Experimental and theoretical comparison of NIRS spectroscopy measurements of cerebral hemoglobin changes

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Firbank, Michael, Clare E. Elwell, Chris E. Cooper, and David T. Delpy. Experimental and theoretical comparison of NIRS spectroscopy measurements of cerebral hemoglobin changes. J. Appl. Physiol. 85(5): 1915–1921, 1998.—Two near-infrared spectroscopy (NIRS) methods are available for measuring changes (Δ) in total cerebral hemoglobin concentration (CHC): 1) a continuous measurement of the changes in total hemoglobin concentration (Δ[Hb]tot) and 2) the difference between two absolute measurements of CHC, each derived from a small, controlled change in inspired O₂ fraction. This paper investigates the internal consistency of these two methods by using an experimental and theoretical comparison. NIRS was used to measure [Hb]tot in five newborn piglets before and after a change in arterial P CO₂. Over the last decade, near-infrared spectroscopy (NIRS) has found widespread application for the monitoring of tissue hemodynamics and oxygenation (7, 11, 24). Because the technique allows the continuous and noninvasive measurement of changes in concentration of oxyhemoglobin (Δ[HbO₂]), deoxyhemoglobin (ΔHb), and changes in the redox state of cytochrome oxidase, its simplest application is as a trend monitor of global tissue oxygenation status. If the Δ[HbO₂] and ΔHb signals are summed, this provides a derived measurement of changes in total hemoglobin concentration (Δ[Hb]tot), which can be used to reflect changes in blood volume.

An important step in the development of NIRS occurred when methods for the measurement of absolute hemodynamic parameters were devised. In 1988, Edwards et al. (6) described the measurement of absolute blood flow by using a small but rapid change in concentration of HbO₂ as an intravascular near-infrared dye. The technique was first applied to the measurement of neonatal cerebral blood flow and, in a subsequent validation, was shown to correlate with the xenon-clearance method (20).

By 1990, a method for the measurement of absolute cerebral hemoglobin concentration (CHC) had been described (25). This method employs a small, but this time slow, change in HbO₂ concentration and, for this reason, is usually referred to as the O₂ method for estimating cerebral blood volume (CBV). To date, independent validation of this method has not been performed, although the technique has been widely used after its initial application in neonatal medicine (15, 22, 26).

It is, therefore, possible to attempt an internal validation by comparing changes in CBV measured from the continuous Δ[Hb]tot measurement against the change calculated from two separate absolute concentration measurements (ΔCHC = CHC₂ – CHC₁). One study (2) attempted such a comparison by using CO₂-induced CBV changes in a small number of newborn infants and reported a significant difference in the values obtained by each method: 0.89 ml·100 g⁻¹·kPa⁻¹ for the absolute O₂ method vs. 0.22 ml·100 g⁻¹·kPa⁻¹ for the continuous-change measurement (Δ[Hb]tot).

The aim of the study reported here was to confirm these observations in a more controlled animal model, and to investigate, from a theoretical basis, the possible reasons for any differences. This paper will describe measurements in which a change in fraction of inspired O₂ (F IO₂) was used to measure CBV (by using the O₂ method) and a change in fraction of inspired CO₂ (FICO₂) was used to change CBV.

NIRS MEASUREMENT THEORY

In a nonscattering medium, the attenuation A of light traveling a distance d is simply given by the equation

$$A = \mu_i C_i d$$  \hspace{1cm} (1)

where each absorber i has a concentration C_i and a specific absorption coefficient µ_i.

