Using near-infrared spectroscopy to measure cerebral metabolic rate of oxygen under multiple levels of arterial oxygenation in piglets

Kenneth M. Tichauer,1,2 Jonathan T. Elliott,1,2 Jennifer A. Hadway,1,3 David S. Lee,4 Ting-Yim Lee,1,2,3 and Keith St. Lawrence1,2

1Imaging Division, Lawson Health Research Institute, 2Department of Medical Biophysics, University of Western Ontario, 3Imaging Research Laboratories, Robarts Research Institute, and 4Department of Paediatrics, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada

Submitted 24 December 2009; accepted in final form 1 July 2010

Tichauer KM, Elliott JT, Hadway JA, Lee DS, Lee TY, St. Lawrence K. Using near-infrared spectroscopy to measure cerebral metabolic rate of oxygen under multiple levels of arterial oxygenation in piglets. J Appl Physiol 109: 878–885, 2010. First published July 8, 2010; doi:10.1152/japplphysiol.01432.2009.—Improving neurological care of neonates has been impeded by the absence of suitable techniques for measuring cerebral hemodynamics and energy metabolism at the bedside. Currently, near-infrared spectroscopy (NIRS) appears to be the technology best suited to fill this gap, and techniques have been proposed to measure both cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMRO₂). We have developed a fast and reliable bolus-tracking method of determining CMRO₂ that combines measurements of CBF and cerebral venous oxygenation [venous oxygen saturation (CSVO₂)]. However, this method has never been validated at different levels of arterial oxygenation [arterial oxygen saturation (SaO₂)], which can be highly variable in the clinical setting. In this study, NIRS measurements of CBF, CSVO₂, and CMRO₂ were obtained over a range of SaO₂ in newborn piglets (n = 12); CSVO₂ values measured directly from sagittal sinus blood samples were collected for validation. Two alternative NIRS methods that measure CSVO₂ by manipulating venous oxygenation (i.e., head tilt and partial venous occlusion methods) were also employed for comparison. Statistically significant correlations were found between each NIRS technique and sagittal sinus blood oxygenation (P < 0.05). Correlation slopes were 1.03 (r = 0.91), 0.73 (r = 0.73), and 0.73 (r = 0.81) for the bolus-tracking, head tilt, and partial venous occlusion methods, respectively. The bolus-tracking technique displayed the best correlation under hyperoxic (SaO₂ = 99.9 ± 0.03%) and normoxic (SaO₂ = 86.9 ± 6.6%) conditions and was comparable to the other techniques under hypoxic conditions (SaO₂ = 40.7 ± 9.9%). The reduced precision of the bolus-tracking method under hypoxia was attributed to errors in CSVO₂ measurement that were magnified at low SaO₂ levels. In conclusion, the bolus-tracking technique of measuring CSVO₂, and therefore CMRO₂, is accurate and robust for an SaO₂ > 50% but provides reduced accuracy under more severe hypoxic levels.

cerebral venous oxygenation; newborn piglet

RELIABLE MONITORING of cerebral hemodynamics and energy metabolism is an essential aspect of critical care of neurology patients. Unfortunately, conventional clinical methods of measuring these parameters are unsuitable for use at the bedside of critically ill newborns (9). Accordingly, much interest has been garnered by near-infrared spectroscopy (NIRS) as it provides safe and portable techniques for measuring cerebral blood flow (CBF) and cerebral blood oxygenation (34). Over the last decade, a number of groups have combined NIRS hemodynamic measurements with oxygenation measurements to calculate the cerebral metabolic rate of oxygen (CMRO₂) in infants (11, 16, 29, 36); however, the accuracies and limitations of the different techniques have not been extensively investigated. All of these techniques are based on the Fick principle and employ NIRS measurements of CBF and the cerebral venous oxygen saturation (CSVO₂) to determine CMRO₂ (28). Since the calculation of CMRO₂ is reliant on multiple independent measurements, it is crucial that these measurements are of the highest possible accuracy.

The accuracy of CSVO₂ measurements derived from second-derivative NIRS (20) and a bolus-tracking technique—using the NIR absorber indocyanine green (ICG) as the bolus (4, 30)—was investigated (3, 28, 29). With this approach, cerebral blood volume (CBV) calculated from the bolus-tracking technique (4) is used to normalize the deoxyhemoglobin (HHb) measurement from the second-derivative NIRS technique (20) and CSVO₂ is calculated with a fixed arterial-to-venous blood volume ratio (FAVR) (22). In addition, measurements of CSVO₂ were combined with CBF measurements—also derived from the bolus-tracking technique—to investigate the contribution of CSVO₂ accuracy to measurements of CMRO₂. The attraction of this technique is that it provides measurements of CBF, CBV, CSVO₂, and CMRO₂ from a single bolus of ICG.

