Simultaneous pO₂ and HbO₂ measurement to determine absolute HbO₂ concentration for *in vivo* near infrared spectroscopy

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Abstract. *Purpose:* Near infrared spectroscopy (NIRS) monitors absolute changes in concentration of chromophores from an unquantified baseline. A change in oxygenation is required to obtain information. Clinically, knowing initial chromophore concentration is desirable. We sought absolute initial concentrations by combining NIRS measurements with simultaneous Eppendorf histograph measurement (absolute values of tissue partial pressure of oxygen (pO₂)). *Methods:* There were 22 trials on 9 occasions in 2 adult volunteers. Following local anaesthetic, the histograph probe was inserted into the forearm muscle and NIRS optodes applied. A sphygmomanometer cuff was inflated over the upper arm to produce ischaemia and hypoxia. Change in pO₂ was determined as a percentage of the initial pO₂. Change in HbO₂ was measured, and the initial HbO₂ calculated by assuming the percentage change in HbO₂ equaled the percentage change in pO₂. *Results:* Mean percentage change in pO₂ was 20.7 ± 9.6 . Mean change in HbO₂ was $22.4 \pm 3.4 \mu\text{mol/l(tissue)}$. Calculated initial HbO₂ was $146.4 \pm 108.7 \mu\text{mol/l(tissue)}$, range 51 to 508 $\mu\text{mol/l(tissue)}$. Correlation (r^2) between pO₂ and HbO₂ was 0.97 ± 0.03 . *Conclusion:* Although changes in pO₂ and HbO₂ were highly correlated, calculation of initial absolute concentration of HbO₂ was not achieved by this method. A reliable method of obtaining absolute concentrations of HbO₂ *in vivo* remains to be found.

Keywords: Near infrared spectroscopy, histography, oxygen consumption, forearm

1. Introduction

Near infrared spectroscopy (NIRS) is an emerging technology that provides continuous, non-invasive, bedside measurement of absolute change in concentration of both oxygenated and deoxygenated haemoglobin, and change in the redox status of cytochrome aa₃ [2,6,9], the terminal enzyme in the electron transfer chain. When NIRS reaches its potential, it will provide a wealth of information on cerebral hemodynamics and energy utilization at the cellular level in the brain. It will also allow us to rationally define our clinical management protocols in a wide range of conditions including birth asphyxia, prematurity, traumatic head injury, near-drowning, and cardiopulmonary bypass [7]. However, techniques still need to be developed which will allow comparison of measurements on repeat testing of single subjects and between individuals. To achieve this goal, it is necessary to determine the absolute concentrations of oxygenated and deoxygenated haemoglobin. Others have used the second derivative of the water spectrum in the near infrared region to calculate absolute deoxy-haemoglobin concentration [3], but the calculation is based on an assumed water concentration in the brain of 85%, when water concentration varies between 80 and 90%.

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The Eppendorf histogram is the gold standard for measuring tumor oxygenation [4]. It is a mildly invasive monitoring tool that gives a direct measurement of tissue pO₂ obtained via microelectrodes contained in a stainless steel tissue probe. The current that flows between the electrodes is used to calculate the positive pressure of oxygen. This permits direct determination of absolute oxygen tensions *in situ* [8]. The volume of tissue being assessed is approximately 30 cells.

We performed a study to determine whether the data obtained from the Eppendorf pO₂ Histogram could be combined with NIRS data to calculate absolute initial values for the concentrations of oxy and de-oxyhaemoglobin.

2. Method

This was a prospective observational study. Subjects were healthy adult males. The study was approved by the University of British Columbia Human Research Ethics Committee and subjects provided informed consent.

NIRS optodes were placed 30 mm apart on the volar aspect of the forearm of healthy adult males.

