

Investigation of *in-vivo* measurement of cerebral cytochrome-*c*-oxidase redox changes using near-infrared spectroscopy in patients with orthostatic hypotension.

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Abstract

We have previously used a continuous four wavelength near infrared spectrometer to measure changes in the cerebral concentrations of oxy- ($\Delta[\text{HbO}_2]$) and deoxy- haemoglobin ($\Delta[\text{HHb}]$) during head-up tilt in patients with primary autonomic failure. The measured changes in light attenuation also allow calculation of changes in the concentration of oxidised cytochrome c oxidase ($\Delta[\text{oxCCO}]$), and this paper analyses the $\Delta[\text{oxCCO}]$ during the severe episodes of orthostatic hypotension produced by this experimental protocol. We studied 12 patients during a passive change in position from supine to a 60° head-up tilt. The challenge caused a reduction in mean blood pressure of 59.93 (± 26.12) mmHg (Mean (\pm SD), $p < 0.0001$), which was associated with a reduction in the total concentration of haemoglobin ($\Delta[\text{HbT}] = \Delta[\text{HbO}_2] + \Delta[\text{HHb}]$) of 5.02 (± 3.81) μM ($p < 0.0001$) and a reduction in the haemoglobin difference concentration ($\Delta[\text{Hb}_{\text{diff}}] = \Delta[\text{HbO}_2] - \Delta[\text{HHb}]$) of 14.4 (± 6.73) μM ($p < 0.0001$). We observed a wide range of responses in $\Delta[\text{oxCCO}]$. 6 patients demonstrated a drop in $\Delta[\text{oxCCO}]$ ($0.17 \pm 0.15 \mu\text{M}$); 4 patients demonstrated no change ($0.01 \pm 0.12 \mu\text{M}$) and 2 patients showed an increase in $\Delta[\text{oxCCO}]$ ($0.21 \pm 0.01 \mu\text{M}$). Investigation of the association between the changes in concentrations of haemoglobin species and the $\Delta[\text{oxCCO}]$ for each patient show a range of relationships. This suggests that a simple mechanism for crosstalk, which might produce artefactual changes in $[\text{oxCCO}]$, is not present between the haemoglobin and the oxCCO NIRS signals. Further investigation is required to determine the clinical significance of the changes in $[\text{oxCCO}]$.

Keywords: near-infrared spectroscopy, cytochrome c oxidase, cerebral oxygenation, autonomic failure

Introduction

Cytochrome c oxidase (CCO) is the final electron acceptor of the mitochondrial electron transfer chain and catalyses the metabolism of molecular oxygen. It is responsible for > 95% of the oxygen consumption in the body (Richter and Ludwig 2003) and drives adenosine triphosphate (ATP) synthesis. The enzyme contains four redox active metal centres (heme a, heme a₃, copper A and copper B). The copper A (Cu_A) redox centre of CCO has a distinct redox sensitive absorbance band in the near-infrared spectrum between 700-1000 nm; if one assumes that the total concentration of CCO does not change within the experimental time period then this allows changes in its redox state to be detected non-invasively using *in-vivo* near-infrared spectroscopy (NIRS) (Jobsis 1977, Jobsis *et al* 1977, Beinert *et al* 1980).

The technique of cranial *in-vivo* NIRS was first described by Jobsis (Jobsis 1977) and is based on the relative transparency of tissue to near-infrared light between 700-1000 nm. Within this wavelength range, optical attenuation depends on the concentrations of light absorbing chromophores including oxygenated haemoglobin ([HbO₂]), deoxygenated haemoglobin ([HHb]) and oxidised cytochrome c oxidase ([_{ox}CCO]). Changes in attenuation can be converted into changes in chromophore concentration, thus providing continuous non-invasive measurement of changes in tissue oxygenation and metabolism.

NIRS measurements of Δ [_{ox}CCO] have the potential to provide a unique method for measuring changes in the intracellular redox state, both non-invasively and with good time resolution. The clinical relevance of changes in the redox state of CCO has been investigated in animal models where a strong association has been demonstrated between cellular energy status and the Δ [_{ox}CCO] (Tsuji *et al* 1995, Matsunaga *et al* 1998, Springett *et al* 2003) and in human patients including neonates (Edwards *et al* 1991), children (Skov and Greisen 1994) and adults (Nollert *et al* 1995, Nollert *et al* 1998, Kakihana *et al* 2002) in whom changes in [_{ox}CCO] have been shown to correlate with neurological dysfunction after cardiac surgery. We have also recently described changes in [_{ox}CCO] measured using NIRS during profound arterial de-saturation associated with apnoeas in obstructive sleep apnoea patients and have discussed the clinical potential of this measurement to monitor cellular oxygen metabolism in the brain (McGown *et al* 2003, Tachtsidis *et al* 2004).

However, although the specific absorption coefficients for oxidised cytochrome c oxidase (_{ox}CCO) in the near infrared region are of a similar magnitude to those of both HbO₂ and HHb, the concentration of the CCO centre is less than 10% of that of haemoglobin, and hence its detection is no trivial matter. Historically, particular doubts have been raised as to whether the measured concentration changes really arise from changes in the redox state of CCO, or are artefacts of the algorithms used to convert the measured attenuation changes into chromophore concentration changes (Matcher *et al* 1995).