To this point, the proposed method of measuring CMRO₂ with NIRS has been validated in piglets over a range of metabolic states (32) and after transient hypoxia-ischemia (31). It was unknown, however, how the technique would perform at different levels of arterial oxygenation (hyperoxic to hypoxic). In fact, there is some speculation as to its reliability since one study demonstrated that the accuracy of the HHb concentration measurement may be compromised under hypoxic conditions (17). There is also evidence of high intersubject variability in the arterial-to-venous blood volume ratio (33). The validity of this technique could have significant clinical relevance for newborn intensive care, particularly in preterm infants who are prone to have fluctuating levels of arterial oxygen saturation (SaO₂). Even healthy neonates present a wide range of arterial oxygenations, and they often encounter hyperoxia when provided with oxygen supplementation (25).

The purpose of the present study was to validate our bolus-tracking CSVO₂ method over a range of arterial blood oxygen saturations in newborn piglets, using oxygenation measurements from the superior sagittal sinus as a gold standard. For comparison, CSVO₂ was also measured by two alternate NIRS techniques that have been applied clinically: the head tilt (HT) and partial venous occlusion (PVO) techniques (29, 37). These techniques are independent of an assumed FAVR and are not...
affected by the same potential sources of error under hypoxic conditions (17). CMRO₂ was calculated with the CSVo₂ measurements from each NIRS technique, as well as from sagittal sinus O₂ measurements, in combination with the CBF measurements acquired from the ICG bolus-tracking method to demonstrate how errors in CSVo₂ propagate into CMRO₂ measurements.

MATERIALS AND METHODS

Animal preparation. The study was approved by the Animal Use Subcommittee of the Canadian Council on Animal Care at the University of Western Ontario. After delivery from a local supplier, anesthesia was induced in newborn (<3 days of age) Duroc piglets (n = 12) with 5% isoflurane, which was reduced to 3% during surgery. Piglets were tracheotomized and mechanically ventilated, and cannulas were inserted into an ear vein for ICG injection and into a femoral artery for monitoring blood pressure and collecting arterial blood samples for gas and glucose analysis. The posterior scalp was resected, and the sagittal and lambdoid sutures were identified; a small burr hole was drilled 1 cm posterior to the lambda structure, and a cannula was inserted into the superior sagittal sinus for collection of cerebral venous blood samples. After surgery, isoflurane was reduced to 1.75% and piglets were placed in a frame and allowed to stabilize for 1 h before the experiment was begun.

Experimental procedure. All piglets were ventilated at three different levels of inspired oxygen (FIO₂): 50%, 21%, and 10% oxygen, consecutively, in a medical air mixture to represent hyperoxia, normoxia, and hypoxia, respectively. At each level of FIO₂, NIRS was used to collect one measurement of FAVR-CSVo₂ (which required a 0.2 mg/kg intravenous 1-ml bolus of ICG) and three measurements of both HT-CSVo₂ and PVO-CSVo₂. A blood sample was collected from the superior sagittal sinus for blood gas analysis immediately after NIRS measurements at each level of FIO₂. Blood oxygenation (SS-SVo₂) was measured by hemoximetry (Radiometer OSM3, Copenhagen, Denmark). The data was set to <5 min to carry out the full data collection procedure at each level of oxygenation. After each alteration of FIO₂, piglets were allowed to acclimate for 10 min before subsequent measurements were collected.

Arterial Pco₂ was maintained between 38 and 42 Torr throughout each experiment by adjusting the rate of mechanical ventilation. Blood glucose was maintained between 3 and 8 mM by intermittent 0.2 mg/kg intravenous 1-ml bolus of ICG) and three measurements of both HT-CSVo₂ and PVO-CSVo₂. A blood sample was collected from the superior sagittal sinus for blood gas analysis immediately after NIRS measurements at each level of FIO₂. Blood oxygenation (SS-SVo₂) was measured by hemoximetry (Radiometer OSM3, Copenhagen, Denmark). The data was set to <5 min to carry out the full data collection procedure at each level of oxygenation. After each alteration of FIO₂, piglets were allowed to acclimate for 10 min before subsequent measurements were collected.

NIR measurements from each NIRS technique and from the sagittal sinus blood samples (SS-SVo₂) (28):

\[
\text{CMRO}_2 = \text{CBF} \cdot \beta \cdot (\text{Hb} - \text{CSVo}_2).
\]  

where β is the oxygen carrying capacity of hemoglobin (1.39 ml O₂/g Hb for fetal hemoglobin). The bolus-tracking method of measuring CBF was used in all CMRO₂ calculations. The fraction of arterial blood in the CBF was also measured independently by replacing CSVo₂ with SS-SVo₂ in Eq. 1 and solving for α.

Error analysis of FAVR-CSVo₂. An error analysis was carried out to determine the sensitivity of FAVR-CSVo₂ to errors in the measured parameters used in its calculation (Eq. 3). The analysis was conducted on various levels of SaO₂. The effect of an error in α was assessed by comparing three sets of FAVR-CSVo₂ values that were calculated over a range of SaO₂ from 30% to 100% with average values of CBF, cerebral HHb concentration, and tHb concentration from the study.