The Eppendorf system was calibrated using 0.9% saline solution equilibrated with air or 100% nitrogen. After instilling 1% local anaesthetic, a 21 gauge scalp vein needle was inserted subcutaneously 50 mm lateral to the optodes and an Eppendorf pO₂ tissue probe was introduced into the tissues via the lumen of the scalp vein needle. The histogram reference electrode was secured over the palmar aspect of the wrist.

Four periods of ischaemia were generated using a sphygmomanometer cuff inflated to 30 mm above systolic blood pressure to achieve occlusion of blood flow through the upper arm. Each period lasted 4 minutes and was followed by 6 minutes of recovery with the cuff deflated. The histogram probe remained static within the tissue (it normally advances automatically through the tissue but this feature was overridden). The pO₂ values were recorded at 10-second intervals and NIRS data at 2-second intervals.

2.1. Derivation of absolute HbO₂

Since the patterns of change in HbO₂ were nearly identical to those in pO₂, we assumed that the percentage change in pO₂, an absolute value, was equal to the percentage change in HbO₂. Since the absolute value of change in concentration in HbO₂ is known from NIRS, we were able to calculate the initial concentration of HbO₂ as shown in this example:

Example: Given

- a 15% decrease in pO₂ during occlusion,
- a relative value of +5.0 μmol/l HbO₂ above baseline at the start of occlusion, and
- a relative value of –20.0 μmol/l HbO₂ (below baseline) at the end of occlusion,

we can see that the total change of 25 μmol/l HbO₂ is a 15% change. Thus:

$$\frac{\Delta \text{HbO}_2 \text{ during occlusion}}{\% \text{ change in pO}_2} = \frac{\text{HbO}_2 \text{ at start of occlusion}}{100\%}$$

yields:

$$\frac{25}{15} = \frac{\text{HbO}_2 \text{ at start of occlusion}}{100}.$$

Fig. 1. Overlay of HbO₂ decline and pO₂ decline during ischemia.

Therefore:

$$\text{HbO}_2 \text{ at start of occlusion} = 25 \cdot 100/15 = 166.7 \mu\text{mol/l.}$$

Once the occlusion starting HbO₂ value was known the absolute values of HbO₂ concentrations could be calculated for the remaining data:

$$\begin{aligned} \text{initial HbO}_2 &= 166.7 \mu\text{mol/l,} \\ \text{baseline HbO}_2 &= 166.7 - 5.0 = 161.7 \mu\text{mol/l,} \\ \text{final HbO}_2 &= 166.7 + (-20 - 5.0) = 141.7 \mu\text{mol/l.} \end{aligned}$$

The HbO₂ values at the beginning and end of each subsequent ischaemic event were determined two ways:

- (1) using the baseline value calculated from the first ischaemic event,
- (2) for each event individually.

The percent change in HbO₂ was then compared to the percentage change in pO₂ for each ischaemic event.

3. Results

Twenty-two paired data sets were obtained. The rates of change of the parameters during ischaemia were