As a result of this it has been hypothesised that large changes in the concentrations of oxy- and deoxy- haemoglobin might induce spurious changes in the [_{ox}CCO] signal (Matcher *et al* 1995). Cooper and colleagues (Cooper *et al* 1999) addressed this concern using mitochondrial inhibitors and perfluorocarbon blood exchange in a neonatal animal model and showed that the measured _{ox}CCO signal was stable despite large changes in [HbO₂] and [HHb]. It is clearly difficult to produce large changes in cerebral haemoglobin concentrations in healthy humans but some disease states expose patients to these types of changes on a regular basis.

In healthy subjects in a move from supine to an upright position blood pressure is maintained via autonomic reflex activity causing an increase in systemic vascular resistance in response to gravitational displacement of blood to the lower part of the body and increased filtration from capillaries into the interstitial space (Lye and Walley 1998). Patients with primary autonomic failure (PAF) are unable to modulate vascular tone in response to the upright body position because of the absence of this reflex activity (Mathias 2003). Postural tolerance varies between patients and postural hypotension associated with PAF can result in syncope or pre-syncope symptoms such as light headedness and blurred vision that may reflect reduced cerebral perfusion (Mathias *et al* 1999).

We have previously described large and consistent changes in cerebral tissue oxygenation, resulting from passive head-up tilt in this patient group (Hunt K. *et al* 2006). In that study we confirmed that there is a significant decrease in cerebral oxygenation during postural hypotension in patients with autonomic failure.

The aim of this paper is to investigate the changes in [_{ox}CCO] during these episodes of reduced cerebral oxygenation produced by blood pressure driven changes in oxy- and deoxy-haemoglobin concentrations.

Methods

Patients

12 patients with primary autonomic failure (8 men and 4 women; age 42 to 79 years; mean 63.2 years) were investigated following local ethics committee approval and written informed consent.

Near-infrared spectroscopy

Optical attenuation data was collected at 6Hz using a continuous wave, four wavelength (775, 813, 853 and 910nm) near-infrared spectrometer (NIRO 300, Hamamatsu Photonics KK, Japan). Changes in [HbO₂], [HHb] and [_{ox}CCO] were calculated offline using the modified Beer-Lambert law (Delpy *et al* 1988) and the UCL4 wavelength algorithm (Matcher *et al* 1995) with an age-corrected differential pathlength factor calculated as $5.13 + (0.07 \times A^{0.81})$, where A is the age in years (Duncan *et al* 1996). This algorithm converts changes in optical attenuation to changes in [HbO₂], [HHb] and [_{ox}CCO] using multi-linear regression and the specific extinction coefficients for HbO₂, HHb, and _{ox}CCO measured on purified solutions of the compounds corrected for the wavelength dependence of the pathlength in tissue (Cope 1991).

The NIRO 300 spectrophotometer uses four discrete wavelengths, with the UCL4 algorithm, which in matrix notation has the form:

$$\begin{bmatrix} \Delta[HHb] \\ \Delta[HbO_2] \\ \Delta[{}_{ox}CCO] \end{bmatrix} = \frac{1}{L} \times \begin{pmatrix} 1.50 & -1.02 & -0.78 & 0.66 \\ -0.72 & -0.66 & 0.28 & 1.70 \\ -0.19 & 0.90 & 0.35 & -1.00 \end{pmatrix} \times \begin{pmatrix} \Delta A_{775nm} \\ \Delta A_{813nm} \\ \Delta A_{853nm} \\ \Delta A_{910nm} \end{pmatrix} \quad (1)$$

where L represents the total optical pathlength (cm), ΔA represents the change in detected attenuation measured in optical density (OD) at the wavelength given in the subscript, and the numerical matrix (mM·cm/OD) is the inverse of a matrix containing the specific extinction coefficients for HbO₂, HHb and _{ox}CCO multiplied by the correction factors for the wavelength dependence of the optical pathlength at each wavelength (Essenpreis *et al* 1993). The calculated concentration changes in Equation 1 are in mM.

By using a pre-defined optical pathlength, this algorithm implicitly assumes a particular value for the effects of tissue scattering. When scattering is also considered, the absorption coefficient and the attenuation are related nonlinearly. However, over a small wavelength range and when the change in the absorption coefficient is small, a linear relationship is a good first order approximation. This algorithm exploits this and makes it possible for us to apply the modified Beer-Lambert law to convert optical attenuation changes to chromophore concentration changes as shown in Eq.1 (Cope 1991).

Changes in total haemoglobin concentration ($\Delta[HbT]$), which can be used as an indicator of changes in blood volume were calculated from the sum of $\Delta[HbO_2]$ and $\Delta[HHb]$. The

difference between $\Delta[\text{HbO}_2]$ and $\Delta[\text{HHb}]$ was calculated subsequently; this is termed the change in haemoglobin difference concentration ($\Delta[\text{Hb}_{\text{diff}}]$), which can provide an indication of tissue ischemia (Kirkpatrick *et al* 1998).