The effect of an error in CBF, cerebral HHb concentration, tHb concentration, or SaO₂ was also investigated. A similar procedure was invoked by using a blood volume ratio of 0.25 and 0.75 to generate three different sets of FAVR-CSVo₂ values that were calculated over a range of SaO₂ from 30% to 100% with average values of CBF, cerebral HHb concentration, and tHb concentration from the study.
to determine statistical significance of correlations between the indi-
subjects variable. A straightforward statistical test could not be used
subjects variable and the measurement technique as the between-
determine the agreement of the correlation with the line of identity.

distribution of slopes against the null hypothesis (i.e., slope
applied to the data from each piglet, individually. Second, a significant
estimating equation technique was utilized (38). First, a linear fit was
of piglets and the number of data points, a variation of the generalized
animals; therefore, all of the data points could not be assumed to be
sure measurements because there were multiple data points from the same

fraction of inspired oxygen (FIO2) level because of the inability to sample blood
from the sagittal sinus in 2 piglets. *

blood measurements at different levels of FIO2 were investigated by
(50%, 21%, and 10%). The three groups are classified as hyperoxic, normoxic, and hypoxic because of the measured levels of SaO2 and PO2. As expected, both SaO2 and arterial PO2 dropped significantly as FIO2 was reduced (P < 0.05). No statistically significant differences were found between average arterial PCO2, mean arterial pressure, or temperature values at the different levels of FIO2. However, heart rate rose significantly (P < 0.05) at each consecutive level of reduced FIO2, while pH was significantly lower at 10% FIO2, than at 50% and 21% FIO2.

Figure 1 displays correlations and Fig. 2 displays Bland-
Altman plots (2) between SS-SvO2, and CSvO2, from each of the three NIRS techniques. Statistically significant correlations
were found for all comparisons (P < 0.05) and slopes of all
correlations were not significantly different from the line of
identity. The slopes, intercepts, and coefficients of linear re-
gression for the three techniques were 1.03, −3.3%, and r = 0.91 for FAVR (Fig. 1, left); 0.73, 18.3%, and r = 0.73 for HT (Fig. 1, center); and 0.73, 12.0%, and r = 0.81 for PVO (Fig. 1, right). With respect to the FAVR-CSvO2 data, a relationship
was observed between the variance in the data and the level of
oxygenation (Fig. 2, top). At normoxic and hyperoxic levels of
oxygenation, the 95% confidence interval in the FAVR-CSvO2
data was relatively small (±10.4%), while the 95% confidence
interval increased to ±37.3% at hypoxic oxygenation. Data were
also separated based on an SaO2 threshold of 50%. For SaO2 >
50%, the mean ± 95% confidence interval of the difference
between FAVR-CSvO2 and SS-SvO2 was −0.04 ± 12.87%, while for
SaO2 < 50% the difference was −9.04 ± 42.60% (4 measurements
at FIO2 of 10% resulted in an SaO2 > 50%). No rela-
tionship was found for either of HT- or PVO-CSvO2 tech-
niques; both displayed relatively homogenous 95% confidence
intervals of ±33.7% and ±30.0%, respectively (Fig. 2, middle and bottom).

Figure 3 presents Bland-Altman plots of FAVR-, HT-, and
PVO-CMRo2 compared with SS-CMRo2 (Fig. 3, A, B, and C,
respectively), with data collected at each FIO2 level. The average
differences between each NIRS-CMRo2 measurement and the
gold standard SS-CMRo2 measurement over all FIO2 levels were
−0.02 ± 0.65, −0.35 ± 1.20, and 0.11 ± 0.92 ml
O2·min−1·100 g−1 for the FAVR, HT, and PVO techniques,
respectively. Under hyperoxic and normoxic conditions, the
variation between FAVR-CMRo2 and SS-CMRo2 was consid-

Table 1. Physiological parameters at each level of
of oxygenation

<table>
<thead>
<tr>
<th>Fraction of Inspired Oxygen</th>
<th>Physiological Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% (hyperoxia)</td>
<td>21% (normoxia)</td>
</tr>
<tr>
<td>Arterial oxygen saturation, %</td>
<td>99.9 ± 0.3</td>
</tr>
<tr>
<td>Arterial PO2, Torr</td>
<td>150 ± 36</td>
</tr>
<tr>
<td>Arterial PCO2, Torr</td>
<td>38.3 ± 4.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.03</td>
</tr>
<tr>
<td>Mean arterial pressure, Torr</td>
<td>51.3 ± 12.0</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>161 ± 27</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>38.0 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SD for n = 12 piglets, except that n = 10 at the 10% fraction of inspired oxygen (FIO2) level because of the inability to sample blood from the sagittal sinus in 2 piglets. *P < 0.05 compared with corresponding 50% FIO2 value.

data sets and a positive and negative 10% error was introduced into
each of the CBV, HHb, tHb, or SaO2 measurements for the two error
data sets.