In a scattering medium, such as biological tissue, the scattering acts to increase the path traveled, and, as shown by the diffusion Eq. 1, attenuation is no longer a linear function of the µ_i. For small changes in absorption, however, changes in attenuation can be approximated by

$$\Delta A = \Delta \mu_i C_i \beta d$$  \hspace{1cm} (2)

which gives

$$\Delta C_i = \frac{\Delta A}{\mu_i \beta d}$$  \hspace{1cm} (3)

where β is the factor by which the optical pathlength has been increased due to scattering [the so-called
Experimental Procedure

Five newborn piglets (age <24 h) were studied. Anesthesia was induced by using 5% isoflurane, mechanical ventilation was established, and the isoflurane level was reduced to 1.5%. Ventilation was maintained at FIO2 of 40% (balance N2). The optodes from a Hamamatsu NIRO 500 spectrometer were placed on either side of the head at spacings between 3.8 and 4.5 cm (Table 1). By using pulsed laser diodes at four wavelengths (779, 821, 855 and 908 nm), the spectrometer was used to measure changes in the concentration of cerebral HbO2 and Hb. For newborn piglets, a differential pathlength factor (4.57) was used to convert the data to micromolar units (R. Springett, personal communication). SaO2 and heart rate were measured by using a pulse oximeter (Novametrix 500) via a probe positioned on a skin flap on the right leg. Mean arterial blood pressure was recorded via the umbilical artery, and analog outputs from the oximeter and blood pressure transducer were linked directly to the spectrometer for display and storage alongside the NIRS data. All data were sampled every 2 s.

A computer-controlled gas blender (8) was used to supply a controlled, accurate mixture of O2, N2, and CO2 to the time-cycled ventilator (Vickers model 77) with preset pressure. Initially, FICO2 was set to 0%, and at least six CHC measurements were made by slowly varying FICO2 over several minutes. FICO2 was then increased to either 2.5 or 5%. Once a new stable baseline had been reached, the CHC measurements were repeated. Arterial blood samples were used to measure arterial PCO2 throughout the study.

Experimental Results

An example of the NIRS and SaO2 data collected during a single CHC measurement in one piglet is shown in Fig. 1. In this example, SaO2 was reduced to ~50%. However, only the initial portion of this change (in which SaO2 is >90%) is used for the calculation of CHC. As previously described, CHC is calculated from the gradient of the [Hb]diff and ΔSaO2 plot shown in Fig. 2. Δ[HB]tot is included on this plot to demonstrate that, for small changes in SaO2, the cerebral circulation is not disturbed. At least three CHC measurements were made at each of the two levels of PaCO2 for each piglet.

### Table 1. Changes measured in total hemoglobin concentration by using continuous method and the O2 method for each animal, as well as interoptode spacing and results of statistical power calculation of minimum detectable change in CHC

<table>
<thead>
<tr>
<th>Piglet No.</th>
<th>IOS, cm</th>
<th>CHC1, µM</th>
<th>CHC2, µM</th>
<th>ΔCHC, µM</th>
<th>Δ[Hb]tot, µM</th>
<th>min [ΔCHC]t0, µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.50</td>
<td>55.8±10.3</td>
<td>70.9±2.2</td>
<td>15.1±10.5</td>
<td>14.7±1.1</td>
<td>14.8</td>
</tr>
<tr>
<td>2</td>
<td>4.06</td>
<td>101.5±25.1</td>
<td>113.1±20.4</td>
<td>11.6±32.3</td>
<td>23.4±3.3</td>
<td>36.3</td>
</tr>
<tr>
<td>3</td>
<td>3.82</td>
<td>58.0±15.2</td>
<td>69.0±12.1</td>
<td>10.9±19.4</td>
<td>17.1±2.7</td>
<td>22.0</td>
</tr>
<tr>
<td>4</td>
<td>3.93</td>
<td>83.8±21.8</td>
<td>85.2±17.1</td>
<td>1.4±27.7</td>
<td>12.3±8.3</td>
<td>31.6</td>
</tr>
<tr>
<td>5</td>
<td>3.88</td>
<td>66.5±12.3</td>
<td>70.6±3.3</td>
<td>4.1±12.7</td>
<td>15.0±0.7</td>
<td>17.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. IOS, interoptode spacing; CHC, cerebral hemoglobin concentration; CHC1, CHC2, absolute concentration measurements of CHC; Δ, change in measurement; [Hb]tot, total hemoglobin concentration; min [ΔCHC]t0, minimum detectable change in CHC.
piglet. The summarized results of these measurements are shown in Fig. 3 and detailed in Table 1. The normocapnic PaCO₂ levels for each piglet ranged between 3.8 and 6.8 kPa.

The coefficient of variation for the CHC method was 22.8 ± 3.5% (mean ± SD) compared with 2.8 ± 2.8% for the [Hb]tot method. A statistical calculation (assuming a power of 90% at the 95% significance level) was performed on the CHC data to determine the minimum change in CHC which could be detected (min[ΔCHC]) under these experimental conditions. The values for this min[ΔCHC] are given in Table 1. In four of the five piglets, min[ΔCHC] was higher than the change in [Hb]tot and CHC because of alteration of PaCO₂.

**THEORETICAL MODEL**

To investigate this problem theoretically, we used diffusion theory (1, 18) to calculate the expected attenuation of light from known values of absorption and scattering of tissue and blood and also the source-detector separation. The tissue was assumed to be a uniform slab of 40-mm thickness, with an optode spacing of 40 mm (similar to that used in the experimental study). Data were calculated by using absorption and scattering properties at four wavelengths, chosen to be similar to those used by the NIRO 500. The values used for the µₐ of hemoglobin and water are shown in Table 2. Data are taken from Matcher et al. (17) for blood and from Matcher et al. (16) for water and then converted to natural log base (ln). The µₐ of blood was calculated by assuming a hemoglobin concentration in the blood of 1.68 mM (10.8 g/100 ml) and a mean saturation of 80%. To calculate blood volume, we used a saturation decrease of 3%.

The transport scattering coefficient, µₛ, is defined as µₛ (1 - g), where g is the anisotropy factor of blood. This was assumed to vary linearly with wavelength λ and was calculated from the following expression:

\[
\mu_s = \mu_{s0} + \mu_{s1} \lambda
\]

**Table 2.** Absorption coefficient values for Hb, HbO₂, and H₂O used in theoretical calculations

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>µₐ Hb (mm⁻¹·molar⁻¹)</th>
<th>µₐ HbO₂ (mm⁻¹·molar⁻¹)</th>
<th>µₐ H₂O (mm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>770</td>
<td>0.331</td>
<td>0.156</td>
<td>0.00236</td>
</tr>
<tr>
<td>825</td>
<td>0.182</td>
<td>0.237</td>
<td>0.00269</td>
</tr>
<tr>
<td>847</td>
<td>0.182</td>
<td>0.266</td>
<td>0.00378</td>
</tr>
<tr>
<td>902</td>
<td>0.207</td>
<td>0.312</td>
<td>0.00625</td>
</tr>
</tbody>
</table>

µₐ, Absorption coefficient; Hb, deoxyhemoglobin; HbO₂, oxyhemoglobin.
mated from data measured on human neonatal brain (23), with \( \lambda \) in nanometers

\[
\mu_s' = 1.0 + \frac{0.3(900 - \lambda)}{(900 - 770)}
\]

(5)

An average \( \mu_a \) for tissue was calculated by assuming a fraction of blood vessels \((f_v)\) per unit volume of tissue, using

\[
\langle \mu_a \rangle = (1 - f_v)(\mu_a^w + k) + f_v\mu_a^b
\]

(6)

where \( \mu_a^w \) is the \( \mu_a \) of blood, \( \mu_a^w \) is the absorption of water, and \( k \) is a wavelength-independent constant. For the study, calculations were made by assuming \( f_v = 0.02 \) for the low blood volume and an increase of 15%, giving the higher blood volume fraction as \( f_v \). If the difference is calculated for an initial concentration (i.e., the second concentration will be 4% too small). If the difference is calculated for an initial

\[
\Delta C = (C \cdot 0.96 \cdot 1.15) - C = 0.1C
\]

(8)

In this case, a 10% difference has been measured in instead of the actual 15%.

The change in pathlength with increased blood volume is dependent on the absorption properties of the bloodless tissue. If the background absorption of blood tissue is zero, then a 15% increase in blood volume results in a 15% increase in absorption. However, if the background absorption of the tissue is nonzero, then a change in the blood volume will have a lesser effect on the total absorption.

Figure 4 shows the theoretical estimate of the measured change in hemoglobin concentration for the two techniques as calculated for different background \( \mu_a \) (for differing values of \( k \) in Eq. 6). As expected from the above discussion, for low background absorption, the \( \Delta \text{CHC} \) method underestimates the real value. As the background absorption increases, the continuous \( \Delta [\text{Hb}]_{\text{tot}} \) method gives smaller values.

Effect of \( \mu_s \)

As an example of the effect of a change in \( \mu_s \), Fig. 4 also shows the effect on the blood volume measurements of a 1% drop in \( \mu_s \) simultaneous with an increase in blood volume.

Blood Vessel Diameter

Figure 5 shows the effect on the \( [\text{Hb}]_{\text{tot}} \) measurements of restricting the change in volume to blood vessels of different diameters. As blood becomes concentrated in fewer, larger vessels, the observed concentration change, as measured by both the \( \Delta \text{CHC} \) and \( \Delta [\text{Hb}]_{\text{tot}} \) methods, decreases.

Movement of Optodes

Figure 6 shows the effect of a \( \pm 1 \)-mm optode movement on the measurement of \( \Delta [\text{Hb}]_{\text{tot}} \) and \( \Delta \text{CHC} \) for a
fixed change in blood volume of 15%. This model predicts that optode movement will have a greater effect on the $[\text{Hb}]_{\text{tot}}$ than on the CHC signal; i.e., with zero optode movement, $D[\text{Hb}]_{\text{tot}} = 15\%$ and $D\text{CHC} = 10\%$; however, in the presence of optode movement of 1 mm, the observed $D[\text{Hb}]_{\text{tot}} = 29\%$, whereas the CHC signal will still show a change of 10%. These data have been calculated by assuming a movement of the optodes occurring between measurement of volumes but also assuming that the optode positioning is constant during any given CHC measurement.

**DISCUSSION**

**Experimental Results**

It can been seen clearly that the variability of the CHC values is far greater than the variability of the $D[\text{Hb}]_{\text{tot}}$ data. By using the CHC values obtained at the lower CO$_2$ levels, the sensitivity of the measurement was calculated and was found to be inadequate to accurately resolve the changes in CHC caused by the increase in FICO$_2$. For the data presented, although $\Delta[\text{Hb}]_{\text{tot}}$ always shows an increase with FICO$_2$, CHC does not show a significant change.

The calculation of CHC in Eq. 4 involves finding the regression between $([\text{HbO}_2] - [\text{Hb}])$ and $D\SaO_2$. To avoid changes in blood volume and flow, the changes of $\Delta\SaO_2$ are usually restricted to $<10\%$. These changes are measured by using pulse oximeters with typical accuracy of $\pm 2\%$ (21). Therefore, it is likely that the error on the SaO$_2$ reading may be responsible for a large degree of the noise in the CHC measurement. A further problem can be the limited analog-output step size of pulse oximeters, because some output data only in steps of 1%.

The noise in the measurement of [Hb]$_{\text{diff}}$ is approximately the same as that for measurement of [Hb]$_{\text{tot}}$. However, in the conversion to an absolute concentration, the former is divided by a small factor ($\Delta\SaO_2$) which acts to multiply the noise. This is further compounded by errors in the measurement of SaO$_2$.

Unfortunately, although greater SaO$_2$ changes would potentially lead to more accurate estimations of blood volume, a large change in SaO$_2$ might also result in physiological effects, such as changes in blood volume or flow, which would invalidate the basis of the measurement. Also, for clinical use of the technique, there are obvious limits on the change in FICO$_2$ which can be used without compromising the patient’s health.