The NIRS optodes (one emitting optical fibre and one detector photodiode chip) were placed on the scalp over the frontal region below the hairline, approximately 4 cm above the supra-orbital ridge (taking care to avoid the midline sinuses). An optode spacing of 5 cm was used and light shielding was provided by using an elastic bandage and a black cloth.

Systemic Data

A Portapres® system (Biomedical Instrumentation, TNO Institute of Applied Physics) was used to measure blood pressure non-invasively using a finger cuff. The mean blood pressure (MBP) data was collected to a PC via a serial link at 100Hz sampling rate and was later resampled to 6Hz.

Protocol

Each subject was strapped to an electric tilt table in the horizontal position. After an initial period of acclimatisation, baseline data was collected for 3 minutes with the patient lying supine on the horizontally positioned table. The subject was then tilted over a period of 15 seconds to a 60° head up angle and remained in that position for up to 10 minutes. The head-up tilt was reversed immediately if any patient complained of severe symptoms or if syncope was imminent.

Data acquisition and analysis

Physiological data were downloaded onto a personal computer for later analysis off-line. All data was resampled (Matlab Mathworks Inc) to 0.1Hz (1 point per 10seconds).

The $\Delta[\text{Hb}_{\text{diff}}]$ signal was taken as an index marker against which to compare the other NIRS data. In all patients $\Delta[\text{Hb}_{\text{diff}}]$ dropped with the head-up tilt. 6 patients were returned to the supine position before the end of the 10 minute measured period due to severe pre-syncope symptoms. In some of the remaining 6 patients $\Delta[\text{Hb}_{\text{diff}}]$ reached a plateau or slightly increased after the initial drop. In all patients the analysis period of each signal was restricted to the data between the start and the end point of the $\Delta[\text{Hb}_{\text{diff}}]$ drop.

The start and the end point of the $\Delta[\text{Hb}_{\text{diff}}]$ drop were identified manually for each patient. Data was averaged over the 1 minute prior to the start point of the $\Delta[\text{Hb}_{\text{diff}}]$ drop (baseline) and the 1 minute prior to the end point of the $\Delta[\text{Hb}_{\text{diff}}]$ drop (end of drop). Mean changes between the baseline and the end of drop were compared using Student's t-test with $p \leq 0.05$ required for statistical significance.

Results

The changes from baseline to the end of the $\Delta[\text{Hb}_{\text{diff}}]$ drop period for each patient and the group means are presented in table 1. Group data are presented here as mean \pm standard deviation. The mean duration of the $\Delta[\text{Hb}_{\text{diff}}]$ drop was 6.97 minutes (range 4 to 9.67 minutes).

Figure 1 shows group data for $\Delta[\text{MBP}]$, $\Delta[\text{HbT}]$, $\Delta[\text{Hb}_{\text{diff}}]$, $\Delta[\text{HbO}_2]$, $\Delta[\text{HHb}]$ and $\Delta[\text{oxCCO}]$ over the time course of the analysis. The first 3 minutes represents the pre-tilt baseline period. Note that the duration of the $[\text{Hb}_{\text{diff}}]$ drop varied between patients therefore beyond 4 minutes the number of patients included in the mean gradually decreased as indicated by the lower histogram plot in figure 1.

Investigation of individual $\Delta[\text{oxCCO}]$ revealed wide inter-individual variation. For each patient a t-test was used to compare $\Delta[\text{oxCCO}]$ between the baseline and end of drop. The statistical results are shown in table 1. Three different patterns were identified (data is presented as mean \pm standard deviation). 6 patients demonstrated a drop in $\Delta[\text{oxCCO}]$ ($0.17 \pm 0.15 \mu\text{M}$); 4 patients demonstrated no change ($0.01 \pm 0.12 \mu\text{M}$) and 2 patients showed an increase in $\Delta[\text{oxCCO}]$ ($0.21 \pm 0.01 \mu\text{M}$). Figure 2 shows an example of each pattern of $[\text{oxCCO}]$ change.

In the subset of the 6 patients in which the end of drop in $[\text{oxCCO}]$ was statistically lower than the baseline $[\text{oxCCO}]$ there appears to be a threshold before which $[\text{oxCCO}]$ does not drop. Figure 3 shows the changes in $[\text{oxCCO}]$ plotted against $\Delta[\text{HHb}]$, $\Delta[\text{HbO}_2]$, $\Delta[\text{HbT}]$ and $\Delta[\text{Hb}_{\text{diff}}]$ for this subset of patients. The drop in $[\text{oxCCO}]$ only becomes significant ($0.10 \pm 0.12 \mu\text{M}$, $n=6$, $p=0.049$) 4.33 minutes after the start of the drop in $[\text{Hb}_{\text{diff}}]$ when $\Delta[\text{Hb}_{\text{diff}}]$ has dropped by $14.36 \pm 3.67 \mu\text{M}$ and $\Delta[\text{HbT}]$ by $4.4 \pm 2.73 \mu\text{M}$.