Statistical analysis. SPSS 16.0 (SPSS, Chicago, IL) was used for
all statistical analyses. Interactions between the different NIRS tech-
niques of measuring CSvO2, and CMRO2, compared with sagittal sinus
blood measurements at different levels of FIO2, were investigated by
repeated-measures ANOVAs with the level of FIO2, as the within-
subjects variable and the measurement technique as the between-
subjects variable. A straightforward statistical test could not be used
to determine statistical significance of correlations between the indi-
vidual NIRS CSvO2 measurements and corresponding SS-SvO2
measurements because there were multiple data points from the same
animals; therefore, all of the data points could not be assumed to be independent (12). To account for the difference between the number of
piglets and the number of data points, a variation of the generalized
estimating equation technique was utilized (38). First, a linear fit was
applied to the data from each piglet, individually. Second, a significant
correlation was tested by using a t-test to compare the average of the
distribution of slopes against the null hypothesis (i.e., slope = 0).
Finally, the distribution of slopes was compared with a slope of 1 to
tdetermine the agreement of the correlation with the line of identity.
All group average values in the experiment are presented as means ±
SD, and statistical significance was defined as P < 0.05.

RESULTS

Twelve piglets (5 female, 7 male) were studied with an average
age of 1.7 ± 0.3 days and an average weight of 1.63 ± 0.05 kg.
Complete data sets could not be collected from two animals; in
each case it was impossible to draw a blood sample from the
sagittal sinus catheter at 10% FIO2. Table 1 presents a summary of
the average physiological parameters of the piglets at the
three FIO2 values (50%, 21%, and 10%).

Fig. 1. Correlation between cerebral venous oxygenation calculated from each near-infrared spectroscopy (NIRS) technique and sagittal sinus oxygenation (n = 12). Data are presented for fixed arterial-to-venous blood volume ratio (FAVR)-cerebral venous oxygen saturation (CSvO2) (left), head tilt (HT)-CSvO2 (center),
and partial venous occlusion (PVO)-CSvO2 (right). There are 3 data points for each piglet at each of the 3 levels of inspired oxygen [50%, 21% and 10% fraction
of inspired oxygen (FIO2)]. The dotted lines are the lines of identity, and the solid lines are the linear regressions (FAVR-CSvO2: slope = 1.03, intercept = −3.3%,
r = 0.91, P < 0.05; HT-CSvO2: slope = 0.73, intercept = 18.3%, r = 0.73, P < 0.05; PVO-CSvO2: slope = 0.73, intercept = 12.0%, r = 0.81, P < 0.05).
of cerebral HHb concentration (obtained by second-derivative spectroscopy) averaged over all piglets under hyperoxic, normoxic, and hypoxic conditions. There were no statistically significant differences in CBF or CBV in response to changes in FIO₂ (P > 0.05); however, the larger variances in CBF values collected under normoxia and hypoxia, compared with hyperoxic values, could have masked a possible trend toward increased CBF under normoxia. Further analysis uncovered a minor but significant correlation between CBF and SaO₂ at

Fig. 2. Bland-Altman plots of NIRS-calculated cerebral venous oxygenation and sagittal sinus oxygenation (n = 12). The Bland-Altman plots of each of the correlations from Fig. 1 for FAVR-CSvO₂ (top), HT-CSvO₂ (middle), and PVO-CSvO₂ (bottom) are presented. Measurements collected under FIO₂ levels of 50%, 21%, and 10% are presented as red circles, blue triangles, and green squares, respectively. The dashed lines represent the 95% confidence intervals of the data, which are also colored to represent variance at distinct oxygenation levels.

Fig. 3. Comparison of NIRS cerebral metabolic rate of oxygen (CMRO₂) and sagittal sinus CMRO₂ (n = 12). Bland-Altman plots are shown for CMRO₂ measurements from each NIRS technique and SS-CMRO₂: FAVR (A), HT (B), and PVO (C). Measurements collected under FIO₂ levels of 50%, 21%, and 10% are presented as red circles, blue triangles, and green squares, respectively. The dashed lines represent the 95% confidence intervals of the data, which are also colored to represent variance at distinct oxygenation levels.
For example, the mean value of α at 10% FIO₂ was negative, which is physiologically impossible.

