

QUANTIFICATION OF ADULT CEREBRAL BLOOD VOLUME USING THE NIRS TISSUE OXYGENATION INDEX

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1. INTRODUCTION

Near-infrared spectroscopy (NIRS) is increasingly used as a non-invasive technique for monitoring cerebral oxygenation and haemodynamics^{1, 2}. Simple continuous-wave (CW) NIRS systems utilising differential spectroscopy can measure quantitative changes in oxy- and deoxy- haemoglobin ($\Delta[\text{O}_2\text{Hb}]$, $\Delta[\text{HHb}]$) but only from an arbitrary baseline. Numerous studies of changes in cerebral oxygenation and haemodynamics in adults have been published but only few absolute quantitative measurements have been reported. Recent advances in the NIRS technology have enabled quantitative assessment of haemoglobin concentration in tissue using near-infrared (NIR) phase and time resolved systems; and absolute measurements of tissue saturation using phase, time or spatially resolved spectroscopy (SRS) systems^{3, 4, 5, 6}.

This paper suggests a way to use a commercially available spectrometer, which has both CW and SRS capabilities in order to measure absolute tissue haemoglobin (Hb_{tc}) and hence cerebral blood volume (CBV). The methodology is based on that of Wyatt et al.⁷ who developed a method for measuring absolute CBV, using NIRS measurements during controlled changes in inspired O_2 fraction. By using NIRS measured tissue $\Delta[\text{O}_2\text{Hb}]$ and comparing it to changes in arterial saturation (SaO_2) measured with a pulse oximeter it is possible to calculate absolute Hb_{tc} concentration. This is the so-called ‘desaturation method’ or ‘ O_2 method’ or ‘ SaO_2 method’^{8, 9, 10, 11}. The purpose of the present study was to compare measurements of CBV made using the conventional ‘ SaO_2 method’ with those using a new method employing the SRS derived absolute cerebral tissue oxygenation index (TOI), which will be called the ‘TOI method’.

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2. THEORY

2.1 Hb_{tc} Calculation

The theory for absolute quantification of Hb_{tc} relies upon the induction of a small change in the inspired oxygen. During the manoeuvre the consequent change in cerebral $\Delta[\text{O}_2\text{Hb}]$ is equivalent to the product of the Hb_{tc} and the change in fractional tissue saturation. If CBV is constant then $\Delta[\text{Hb}_{\text{diff}}]$ (i.e. $\Delta[\text{O}_2\text{Hb}] - \Delta[\text{HHb}]$) can be used to derive Hb_{tc}. The 'SaO₂ method' uses a CW NIRS system to monitor the changes in $\Delta[\text{O}_2\text{Hb}]$ and $\Delta[\text{HHb}]$ and a pulse oximeter to measure SaO₂. If cerebral blood flow (CBF), CBV and O₂ consumption remain constant during the manoeuvre the ΔSaO_2 measurement can be related to the tissue saturation. Therefore absolute Hb_{tc} can be obtained using Eq. (1).

Instead of using ΔSaO_2 as an indicator of tissue saturation one can use a direct measurement of tissue saturation, which modern NIRS systems measure. One such system the Hamamatsu NIRO 300 utilises the SRS technique, in which multiple closely separated detectors measure the attenuation slope¹². From these measurements it is possible to calculate scaled absolute haemoglobin concentrations and hence accurately obtain a tissue oxygenation index (TOI) as $k[\text{O}_2\text{Hb}]/[k\text{O}_2\text{Hb} + k\text{HHb}] * 100\%$ where k is a scaling factor dependent upon the tissue scattering coefficient (μ_s). TOI is a measure of oxygen saturation in tissue; therefore one can use Eq. (2) to measure absolute Hb_{tc}.

$$(1) \text{ Hb}_{\text{tc}} (\mu\text{moles/l}) = \frac{\Delta[\text{Hb}_{\text{diff}}]}{2 \cdot \Delta\text{SaO}_2} \quad (2) \text{ Hb}_{\text{tc}} (\mu\text{moles/l}) = \frac{\Delta[\text{Hb}_{\text{diff}}]}{2 \cdot \Delta\text{TOI}}$$

2.2 CBV Calculation

It is important to remember that NIRS measures change in chromophore concentrations in micromolar units. Estimates of blood volume are obtained from these measurements by converting the concentration data into the more conventional clinical units of millilitres/100 grams (see Eq. (3)). This conversion requires knowledge of the red blood cell concentration, which is measured from a venous sample.

$$(3) \text{ CBV}(\text{ml}/100\text{g}) = \frac{[\text{Hb}_{\text{tc}}] \cdot \text{MW}_{\text{Hb}} \cdot 10^{-4}}{d_t \cdot [\text{Hb}_t \cdot 10^{-2}] \cdot \text{CLVHR}}$$

where $\text{MW}_{\text{Hb}}=64500$ is the molecular weight of haemoglobin, $d_t=1.05\text{g/ml}$ is the cerebral tissue density, Hb_t (g/dl) is the haemoglobin concentration obtained from a venous sample, and $\text{CLVHR}=0.69$ is the cerebral to large vessel haematocrit ratio¹³.

