hemodilution after the injection of cyanide, CytO$_2$ signal decreased according to hematocrit value. Wavelength-dependent path length that was measured throughout the experiment changed according to HbT level, which represents the change of hematocrit. There was a linear relationship between HbT, CytO$_2$, and path length after the injection of sodium cyanide.

DISCUSSION

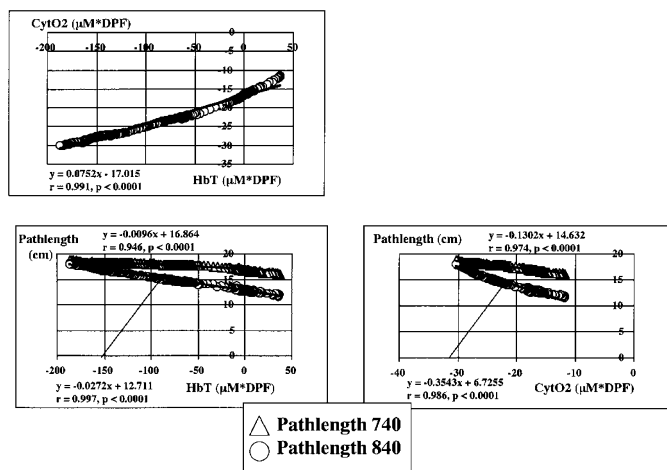Cerebral hypoxia and ischemia are the most common cause of neurologic and neuropsychological deficits in infants and

Table 2. *Blood gas data before hemodilution in group CN*

		During CPB	
	Before CPB	Before cyanide	After cyanide
pH	7.518 ± 0.042	7.473 ± 0.042	7.343 ± 0.090
Paco$_2$ (mm Hg)	35.11 ± 3.96	44.48 ± 5.82	61.88 ± 7.33
Pao$_2$ (mm Hg)	104.43 ± 7.45	414.04 ± 66.83	559.50 ± 36.86
Hct (%)	28.4 ± 2.3	34.2 ± 2.7	32.5 ± 2.7
K (mM)	3.74 ± 0.35	4.30 ± 0.94	8.32 ± 1.17
Glucose (mg/dL)	110.5 ± 17.8	133.1 ± 34.2	75.7 ± 25.8
Lactate (mM)	1.47 ± 0.59	2.64 ± 1.10	20.00 ± 2.69
Sjo$_2$ (%)	79.96 ± 7.68	84.82 ± 8.16	99.85 ± 0.16

Administration of sodium cyanide caused large increases in potassium and lactate, and elevated jugular venous saturation.

Paco$_2$, arterial Pco$_2$; Pao$_2$, arterial Po$_2$; Hct, hematocrit; Sjo$_2$, jugular venous oxygen saturation.

Figure 4. *Top left*, relationship between HbT and CytO$_2$ during hemodilution after cyanide. Data were recorded by CCD system. *Bottom*, change of path length during hemodilution after cyanide. The term "Path length 740 (840)" means the path length that is dependent on wavelength of 740 (840) nm.

children after cardiac repair (2, 3). A method is needed to monitor cerebral oxygenation and guide operative management to prevent severe hypoxia and ischemia during CPB. Presently available methods of monitoring, *e.g.* EEG, measurement of cerebral blood flow, and jugular venous saturation, are unreliable or irrelevant under conditions of deep hypothermia, hemodilution, pH shifts, and circulatory arrest. NIRS would seem to be ideal for the cardiac surgeon because it can monitor cerebral oxygenation continuously during surgery. The cytochrome signal in particular would be a useful indicator of intracellular hypoxia and impending injury. However, the cytochrome signal represents a small component of the total attenuation change and therefore is vulnerable to artifact. Accordingly, the interpretation of NIRS data, especially the CytO$_2$ value, still remains controversial (8, 10, 11).

Several factors, such as hematocrit, pH, temperature, or pump flow, are manipulated over wide ranges during CPB for congenital heart disease. Kurth and Uher (13) have reported previously that hematocrit interferes with the cytochrome signal. Our study confirms such interference in the setting of an animal model with CPB. The results of the current study demonstrate that the CytO$_2$ signal measured by the NIRO 300 device in 5- to 6-wk-old piglets is highly dependent on the hematocrit value.

There have been several previous studies of cross-talk between Hb and cytochrome. Investigators have used cyanide, which is a mitochondrial inhibitor (15, 16). However, it is not feasible to perform such studies completely without CPB. Because cyanide stops the mitochondrial function of the heart simultaneously with the brain, it is impossible to maintain blood flow to the brain after the injection of cyanide without CPB. In previous reports, the heart of the experimental animal was beating throughout the study, keeping the mean blood pressure >40 mm Hg (16). The dose of cyanide (5 mg/kg body weight) is insufficient to completely inhibit mitochondrial function. To ensure complete mitochondrial inhibition with cyanide has been achieved, the experiment has to be performed under CPB as in the current study.

After administration of a saturating quantity of sodium cyanide, mitochondrial oxygen consumption should be completely inhibited and the cytochrome oxidase redox state should be locked in the fully reduced state and independent of intracellular oxygen tension. The CytO$_2$ signal should fall to a nadir level and be independent of cerebral saturation and Hb concentration. In this study, Hb concentration was changed by varying the pump circuit hematocrit, and it was found that the cytochrome signal continued to change with each step of hemodilution after the administration of cyanide. There are two possible explanations for this phenomenon. First, the dose of sodium cyanide may have been insufficient to completely inhibit mitochondrial respiration, that is, cytochrome oxidase was not fully reduced after administration of cyanide and further reduction occurred during hemodilution because of the decrease in oxygen delivery. Second, the NIR system was unable to precisely separate the Hb and cytochrome oxidase components of the change in attenuation, leading to artifactual changes in the cytochrome signal.