Figure 5 displays the sensitivity of the FAVR-CSvO₂ calculation to errors in its constitutive parameters at varying levels of arterial oxygen saturation. In Fig. 5A, the change in FAVR-CSvO₂ attributable to a 10% error in the arterial-to-venous blood compartment ratio (i.e., 0.15:0.85 or 0.35:0.65 vs. 0.25:0.75) is plotted against SaO₂. Under neonatal normoxic conditions (SaO₂ > 80%) (25), a 10% error in the assumed arterial-to-venous ratio resulted in an error in FAVR-CSvO₂ of no greater than 4% (i.e., FAVR-CSvO₂ = 70 ± 4%). Under hypoxic conditions (SaO₂ < 50%), the error was reduced to 2% (i.e., FAVR-CSvO₂ = 30 ± 2%). In Fig. 5B, the error in FAVR-CSvO₂ attributable to a 10% error in tHb, HHb, or CBV (see Eq. 2) is plotted against SaO₂. Under normoxic conditions, this error was associated with an error of 4% (i.e., FAVR-CSvO₂ = 70 ± 4%), while under hypoxic conditions the error due to the error was magnified to ~10% (i.e., FAVR-CSvO₂ = 30 ± 10%).

DISCUSSION

Advances in near-infrared technology have made it possible to attempt cerebral oxygen metabolism measurements in newborns at the bedside (11, 16, 29, 36). There are two key parameters that must be determined accurately to calculate CMRO₂: cerebral venous oxygenation and CBF. Our group has developed a unique NIRS technique for determining CMRO₂ by measuring both of these parameters with an intravenous bolus injection of the NIR absorber, ICG. CBF and CBV are measured by a bolus-tracking method that is based on the same methodology used to assess cerebral hemodynamics by contrast-enhanced computed tomography and magnetic resonance imaging (4, 23). Cerebral venous oxygenation is determined by first normalizing the measurement of absolute HHb concentration by the CBV to calculate average cerebral blood oxygenation. A fixed arterial-to-venous blood volume ratio is then

Table 2. Near-infrared spectroscopy and sagittal sinus blood measurements

<table>
<thead>
<tr>
<th>Fraction of Inspired Oxygen</th>
<th>50% (hypoxia)</th>
<th>21% (normoxia)</th>
<th>10% (hypoxia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral blood flow, ml·min⁻¹·100 g⁻¹</td>
<td>NIRS</td>
<td>58.0 ± 16.7</td>
<td>73.2 ± 29.2</td>
</tr>
<tr>
<td>Cerebral blood volume, ml·min⁻¹·100 g⁻¹</td>
<td>NIRS</td>
<td>4.34 ± 0.75</td>
<td>4.76 ± 1.19</td>
</tr>
<tr>
<td>Cerebral deoxyhemoglobin concentration, μM</td>
<td>NIRS</td>
<td>11.2 ± 2.7</td>
<td>19.2 ± 3.4*</td>
</tr>
<tr>
<td>CSvO₂, %</td>
<td>Blood sample</td>
<td>73.6 ± 6.1</td>
<td>57.4 ± 9.5*</td>
</tr>
<tr>
<td></td>
<td>FAVR-CSvO₂</td>
<td>70.8 ± 8.2</td>
<td>57.8 ± 9.9*</td>
</tr>
<tr>
<td></td>
<td>HT-CSvO₂</td>
<td>71.6 ± 16.3</td>
<td>60.5 ± 17.3*</td>
</tr>
<tr>
<td></td>
<td>PVO-CSvO₂</td>
<td>65.2 ± 16.0</td>
<td>56.1 ± 9.2*</td>
</tr>
<tr>
<td>CMRO₂, ml·min⁻¹·100 g⁻¹</td>
<td>Blood sample</td>
<td>1.49 ± 0.31</td>
<td>2.00 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>FAVR-CMRO₂</td>
<td>1.63 ± 0.31</td>
<td>1.96 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>HT-CMRO₂</td>
<td>1.59 ± 0.88</td>
<td>1.67 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>PVO-CMRO₂</td>
<td>1.89 ± 0.75</td>
<td>2.08 ± 0.71</td>
</tr>
<tr>
<td>α, %</td>
<td>NIRS</td>
<td>16.5 ± 12.9</td>
<td>21.8 ± 24.5</td>
</tr>
</tbody>
</table>

Values are means ± SD for n = 12 piglets, except that n = 10 at the 10% FIO₂ level because of the inability to sample blood from the sagittal sinus in 2 piglets. CSvO₂, cerebral venous oxygen saturation; CMRO₂, cerebral metabolic rate of oxygen; α, fraction of arterial blood in cerebral blood volume; NIRS, near-infrared spectroscopy; FAVR, fixed arterial-to-venous blood volume ratio; HT, head tilt; PVO, partial venous occlusion. *P < 0.05 compared with corresponding 50% FIO₂ value.

nomoxic levels (slope = -1.7 ml·min⁻¹·100 g⁻¹ per % SaO₂, r = 0.44, P < 0.05), but no correlation existed between CBF and SaO₂ under hypoxic conditions. There was, however, a stronger correlation between relative CBF (normalized to CBF measured at FIO₂ of 50%) and relative mean arterial pressure (MAP) (normalized to MAP measured at FIO₂ of 50%) under both normoxic and hypoxic conditions (Fig. 4: slope = 0.92, r = 0.6, P < 0.01). Cerebral HHb concentration increased significantly at each reduction in FIO₂ (P < 0.05).