Table 1
Calculated values of HbO₂ at start and end of 19 ischaemic events

Subject	Start pO ₂ (mmHg)	End pO ₂ (mmHg)	ΔpO ₂	ΔpO ₂ (%)	Start HbO ₂ (μmol/l)	End HbO ₂ (μmol/l)	ΔHb	Baseline HbO ₂ (calc.)
Histog 2	60.5	50.1	10.4	17.2	-1.7	-26.3	24.6	143.0
	54.8	50.7	4.1	7.5	-2.5	-24.9	22.4	298.7
	56.4	43.8	12.6	22.3	5.4	-20.7	26.1	117.0
	50.5	35.2	15.3	30.3	-0.7	-24.2	23.5	77.6
Histog 3	66.9	47.4	19.5	29.1	-4.7	-30.2	25.5	87.6
	51.3	40.6	10.7	24.5	-3.8	-26.6	22.8	131.8
	53.8	43.5	10.3	25.5	-11.8	-27.1	15.3	92.7
	49.4	45.4	4	8.1	-10.8	-32.0	21.2	263.0
Histog 4	62.9	53.0	9.9	15.7	7.2	-9.9	17.1	130.8
	58.1	48.4	9.7	16.7	14.1	-11.7	25.8	164.3
Histog 5	57.3	38.3	19	33.2	-2.9	-22.3	19.4	61.4
	51.8	31.7	20.1	38.8	0.4	-21.3	21.7	59.3
	47.2	30.5	16.7	35.4	-2.0	-19.4	17.4	51.6
	39.8	25.2	14.6	36.7	-6.9	-24.4	17.5	50.7
Histog 6	59.7	42.1	17.6	29.5	-2.0	-23.9	21.9	89.8
	56.0	38.2	17.8	31.8	9.7	-12.3	22	65.0
Histog 7	47.9	41.1	6.8	14.2	-8.1	-27.5	19.4	147.0
	43.9	36.7	7.2	16.4	0.5	-25.2	25.7	154.8
Histog 8	60.9	56.3	4.6	7.5	-6.9	-35.3	28.4	326.7
	71.6	67.6	4	5.6	-7.0	-31.5	24.5	508.1
Histog 9	50.5	38.6	11.9	23.6	2.6	-20.8	23.4	99.1
	41.5	36.4	5.1	12.3	0.8	-25.5	26.3	97.7

HbO₂ -6.82 ± 0.73 μmol/l/min,
Hb +6.99 ± 0.83 μmol/l/min,
HbSum +0.02 ± 0.82 μmol/l/min,
pO₂ -3.32 ± 0.99 mm Hg/min.

The patterns of change in HbO₂ and pO₂ were nearly identical (Fig. 1), and were highly correlated (mean r^2 for ischaemic events was 0.963 ± 0.029).

The percentage change for HbO₂ and pO₂ as well as absolute values for baseline HbO₂, calculated as described above, are shown in Table 1. Calculated absolute value of HbO₂ prior to ischaemic events ranged from 50.7 to 508.1 μmol/l. Note that the "initial" HbO₂ values calculated for the same individual on the same occasion varied widely, although neither the optodes nor the probe had been moved.

4. Discussion

The rates and magnitudes of change of Hb, HbO₂ and pO₂ were consistent and reproducible. There was a minor shift in NIRS baseline between ischaemic events during a given session, perhaps due to incomplete recovery from ischaemia.

Fig. 2. Comparison of absolute change in HbO₂ and percent change pO₂ by event, showing change in HbO₂ to be more consistent than change in pO₂.

Histography and NIRS measure different points in the oxygen utilization cycle but probably these points are temporally close to one another. The patterns of the curves of decline of HbO₂ and pO₂ appeared identical. While this could be a chance finding, the apparent similarity of the rate of decline of tissue pO₂ and HbO₂ could imply that the instruments are both measuring the relative availability of oxygen: NIRS via oxygen transfer from haemoglobin and histography via a change in oxygen concentration within the tissue. More importantly, the patterns of change in HbO₂ and pO₂ are virtually identical in our study, which implies that the two methods are capable of comparable measurements of oxygenation. Furthermore, because these similarities occur we propose that an absolute value of HbO₂ could be derived from the change in tissue pO₂, an absolute measurement, if NIRS and histograph measurements are made simultaneously, but that this cannot be achieved by the method described here. It may be necessary to consider the oxygen dissociation curve in the analysis, the histograph may sample too small a volume to be reliable for comparison with the NIRS, or over-riding the advance of the probe may have resulted in faulty pO₂ data.

5. Conclusions

1. This is the first report of simultaneous measurements of HbO₂ by NIRS and pO₂ by histography. We show comparable rates of decrease of tissue oxygenation during 4 minute periods of ischemia.

Fig. 3. Absolute change in pO₂ vs absolute change in HbO₂.

2. HbO₂ cannot be quantified using a linear correlation for calculation of an absolute value from the percentage change of pO₂.

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