In the current study, sodium cyanide was administered i.v. at a dose of 100 mg/kg, which is 20 times the dose believed to have fully reduced the cerebral cytochrome signal in a previous study on newborn piglets (16). It is generally agreed that the lethal dose is approximately 5–10 mg/kg (17, 18). In our study the lactate concentration increased from 2.64 ± 1.10 to 20.00 ± 2.69 mM, and jugular venous saturation increased from 84.82 ± 8.16% to 99.85 ± 0.16%. These changes confirm the efficacy of sodium cyanide in inhibiting oxygen consumption and are strong evidence that cerebral cytochrome oxidase was fully reduced after administration of cyanide. In addition, the inhibitory dissociation constant of cytochrome oxidase for cyanide is 0.2 μM, which is four orders of magnitude smaller than the average concentration of cyanide in the piglet body (100 mg/kg is approximately 2 mM) (19). We can therefore say with some certainty that the cyanide is at a saturating concentration. Further evidence supporting complete inhibition of oxygen consumption is that jugular venous saturation rose to near 100%. In light of this, it must be concluded that the additional cytochrome oxidase reductions after cyanide administration were artifactual in origin. The assumptions behind the modified Beer-Lambert law are that the optodes do not move with respect to the tissue, the tissue scattering coefficient does not change, and the change in absorption coefficient is small.

This study also examined path length (20–22). The factors that can change path length are optode placement, geometry of the skull, tissue edema, and absorption and scattering of light. The optodes, transmitter and receiver, are separated by a distance of 4.0 cm throughout the experiment, and therefore optode placement does not affect the path length. Regarding geometry of the skull, the piglets used in the current study have skull and extracranial tissue (skin and fat) approximately 4 mm thick. Because the near-infrared light can penetrate to a depth of ≥2 cm with the optode distance of 4 cm, the data should reflect changes in brain tissue (23, 24). However, tissue edema occurs during CPB (25), particularly after the injection of cyanide, and very large changes in CytO$_2$ signal were found during hemodilution. CytO$_2$ signal decreased from −7.0 ± 2.6 μM×DPF at a hematocrit of 32.5 ± 2.7% to −24.3 ± 2.2

$\mu M \times DPF$ at the final hematocrit of $6.7 \pm 0.8\%$. It seems difficult to explain such large changes, which are three times more than the change with administration of cyanide, by tissue edema alone. Full spectral NIRS such as we used in experiment 2 with the CCD system has the added advantage that the differential path length can be measured from the water features using the second differential technique. Furthermore, modeling has shown that full spectral systems can better separate the cytochrome signal from the Hb signals compared with four wavelength systems such as the NIRO 300 (26). Change of path length was indeed found according to the degree of hemodilution in this additional experiment. (There was no change of path length dependent on wavelength of 840 nm before and after the injection of cyanide, although there was approximately a 50% increase of path length dependent on wavelength of 740 nm. Afterward, there were approximately 50 and 20% increases in path length during hemodilution, respectively). Hemodilution increased path length, and for this reason also an anomalous $CytO_2$ signal can be produced. However, inasmuch as all the units are multiplied by DPF, path length affects all the changes in the $CytO_2$ signal.

In group C, $CytO_2$ signal decreased logarithmically during hemodilution and was affected by the pump flow. $CytO_2$ signal decreased 0.6858 $\mu M \times DPF$ in steps with a hematocrit decrease of 1% in group CN. In group C, the negative slope of $CytO_2$ signal was dependent on hematocrit value at each point (10.2371/hematocrit when CPB flow is 100 mL·kg^{-1}·min^{-1}), and this negative slope became steeper with further decrease of hematocrit. When we apply the relation in group CN to the result in group C, a hematocrit of 14.9% (10.2371/0.6858) is necessary to maintain cerebral oxygenation with CPB flow of 100 mL·kg^{-1}·min^{-1}. Interestingly, this hematocrit is almost the same as the minimum safe hematocrit described previously (27, 28), although our own studies suggest that this is not an optimal hematocrit (29, 30). If an algorithm can be developed to correct for hematocrit changes, the $CytO_2$ signal measured by NIRS would more accurately assess cytochrome and could provide important information during CPB (31).

The NIRO 300 calculates HbO_2, HHb, HbT, and $CytO_2$ from the modified Beer-Lambert law and TOI with spatially resolved spectroscopy (32). TOI was not influenced by hematocrit value and showed a positive correlation with pump flow. However, there was no significant relationship between TOI and the jugular venous saturation. Sagittal sinus saturation is lower than the mixed venous saturation (33). This value seems to be close to the TOI value before CPB in the current piglet model. Therefore, TOI as a predictor of cerebral oxygenation may be more reliable than the other variables under conditions of CPB.

In conclusion, TOI may be a useful indicator for brain oxygenation during CPB. The cytochrome signal as presently measured by NIRS is highly dependent on hematocrit. Modeling to correct for this problem is being undertaken, so that an algorithm can be developed to correct the hematocrit changes such as occur under the conditions of CPB.

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