Table 2 also includes CSvO₂ and CMRO₂ measurements from the three NIRS techniques and the average values of α (i.e., the arterial blood fraction). Values of SS-SvO₂ and SS-CMRO₂ were added for comparison (SS-CMRO₂ was calculated with the NIRS CBF measurement). No significant differences were found between any of the NIRS CSvO₂ measurements and SS-SvO₂ at any level of FIO₂ (FAVR-CSvO₂: F₁,22 = 0.294, P > 0.5, power < 0.1; HT-CSvO₂: F₁,22 = 1.200, P > 0.2, power < 0.2; PVO-CSvO₂: F₁,22 = 0.384, P > 0.5, power < 0.1) and a significant effect of FIO₂ was seen for all techniques (P < 0.01, power > 0.9). Similarly, no significant differences were found between NIRS-CMRO₂ and SS-CMRO₂ (FAVR-CMRO₂: F₁,22 = 0.001, P > 0.9, power < 0.1; HT-CMRO₂: F₁,22 = 1.834, P > 0.1, power < 0.3; PVO-CMRO₂: F₁,22 = 0.235, P > 0.6, power < 0.1). Significant interactions were observed between CMRO₂ and FIO₂ for all techniques (FAVR-CMRO₂: F₂,44 = 14.226, P < 0.01, power > 0.99; HT-CMRO₂: F₂,44 = 8.415, P < 0.01, power > 0.9; PVO-CMRO₂: F₂,44 = 6.748, P < 0.01, power < 0.8), which was driven by differences in CMRO₂ at FIO₂ of 21% and 10%. Finally, there was no statistically significant effect of FIO₂ on α, although the variance in the values was quite large.

Fig. 4. Correlation between relative cerebral blood flow (CBF) and relative mean arterial pressure (MAP) under normoxia and hypoxia (n = 12). Relative values of CBF and MAP were calculated by dividing the values at FIO₂ of 10% or 21% by the respective values at FIO₂ of 50%. The solid line depicts the linear regression (slope = 0.91, intercept = 0.15, r = 0.6, P < 0.05).
used to extrapolate CS\textsubscript{O2}. This technique has previously been validated over a range of cerebral metabolic states (32) and after hypoxia-ischemia (31) in newborn piglets. In sick newborn infants, the Sa\textsubscript{O2} can fluctuate over a wide range, despite efforts to maintain it within an accepted normoxic range. Maintaining brain oxygenation under these circumstances becomes a major challenge for neonatal intensive care (19, 25) that is compounded in the presence of hemodynamic instability. The focus of the present study was to validate the CS\textsubscript{O2} component of our CMR\textsubscript{O2} technique over a range of arterial oxygenation levels in piglets. Piglets were studied because they are a commonly used animal model of newborn human neurology (26). Two alternative NIRS techniques that have been applied clinically—the head tilt (HT) method (29) and the partial venous occlusion (PVO) method (37)—were included for comparison. The three NIRS techniques were applied to newborn piglets over a range of cerebral oxygenation levels created by altering F\textsubscript{IO2}. In addition, oxygen saturation in venous blood was determined directly from sagittal sinus blood samples. The sagittal sinus was chosen since it predominantly drains cortical regions comparable to the regions interrogated by our NIRS system (32).

Average CS\textsubscript{O2}, in this study under normoxic conditions was 57 ± 10%, falling within the large range of normal levels found in the literature. Kurth et al. (18) measured a CS\textsubscript{O2} of 49 ± 7%, Hueber et al. (13) measured a value of ~55%, and Iijichi et al. (15) measured a value of 40 ± 8%. The large range is likely to result from the use of different anesthetic regimens. A strong, statistically significant correlation was observed between FAVR-CS\textsubscript{O2} measurements and corresponding sagittal sinus O\textsubscript{2} measurements over a large range of cerebral oxygenation, although the level of agreement varied (Fig. 1, left). The correlation was demonstrated to be markedly stronger under hyperoxic, normoxic, and mildly hypoxic conditions (Sa\textsubscript{O2} > 50%) than under more severe hypoxic conditions (Sa\textsubscript{O2} < 50%). A number of potential reasons may explain the diminished precision of the FAVR-CS\textsubscript{O2} measurement at low F\textsubscript{IO2}. First, hypoxia may change the respective ratio of the arterial and venous blood volumes from the assumed ratio of 25% arterial blood and 75% venous blood. Watzman et al. (33) reported large variances (SD > 20%) in measured arterial-to-venous blood volume ratios in children with varying levels of Sa\textsubscript{O2}. The arterial blood fraction, α, was calculated in the present study from the respective ratios of Sa\textsubscript{O2} and SS-Sv\textsubscript{O2} to the NIRS-measured cerebral tissue blood oxygenation, FAVR-CS\textsubscript{SbO2} (Table 2). In agreement with Watzman et al., a considerable range in α was observed and the variance in α increased with reduced F\textsubscript{IO2}. However, we also determined that the FAVR-CS\textsubscript{O2} calculation was considerably insensitive to errors in α, particularly at lower levels of oxygenation (Fig. 5A). Therefore, errors in the assumed α value are unlikely to have caused the large errors observed in FAVR-CS\textsubscript{O2} under hypoxic conditions. Conversely, the insensitivity of FAVR-CS\textsubscript{O2} to errors in α demonstrated that small errors in tissue blood oxygenation measurements would result in very large errors in α estimations. For example, the results of Fig. 5A indicate that a 1% error in each of CS\textsubscript{bO2}, Sa\textsubscript{O2}, and SS-Sv\textsubscript{O2} measurements would cause an error in α as great as 20%. This may explain the large variances in α values reported in the present study and by Watzman et al., and it suggests that the FAVR technique is potentially a stable approach.