3 PARTICIPANTS AND PROCEDURE

3.1 Participants

Data were recorded during three consecutive graded arterial hypoxaemias in 12 healthy volunteers of mean \pm SD age 32 ± 4 years (the local ethics committee approved the protocol for the study, and all subjects gave informed consent for participation).

3.2 Instrumentation

A continuous wave near-infrared spectrometer (NIRS), with a sampling rate of 6Hz (NIRO 300, Hamamatsu Photonics KK) was used to measure absolute cerebral TOI over the frontal cortex using the SRS technique¹², together with changes in $\Delta[\text{O}_2\text{Hb}]$ and $\Delta[\text{HHb}]$ by using the modified Beer-Lambert law¹⁴. The optodes were placed on the forehead (taking care to avoid the midline sinuses) and were shielded from ambient light by using an elastic bandage and a black cloth. An optode spacing of 4cm was used and optical attenuators were used where necessary to optimise the signal to noise ratio. The differential pathlength factor (DPF) applied was 6.26¹⁵.

A modified anaesthetic machine supplied a controlled mixture of nitrogen and oxygen to the subject via a face mask. Arterial saturation was monitored from the ear with a pulse oximeter (Novametrix 500) modified to measure in a beat-to-beat mode. Inspired oxygen (FiO_2) was monitored continuously via the Merlin modular monitor (Hewlett Packard). A Portapres® system (TNO Institute of Applied Physics), was used to continuously and non-invasively measure blood pressure from the finger.

3.3 Procedure

All measurements were made with the volunteers sitting comfortably in an armchair. The subject initially breathed normal air for 3-5min. The FiO_2 in the circuit was then gradually reduced to 10-15% by mixing air and nitrogen, until a baseline SaO_2 of 90% was achieved. At that point the subject was given 100% O_2 for five consecutive breaths. This was repeated a total of three times for each subject (see Fig 1).

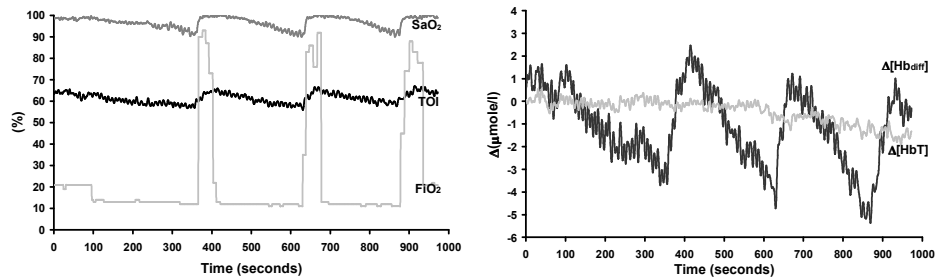


Figure 1. Representative data collected from one volunteer during the three hypoxic episodes. For illustrative purposes data has been filtered to remove heart rate oscillations.

3.4 CBV Measurement

Software has been written in MatLab to calculate absolute CBV using each episode of hypoxaemia. We first set a baseline range for the changes in cerebral total haemoglobin ($\Delta[\text{HbT}] = \Delta[\text{O}_2\text{Hb}] + \Delta[\text{HHb}]$). Within this range, the software identifies the corresponding $\Delta[\text{Hb}_{\text{diff}}]$ and TOI and calculates the absolute cerebral Hb_{tc} from the slope of the plot of these variables using linear regression. With a known haemoglobin concentration (g/dl) from a venous blood sample, the absolute cerebral Hb_{tc} is converted into absolute CBV.

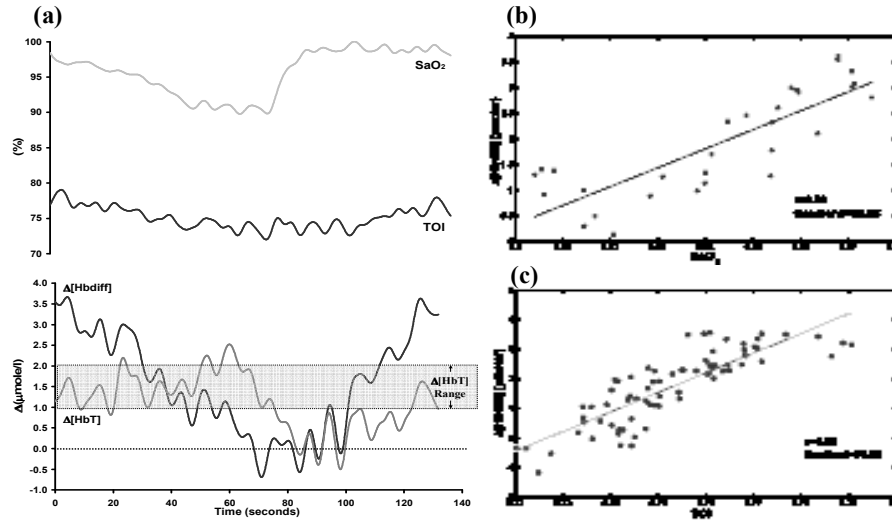


Figure 2. Typical single hypoxia analysis for one volunteer. (a) Changes in SaO₂, TOI, Hb_{diff} and HbT after smoothing to remove cardiac oscillations, a baseline range of the changes in HbT is set, within this range, the software identifies the corresponding [Hb_{diff}], SaO₂ and TOI data points. (b) For the ‘SaO₂ method’ using the pulse oximeter measured SaO₂ a correction has to be made for any temporal differences and only the de-saturation period is considered for analysis, the software calculates the absolute cerebral Hb_c from the slope of the plot of Δ[Hb_{diff}] against Δ[SaO₂] using linear regression. (c) For TOI analysis all the hypoxic swing data is considered and the software calculates the absolute cerebral Hb_c from the slope of the plot of Δ[Hb_{diff}] against Δ[TOI] using linear regression.

3. RESULTS

From a total of 36 hypoxic swings only 21 measurements (9 volunteers) proved suitable for calculation of CBV using the ‘SaO₂ method’ and 27 measurements (10 volunteers) proved suitable for calculation of CBV using the ‘TOI method’. A criterion for rejection was the linearity correlation coefficient *r*. If *r* was less than 0.8, that hypoxic swing was excluded from the analysis.

The mean ±SD CBV was 2.23±1.06ml/100g using the ‘SaO₂ method’ and 2.62±0.61ml/100g using the ‘TOI method’. For all the 36 hypoxic swings the intra-subject coefficient of variation using the ‘SaO₂ method’ ranged from 12 to 83% (mean 35%) and using the ‘TOI method’ ranged from 3 to 21% (mean 11%).

Table 1. Summary of results (mean values ±SD).

	CBV±SD (ml/100g)	Hb _{tc} ±SD (μmoles/l)	Inter-subject Coefficient of variation
‘SaO ₂ method’ (n=21)	2.23±1.06	38.4±18.6	48%
‘TOI method’ (n=27)	2.62±0.61	45.0±10.8	23%

4. DISCUSSION

This study has described an alternative method for measuring absolute Hb_{tc} through the use of a single NIRS system capable of both differential and SRS spectroscopy measurements and we have compared this methodology with the previously described 'SaO₂ method'. The absolute Hb_{tc} and CBV measurements calculated here are comparable with previously published data^{8, 16}. Most importantly we have shown that using the 'TOI method', the data is more robust with lower intra-subject and inter-subject coefficients of variation.

The limitations of the 'SaO₂ method' have been described elsewhere¹⁷ the most important being the low sampling rate and instrumental noise of the pulse oximeter. Limitations of NIRS include the inter-subject variability in DPF which has been shown to be on the order of 12-17%¹⁵. However, in this study, DPF acts purely as an equal scaling factor on the $\Delta[Hb_{diff}]$ and hence Hb_{tc} data to convert the units from micromoles/centimeter to micromoles and as such will not contribute to the discrepancy between the two measurements. There is also criticism regarding the tissue specificity of the NIRS instrumentation. For single optode measurements the influence of the extracranial compartment is inevitable. In a recent study Choi and colleagues⁶ reported significant differences in the absolute haemoglobin measurements of the different layers of the adult head (scalp/skull and brain) using an NIRS multi-distance frequency domain method. NIR interrogates the whole head including scalp, skull and brain resulting in an averaged blood volume, which may be lower than in the brain itself. We are currently investigating this effect using a multi-layer model.

The estimated mean CBV is smaller than that obtained from positron emission tomography (PET)¹⁸ or single-photon emission-computed tomography (SPECT)¹⁸ studies. A further possible error in the calculation of CBV is the CLVHR where there are some differences in the literature values. Lammertsma et al.¹³ measured the cerebral-to-large vessel haematocrit ratio as 0.69, whereas Sakai et al.¹⁹ found a value of 0.76. The value measured depends on the distribution of vessel sizes considered. Sakai et al.¹⁹ also found that the haematocrit may change with blood volume.

The ideal method of measuring CBV would be accurate over a broad range of haemodynamic conditions, reproducible, sensitive to changes in physiologic or pathologic states, non-invasive, and readily incorporated into current clinical protocols. Unfortunately none of the current methods for measuring CBV are satisfactory. CBV may be measured in human subjects by using PET or SPECT. These methods require the use of radioisotopes and are not bedside tests. Magnetic resonance imaging (MRI) can also be used, however the long scan times of equilibrium T1-weighted MRI imposes a major limitation and typical clinical scans using T2-weighted, gradient echo MRI yield only relative CBV changes²⁰. NIRS, which was first described in 1977²¹ has been used to monitor changes in $\Delta[HbT]$ on an arbitrary scale, but recent NIRS developments enable absolute measurements of tissue saturation and Hb_{tc} to be made. These methods avoid the use of radioisotopes and X-rays and may be performed at the bedside.

In the current study we have demonstrated an alternative method, which we call the 'TOI method' to calculate absolute Hb_{tc} and CBV using a single NIRS instrument during small hypoxic swings. We conclude that the 'TOI method' is better than the previous used 'SaO₂ method' with the major advantage being the use of a single system.

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