Intersubject variability in DPF has been shown to be on the order of 12–17% (5). However, in these studies, DPF acts purely as an equal scaling factor on both the $\Delta\text{CHC}$ and $D[\text{Hb}]_{\text{tot}}$ data to convert the units from micromoles/centimeter to micromoles and, as such, will not contribute to the discrepancy shown between the two measurements.

**Theoretical Results**

The effect of background tissue absorption can be understood by considering the fact that, when blood volume increases, the tissue volume sampled by the light decreases. Hence, any attenuation caused by tissue absorption will decrease. This acts to lessen the overall increase in attenuation with blood volume and will thus tend to lead to an underestimate of the hemoglobin concentration change. Increasing the number of wavelengths used would make the fitting to the Hb spectral shape more accurate and thus decrease this error (17).

The theoretical modeling of changes in $\mu_s$ demonstrates a significant effect on the measurement of CHC. However, it is difficult to think of physiological circumstances under which such a change in $\mu_s$ might be observed. It would seem unlikely that the $\mu_s$ of tissue would change by more than a fraction of a percent under normal physiological circumstances. The overall $\mu_s$ might vary as the blood volume changes, due to a change in the $\mu_s$ of blood. Kienle et al. (12) measured
the anisotropy factor of blood g at 820 nm as 0.993 and its $\mu_s$ as 5.5/mm for a hematocrit of 0.01 (which agrees well with Mie theory calculations for red blood cells). This corresponds to a $\mu_s$ of 134/mm for a hematocrit of 0.41 and a $\mu_s$ of 0.94/mm. Because the transport $\mu_s$ of neonatal brain tissue is ~1.0/mm (23), the change in $\mu_s$ due to a small increase in blood volume will be negligible. Changes in scattering caused by the depolarization of neurons both in the normal brain during cortical activation and under pathological conditions of stroke are presently the subject of much investigation (3, 9). Typically the magnitude of any changes seen under these conditions is very small, particularly compared with changes resulting from a change in $\mu_s$; hence, such changes would also have a negligible effect on the described model.

There is some uncertainty in the value of g for blood, and since it is so close to 1.0, uncertainty in g will lead to large uncertainty in $\mu_s$. However, because a blood volume increase of 15% is only 0.3% of the total (~2%) volume, the maximal decrease in scattering would be 0.3% if blood were nonscattering. A 1% increase in scattering could be caused only if blood had a $\mu_s$ 5 times that of the surrounding tissue, which is clearly not the case.

As expected, the modeling of the effect of vessel diameter demonstrates that the measured hemoglobin concentration change decreases as the blood becomes concentrated in fewer, larger vessels. However, because the majority of vessels inside the brain are <0.2-mm diameter, this is unlikely to have a major overall effect. It is possible, however, that a significant change in blood volume confined to the larger pial arteries and veins on the brain surface could lead to an underestimate of the hemoglobin concentration change.

The effect of optode movement is clearly more significant in the measurement of $\Delta[Hb]_{tot}$ than $\Delta CHC$. In practice, it is probable that the optodes will be subject to continuous small random movements, which will give rise to greater noise and uncertainty in the measurement. This effect is likely to be larger for the CHC measurement, for the reasons previously stated, because $\Delta CHC$ is derived from a difference of two measurements.

In conclusion, the measurement of absolute hemoglobin concentration has been shown to be inherently more liable to noise than measurements of changes in hemoglobin concentration.

The absolute value of CHC measured with this technique is dependent on the $\mu_s$ of the tissue surrounding the blood vessels. The $\Delta[Hb]_{tot}$ measurement is less sensitive to the background $\mu_s$, but it is affected more by any changes that occur in the $\mu_s$ of the tissue. Measures which might lead to an improved understanding of the CHC data would be 1) to monitor the optical pathlength during the experiment, 2) to improve the accuracy of the pulse oximetry, and 3) to use more wavelengths to improve fitting to the hemoglobin absorption spectra.

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