A second potential source of error in FAVR-CS\textsubscript{O2} measurements under hypoxic conditions is a bias in the NIRS measurement of HHb concentration, a measurement that is central to the calculation of FAVR-CS\textsubscript{O2} (Eq. 2). Klassen et al. (17) demonstrated that at F\textsubscript{IO2} levels of 9% in humans the calculated differential path length of light determined from the second-derivative technique could be overestimated by as much as 15% in the wavelength range used for calculating HHb. An overestimation of this magnitude would result in a systematic underestimation in HHb concentration of 15% and, therefore, an overestimation of FAVR-CS\textsubscript{O2}. It is possible that the slight overestimation observed in FAVR-CS\textsubscript{O2} between SS-Sv\textsubscript{O2} values of 25 and 40% (Fig. 1, left) may have been caused by this effect; however, the errors in FAVR-CS\textsubscript{O2} observed at lower oxygenations were not systematic, suggesting that the errors stemmed from more random effects.

A more likely source of the larger variance observed in FAVR-Sv\textsubscript{O2} under severely hypoxic conditions is the increased...
sensitivity of the parameter to measurement errors in CBV, cerebral Hb concentration, \(S_{aO_2}\), and \(tHb\) concentration—components of the FAVR-CSvO\(_2\) calculation—that can be observed at low \(S_{aO_2}\) (Fig. 5B). Because of the relationship between CSbO\(_2\) and \(S_{aO_2}\) in Eq. 1, the closer in value CSbO\(_2\) and \(S_{aO_2}\) become—as was the case under hypoxic conditions—the larger an effect errors in either measurement will have on FAVR-CSvO\(_2\). Since \(S_{aO_2}\), \(tHb\) concentration, \(HHb\) concentration, and CBV are all parameters contributing to this difference calculation, the cumulative effects of normal experimental errors in these parameters coupled with the increased sensitivity of the difference calculation are the most likely cause of the increased variances in FAVR-CSvO\(_2\) under hypoxia.

Unlike the FAVR-CSvO\(_2\) technique, the confidence interval of the HT and PVO methods for determining CSvO\(_2\) did not alter with level of oxygenation. However, the precision and accuracy of these techniques at all levels of oxygenation were comparable to the poorer results for the FAVR-CSvO\(_2\) technique obtained under severely hypoxic conditions (Fig. 2). Both the HT and PVO techniques are direct measures of CSvO\(_2\) (i.e., do not require a FAVR assumption) and are calculated solely on changes in the concentrations of oxyhemoglobin (\(\Delta HbO_2\)) and deoxyhemoglobin (\(\Delta HHb\)) during a blood volume manipulation. These hemoglobin measurements were determined by fitting the NIR absorption spectrum in a wavelength range not expected to be affected by hypoxia (17). Therefore, the ability to accurately measure HT- and PVO-CSvO\(_2\) is primarily dependent on the ability to cause a change in CBV with a magnitude that adequately exceeds the noise level in both \(\Delta HHb\) and \(\Delta HHb\) channels and that pertains only to the cerebral venous volume. To minimize contamination of extravascular vessel expansion during HT or PVO manipulation, the ratio of \(\Delta HHb\) and \(\Delta HHb\) from the first 5 s of manipulation was used to calculate CSvO\(_2\). This brief time period has been previously proposed to mitigate delayed compensatory changes in the arterial blood volume (37). The noise-related error source can be partially addressed by only accepting data for which a minimum change in total hemoglobin concentration (\(\Delta HbT = \Delta HbO_2 + \Delta HHb\)) was witnessed. Wong et al. (35) observed that the coefficient of correlation between PVO-CSvO\(_2\) and SS-CSvO\(_2\) improved with increasing \(\Delta HbT\) in newborn lambs. For consistency, we applied a similar threshold (\(\Delta HbT > 4 \muM\)) to the data in the present study, which improved the coefficients of correlation for the HT and PVO techniques from 0.73 and 0.81 to 0.81 and 0.84, respectively. Similarly, the slope of the correlations improved from 0.73 to 0.73 to 0.81 and 0.83, respectively. However, even with the \(\Delta HbT\) threshold, the FAVR-CSvO\(_2\) technique still outperformed the HT and PVO techniques under hyperoxic and normoxic conditions.