Table 1. Changes from baseline to the end of $[\text{Hb}_{\text{diff}}]$ drop in the NIRS signals and MBP during head-up tilt in all patients.

Patients	$\Delta[\text{MBP}]$ (mmHg)	$\Delta[\text{HbT}]$ (μM)	$\Delta[\text{Hb}_{\text{diff}}]$ (μM)	$\Delta[\text{oxCCO}]$ (μM)
1	-83	-4.76	-9.71	-0.00 [‡]
2	-39	-1.62	-14.07	-0.40 [§]
3	-61	-1.48	-17.94	-0.25 [§]
4	-86	-5.97	-22.54	-0.22 [§]
5	-41	-1.59	-11.92	-0.07 [§]
6	-56	-5.19	-10.79	-0.16 [§]
7	-59	-2.58	-8.00	-0.03 [‡]
8	-50	-3.06	-13.95	0.18 [†]
9	-102	-6.72	-22.66	-0.09 [§]
10	-21	-0.93	-3.87	-0.04 [‡]
11	-49	-13.36	-23.33	0.24 [†]
12	-6	-3.16	-6.07	-0.02 [‡]
Mean ($\pm\text{SD}$)	-54 (± 27) [□]	-4.23 (± 3.69) [□]	-13.74 (± 6.63) [□]	-0.05 (± 0.18)

(Group analysis [□] $p < 0.001$)

($\Delta[\text{oxCCO}]$ individual analysis [§] Significant drop, $p \leq 0.05$; [‡] No change, $p > 0.05$; [†] Significant increase, $p \leq 0.05$)

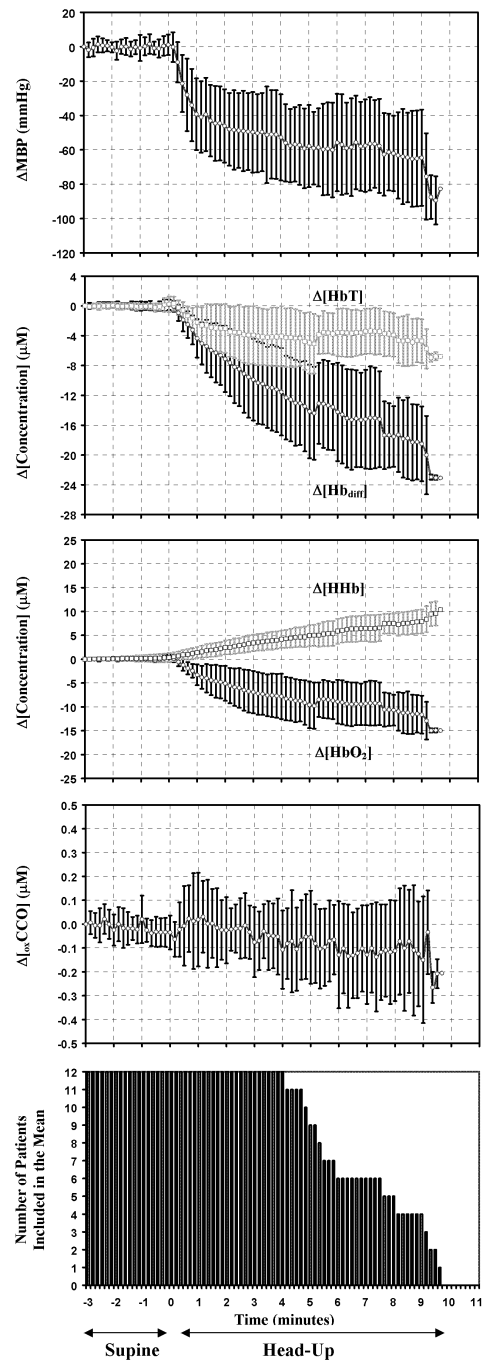


Figure 1. Group means and standard deviations for changes in, mean blood pressure ($\Delta[\text{MBP}]$), total haemoglobin concentration ($\Delta[\text{HbT}]$), haemoglobin difference concentration ($\Delta[\text{Hb}_{\text{diff}}]$), oxy-haemoglobin concentration ($\Delta[\text{HbO}_2]$), deoxy-haemoglobin concentration ($\Delta[\text{HHb}]$) and the concentration of oxidised cytochrome c oxidase ($\Delta[\text{oxCCO}]$). Point 0 represents the start of the $\Delta[\text{Hb}_{\text{diff}}]$ drop. The lower histogram plot shows the number of patients included in the group mean.

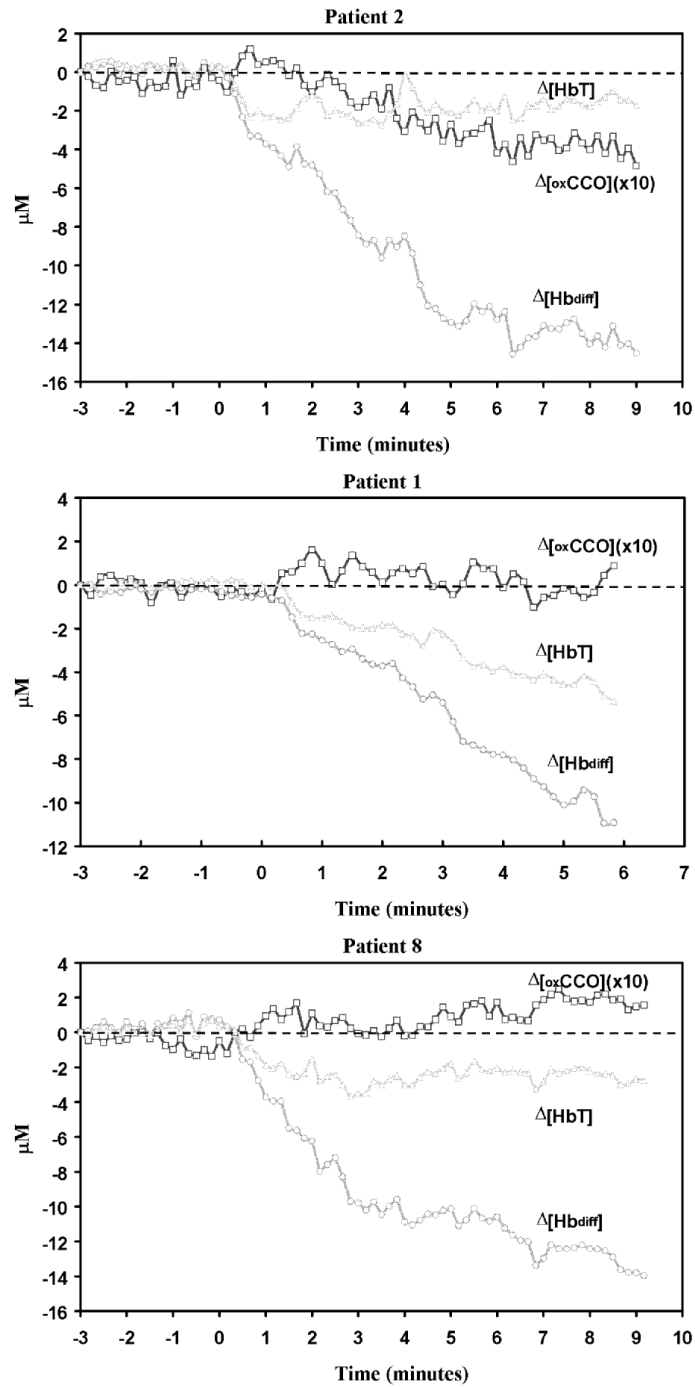


Figure 2. Plots of $\Delta[\text{Hb}_{\text{diff}}]$, $\Delta[\text{HbT}]$ and $\Delta[\text{oxCCO}](\times 10)$ against time for: Patient 2 who showed a significant drop in $\Delta[\text{oxCCO}]$ ($p=0.0001$); Patient 1 who showed no change in the $\Delta[\text{oxCCO}]$ ($p=0.369$) and Patient 8 who showed a significant increase in $\Delta[\text{oxCCO}]$ ($p=0.00001$). Point 0 represents the start of the $\Delta[\text{Hb}_{\text{diff}}]$ drop.

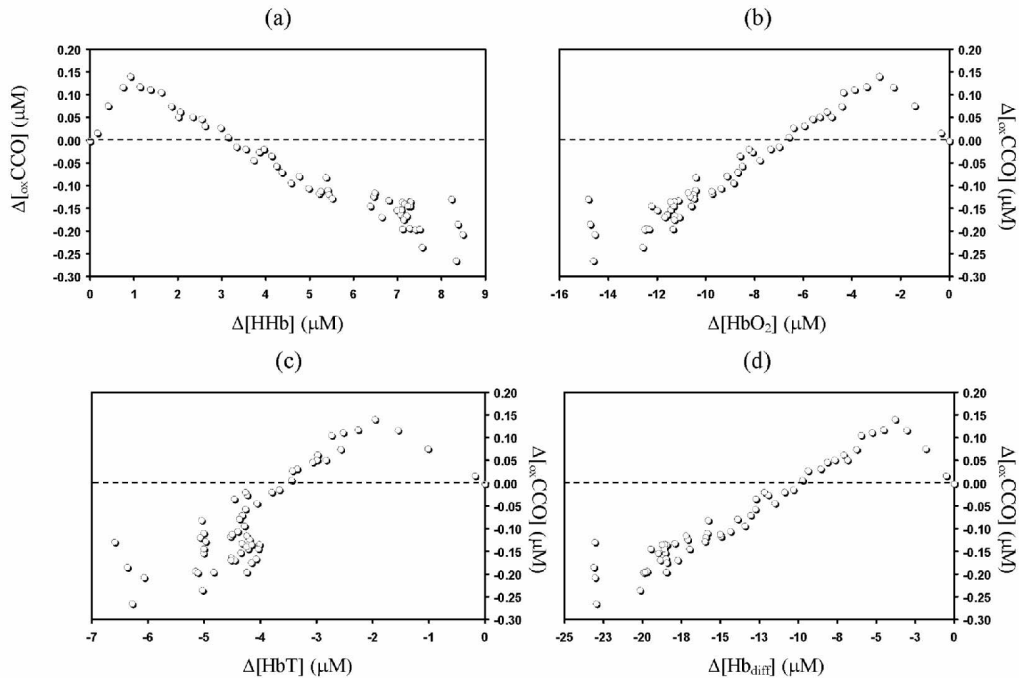


Figure 3. Group mean plots of the sub group of patients that demonstrated a significant drop in $\Delta[\text{oxCCO}]$. The changes in $[\text{oxCCO}]$ are normalised to the beginning of drop in $\Delta[\text{Hb}_{\text{diff}}]$ and are plotted against (a) $\Delta[\text{HHb}]$; (b) $\Delta[\text{HbO}_2]$; (c) $\Delta[\text{HbT}]$ and (d) $\Delta[\text{Hb}_{\text{diff}}]$.

Discussion

This paper describes changes in cerebral $[\text{oxCCO}]$, and haemoglobin concentrations measured using NIRS during passive head up tilt in patients with primary autonomic failure. A number of studies have used NIRS to investigate patients suffering from orthostatic hypotension during head-up tilt with the majority describing changes in cerebral haemoglobin concentration and tissue oxygenation (Colier *et al* 1997, Harms *et al* 2000, van Lieshout *et al* 2003, Hunt K. *et al* 2006). To our knowledge this is the first study reporting the changes in $[\text{oxCCO}]$ resulting from orthostatic hypotension in this group of patients.

During head up tilt there was a severe drop in blood pressure, which led to a substantial reduction in $\Delta[\text{Hb}_{\text{diff}}]$ and $\Delta[\text{HbT}]$ in all subjects. This consistent and large reduction in $\Delta[\text{Hb}_{\text{diff}}]$ and $\Delta[\text{HbT}]$ provides a good opportunity to investigate the relationship between the haemoglobin changes and the $\Delta[\text{oxCCO}]$ response measured using NIRS. Our results show a range of $\Delta[\text{oxCCO}]$ responses during the large decrease in $\Delta[\text{Hb}_{\text{diff}}]$ and $\Delta[\text{HbT}]$. 6 patients show a significant drop, 4 patients show no change and 2 patients show a significant increase in the $\Delta[\text{oxCCO}]$ signal. In the 6 patients subgroup we observed a point after which the drop in $\Delta[\text{oxCCO}]$ becomes significant; this indicates the presence of a threshold between the start of changes in $[\text{Hb}_{\text{diff}}]$ and $[\text{HbT}]$ and those in $[\text{oxCCO}]$. However given that one patient (Patient 11) demonstrated a significant increase in $[\text{oxCCO}]$ associated with a drop in $[\text{Hb}_{\text{diff}}]$ greater than our suggested threshold value during head-up tilt, then this value is not necessarily a “trigger” level for the reduction in $[\text{oxCCO}]$.

When interpreting NIRS CCO data it is important to remember that the CuA redox status responds to multiple factors including the level of reduction of cytochrome c, the proton motive force across the mitochondrial membrane, pH and oxygen tension (Cooper *et al* 1997a). In-vitro studies demonstrate that even at normoxia, the CuA centre in cytochrome oxidase is not fully oxidised (Cooper *et al* 1994) and responds to changes in flux through the

enzyme (Cooper *et al* 1997b). This is consistent with measurements from animal models suggesting that the baseline oxidation of CuA (mean±SD) is 82.0±16.6% in the adult rat brain (Cooper *et al* 1998) and at 67.3±18.3% in the piglet brain (Springett *et al* 2000). Indeed, whilst most reports have focused on the effect of decreasing oxygen delivery on the reduction of CuA (Cooper *et al* 1998, Springett *et al* 2003) other NIRS studies have consistently shown small oxidations are measurable in the neonatal animal brain (Quaresima *et al* 1998) and most recently in the human brain during functional activation (Uludag *et al* 2004). Changes in the CuA redox state are therefore possible in both directions (oxidation or reduction).

Optical techniques have the potential to measure changes in the redox state of the electron transport chain directly, but great care must be taken to separate accurately the CCO signal from the haemoglobin signals. To show that crosstalk is minimal in the system, it is necessary to show that the haemoglobin signals (in any noncollinear combination) can be varied without affecting the $[\text{oxCCO}]$ signal. Computer simulation work by Matcher and colleagues (1995) (Matcher *et al* 1995) investigated the issue of erroneous chromophore calculations using an analytical solution of the diffusion equation based on a homogenous infinite slab of tissue. Using this analytical model, they compared the modelled and estimated changes of chromophore concentrations and concluded that the amplitude of the spurious $[\text{oxCCO}]$ changes fell as the number of wavelengths increased. In this study we used 4 wavelengths to calculate the chromophore concentrations; Matcher *et al* (1995) (Matcher *et al* 1995) stated that the UCL4 algorithm could produce small $\Delta[\text{oxCCO}]$ approximately mirroring $\Delta[\text{HHb}]$ particularly during episodes where total haemoglobin concentration changes. Figure 3(a) shows the group mean $\Delta[\text{oxCCO}]$ plotted against the $\Delta[\text{HHb}]$. It is obvious that $[\text{oxCCO}]$ started to drop much later than $[\text{HHb}]$ and did not mirror its trend. Clearly there is not a linear relationship between the changes in $[\text{HHb}]$ and $[\text{oxCCO}]$. If the changes in $[\text{oxCCO}]$ were purely due to crosstalk then $\Delta[\text{oxCCO}]$ should track $\Delta[\text{Hb}_{\text{diff}}]$ and $\Delta[\text{HbT}]$. In our data, however, this is not the case. The nature of the changes in the $[\text{oxCCO}]$ described suggests that, if crosstalk is present here, there is no simple mechanism for crosstalk between the haemoglobin and oxCCO signals. We are currently investigating the relationship between the haemoglobins and the cytochrome NIRS signals using diffusion modelling based on a layered tissue structure.

Whilst it is relatively easy to measure changes in the concentrations of oxy- and deoxy-haemoglobin, interpretation of the results is less straight forward. Haemoglobin species are present in arterial, venous and capillary compartments and concentration changes are therefore affected not only by changes in oxygen delivery, utilisation and blood flow but also by changes in the relative proportions of the vascular compartments contributing to the signal. In addition, when non-invasively monitoring cranial $\Delta[\text{HbO}_2]$ and $\Delta[\text{HHb}]$ with differential spectroscopy, there is likely to be a degree of ‘contamination’ of the cerebral signal by haemoglobin species in the skin vasculature. Changes in the redox state of CCO will be affected primarily by mitochondrial oxygen concentration (Cooper *et al* 1994) and may be less prone to extracerebral ‘contamination’, as CCO is not present in the red blood cells and is present in low concentrations in the skin compared to brain tissue (Drabkin 1951). Therefore this measurement has the potential to provide a non-invasive indicator of changes in cerebral cellular oxygen delivery and utilisation.

Madsen *et al* (Madsen *et al* 1998) induced orthostatic hypotension from prolonged head-up tilt in a group of healthy volunteers and found that six of them exhibited presyncopal symptoms which were accompanied by a significant reduction in the $\Delta[\text{oxCCO}]$ as measured with a four wavelength NIRS system. Here we investigate a group of PAF patients who are likely to have abnormal cerebral vascular regulation and who might therefore be expected to exhibit a reduction in $[\text{oxCCO}]$ during severe orthostatic hypotension. This was not observed in all patients and the reasons for this are unclear. This patient group exhibits disease heterogeneity (Schondorf *et al* 2001a, Schondorf *et al* 2001b) and variable abnormalities of cerebral autoregulation (Novak *et al* 1998, Hesse *et al* 2002, Hetzel *et al* 2003). However, we cannot comment further on this issue as we made no assessment of cerebral autoregulation in this study; clearly further studies in a larger patient group are required.

There is a modest amount of published research work examining the NIRS measured changes in $[\text{oxCCO}]$ in human adult patients with most studies being intra-operative. Nollert and colleagues (Nollert *et al* 1995) investigated 41 patients undergoing cardiac bypass during surgery and observed a significant reduction in the $\Delta[\text{oxCCO}]$ in all patients. They found that the four patients with reversible postoperative neuropsychological deficit, as diagnosed using a mini-mental state examination, exhibited significantly larger decrease in $\Delta[\text{oxCCO}]$ than the remainder of the group. Kakihana *et al* (Kakihana *et al* 2002) investigated 66 patients who underwent thoracic aortic surgery. They found a variability in the $\Delta[\text{oxCCO}]$ and demonstrated a correlation between the intra-operative measurement of $\Delta[\text{oxCCO}]$ and neurological outcome. They concluded that changes in the redox state of CCO during thoracic aortic surgery were a good predictor of postoperative cerebral outcome. In previous work studying patients with obstructive sleep apnoea we demonstrated a significant reduction in $[\text{oxCCO}]$ during deep apnoeas associated with arterial de-saturations down to 55% (Tachtsidis *et al* 2004).

In our most recent work in sleep apnoea patients (Tachtsidis *et al* 2004) - using the same NIRS instrumentation as discussed in this study - we found that during these apnoeas $\Delta[\text{Hb}_{\text{diff}}]$ dropped consistently in all patients, but two different patterns of $\Delta[\text{HbT}]$ were observed. We divided the patients into two groups on the basis of these changes. In Group 1 $\Delta[\text{HbT}]$ increased steadily throughout the apnoea and $\Delta[\text{oxCCO}]$ appeared to remain constant for several seconds after which it dropped and levelled off. In Group 2 $\Delta[\text{HbT}]$ initially dropped and then rose while the $\Delta[\text{oxCCO}]$ dropped essentially simultaneously with $\Delta[\text{Hb}_{\text{diff}}]$ with the slope of the decrease changing after several seconds. As with this current study these changes in $[\text{oxCCO}]$ were not linearly correlated with the haemoglobin concentration changes. In figure 4(a) we reproduce the sleep apnoea patients results for Group 1 from Tachtsidis *et al* (Tachtsidis *et al* 2004) where we plot $\Delta[\text{oxCCO}](\times 10)$ and $\Delta[\text{HbT}]$ against $\Delta[\text{Hb}_{\text{diff}}]$ and compare it with the results from the patients in this study who showed a significant drop in $\Delta[\text{oxCCO}]$ (see figure 4(b)). Although the patient pathology is different, both these experimental protocols produce a large reduction in $[\text{Hb}_{\text{diff}}]$. In the sleep apnoea patients $\Delta[\text{HbT}]$ increased but in the PAF patients $\Delta[\text{HbT}]$ decreased. However the $\Delta[\text{oxCCO}]$ signal showed a reduction in both patient groups, which appears to occur beyond some threshold. The data shown in figure 4 provide evidence that the NIRS measured changes in the $[\text{oxCCO}]$ are not simply a product of erroneous chromophore calculations produced by the algorithm.

In summary, we describe the cerebral changes in haemoglobin concentrations and $[\text{oxCCO}]$ in a group of primary autonomic failure patients during head-up tilt. The NIRS measurement of CCO redox changes in patients has the potential to provide a useful, non-invasive real time marker of alterations in mitochondrial oxygen availability and identify failing mitochondrial metabolism after brain injury. Our results suggest that we are able to measure an independent oxCCO signal and to our knowledge this is the first time that the $\Delta[\text{oxCCO}]$ in PAF patients have been reported. During head-up tilt a subset of our patients exhibited a reduction in $[\text{oxCCO}]$ past a certain threshold. This might be indicative of changes in cerebral cellular redox state; however further work is required to determine the clinical relevance of these findings.

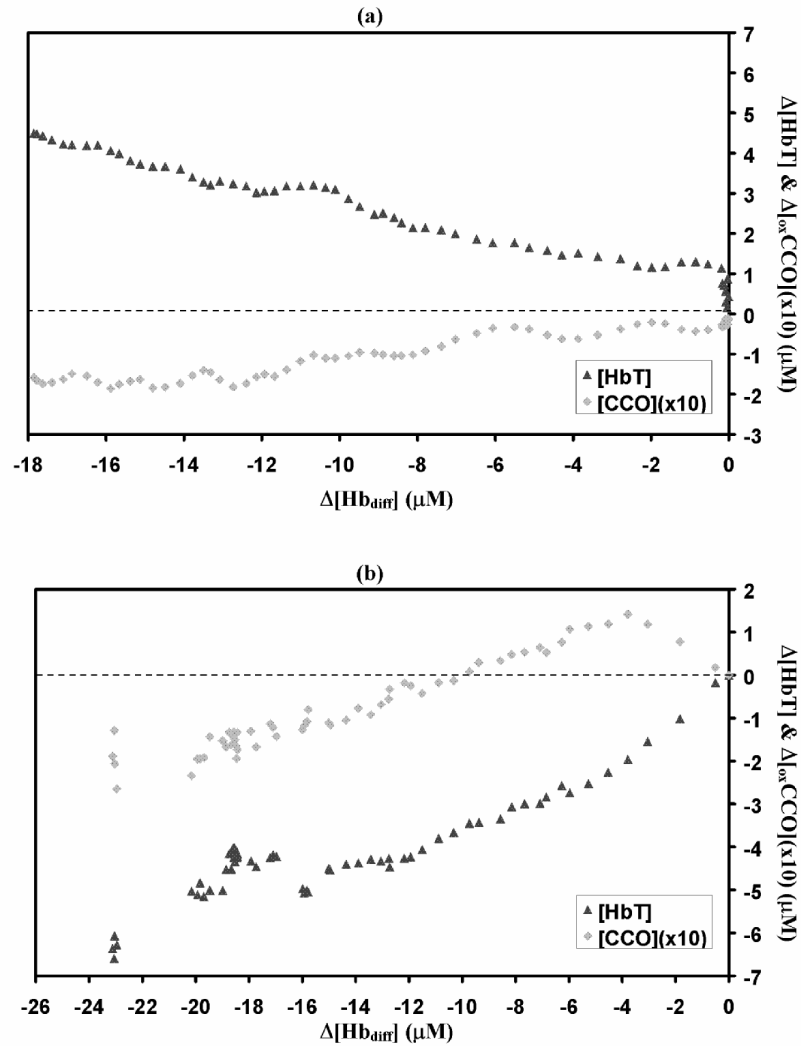


Figure 4. (a) Mean changes in cerebral [HbT] and [oxCCO] versus [Hb_{diff}] in sleep apnoea patients (reproduced from (Tachtsidis *et al* 2004)); (b) Mean changes in cerebral [HbT] and [oxCCO] versus [Hb_{diff}] in the primary autonomic failure patient group that showed a significant drop in $\Delta[\text{oxCCO}]$.

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