All NIRS-derived CMR\(_O_2\) measurements agreed well with the SS-CSvO\(_2\)-calculated CMR\(_O_2\), (Table 2). Comparing each NIRS CMR\(_O_2\) measurement to the corresponding SS-CMR\(_O_2\) measurement demonstrated that, as expected from the CSvO\(_2\) results, the FAVR technique outperformed the others under hyperoxic and normoxic conditions, while all three techniques performed equivalently under hypoxic conditions (see RESULTS). The interest in displaying the CMR\(_O_2\) data was to present the propagation of CSvO\(_2\) measurement errors into CMR\(_O_2\) measurements. For an \(S_{aO_2}\) > 50%, the 95% confidence interval of ±12.9% in FAVR-CSvO\(_2\) corresponded to a confidence interval of ±0.65 ml O\(_2\)min\(^{-1}\)-100 g\(^{-1}\) in CMR\(_O_2\), whereas for \(S_{aO_2}\) < 50%, the confidence interval of ±42.6% corresponded to a confidence interval of ±2.4 ml O\(_2\)min\(^{-1}\)-100 g\(^{-1}\).

A limitation of the present study was the absence of an expected hyperemic effect of hypoxia (28). In the present study, a correlation was observed between CBF and \(S_{aO_2}\) under normoxic conditions; however, no such effect was observed under hypoxia, and average CBF under hypoxia was similar to hyperoxic levels. One possible explanation for this absence is that the technique used to measure CBF failed under hypoxia; however, there is considerable evidence that this was not the case. The technique has been validated in three separate studies during hypercapnic hyperemia (4, 10), hypocapnia (10), and endothelin-induced cerebral ischemia (8) by demonstrating a good agreement with CBF measurements obtained with CT Perfusion (GE Healthcare, Milwaukee, WI). In turn, CT Perfusion has been validated against microspheres (5, 6, 23) and is widely used clinically. The reproducible hyperemic response to hypacapnia observed by our group and others (30) suggests that the ICG bolus-tracking technique should be adequate for detecting hyperemia resulting from hypoxia. Furthermore, ICG-based CBF techniques provide data with high signal-to-noise ratios (>100:1), demonstrating a much higher success rate than hemoglobin manipulation techniques (24), and our group has improved the reproducibility of the ICG bolus-tracking technique by using an in-house-developed deconvolution algorithm to account for indicator recirculation, resulting in a coefficient of variation of 10% in piglets (4). A more likely explanation for the absence of an observable hyperemic response to hypoxia stems from the use of the anesthetic agent isoflurane. Isoflurane is known to be a potent vasodilator (1, 14), an effect which could have masked any hyperemic stimulus from hypoxia. This explanation was corroborated by the significant correlation between CBF and MAP (Fig. 4), suggesting that the piglets were close to maximal vasodilation even under normoxia, which led to pressure-passive CBF.

In the present study, the validity of our CBF measurement under hyperoxic and normoxic levels was supported by the accuracy of the CSvO\(_2\) at these levels. The CBV measurement, which was calculated with the same theory as the CBF measurement, is an integral part of the CSvO\(_2\) measurement. The excellent correlation between the CSvO\(_2\) and the SS-CSvO\(_2\) at hyperemic, normoxic, and mild hypoxic levels suggests that the CBV, and by association the CBF measurements, were robust in this clinically relevant range (25).

In summary, this study suggests that the ICG-based technique for measuring CSvO\(_2\) and CMR\(_O_2\) is preferable to the HT and PVO techniques under hyperoxic, normoxic, and mild hypoxic conditions (\(S_{aO_2} > 50\%\)); however, under conditions of more severe hypoxia (\(S_{aO_2} < 50\%\)) the ICG-based technique tends to break down. Despite the increased variability of the FAVR CSvO\(_2\) measurements under hypoxia, this technique would appear to be well suited for clinical application since the requirement for CMR\(_O_2\) measurements during severe hypoxia may be limited. In general, newborns presenting with hypoxia would immediately be given supplemental oxygen to restore oxygenation to normal levels, with the exception of newborns with cyanotic cardiac malformations (27). The ability to quickly measure CMR\(_O_2\) may prove useful for solving the current dilemma of determining the optimal oxygenation level in premature infants (25).
GRANTS
This work was supported by the Canadian Institutes of Health Research (CIHR).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES