Near-infrared spectroscopic quantification of changes in the concentration of oxidized cytochrome *c* oxidase in the healthy human brain during hypoxemia

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Abstract. The near-IR cytochrome *c* oxidase (CCO) signal has potential as a clinical marker of changes in mitochondrial oxygen utilization. We examine the CCO signal response to reduced oxygen delivery in the healthy human brain. We induced a reduction in arterial oxygen saturation from baseline levels to 80% in eight healthy adult humans, while minimizing changes in end tidal carbon dioxide tension. We measured changes in the cerebral concentrations of oxidized CCO (Δ[oxCCO]), oxyhemoglobin ($\Delta[HbO_2]$), and deoxyhemoglobin (Δ[HHb]) using broadband near-IR spectroscopy (NIRS), and estimated changes in cerebral oxygen delivery ($ecDO_2$) using pulse oximetry and transcranial Doppler ultrasonography. Results are presented as median (interquartile range). At the nadir of hypoxemia $ecDO_2$ decreased by 9.2 (5.4 to 12.1)% ($p<0.0001$), Δ[oxCCO] decreased by 0.24 (0.06 to 0.28) micromoles/l ($p<0.01$), total hemoglobin concentration increased by 2.83 (2.27 to 4.46) micromoles/l ($p<0.0001$), and change in hemoglobin difference concentration ($\Delta[Hbdiff]=\Delta[HbO_2]-\Delta$[HHb]) decreased by 12.72 (11.32 to 16.34) micromoles/l ($p<0.0001$). Change in $ecDO_2$ correlated with Δ[oxCCO] ($r=0.78$, $p<0.001$), but not with either change in total hemoglobin concentration or Δ[Hbdiff]. This is the first description of cerebral Δ[oxCCO] during hypoxemia in healthy adults. Studies are ongoing to investigate the clinical relevance of this signal in patients with traumatic brain injury. [DOI: 10.1117/1.2718541]

Keywords: cytochrome *c* oxidase; near infrared spectroscopy; cerebral monitoring.

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1 Introduction

Cytochrome *c* oxidase (CCO) is the terminal electron acceptor of the mitochondrial electron transfer chain and catalyzes over 95% of oxygen metabolism, thereby driving adenosine triphosphate (ATP) synthesis.[1] The CCO redox state reflects the balance between electron donation from cytochrome *c* and oxygen reduction to water. Although many factors can influence the CCO redox state,[2] the most significant is the availability of molecular oxygen.[3]

The difference spectrum between oxidized and reduced CCO has a distinct band in the near-IR region, which can be measured using near-IR spectroscopy[4,5] (NIRS). Assuming the total concentration of CCO remains constant during an experiment, changes in the NIRS CCO signal represent changes in the CCO redox state. This signal has the potential to provide a noninvasive marker of changes in mitochondrial oxygen delivery and utilization, and might facilitate detection of ischemic thresholds and guide subsequent clinical interventions.

The *in vivo* use of NIRS was first described by Jobsis[5] in 1977, and it has been used in animals and humans to measure change in concentration of oxyhemoglobin ($\Delta[HbO_2]$), deoxy-hemoglobin (Δ[HHb]), and oxidized cytochrome *c* oxidase (Δ[oxCCO]).[6–11] NIRS exploits the fact that biological tissue is relatively transparent to near-IR light between 700 and 900 nm, enabling interrogation of structures beneath the tissue surface.[5] Biological tissue is a highly scattering medium, complicating the calculation of chromophore concentration, but if the average path length of light through tissue is known, the modified Beer-Lambert law, which assumes constant scattering losses, enables calculation of absolute changes in chromophore concentration.[12]

Specific extinction coefficients of the oxidized-reduced CCO difference spectrum in the near-IR (NIR) region are similar in magnitude to those of oxy- and deoxyhemoglobin,[2] but the concentration of CCO in the brain is approximately one order of magnitude less than these other two

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chromophores.[13] This complicates its detection and raises the possibility that NIRS measured changes in Δ[oxCCO] might be subject to artefacts resulting from measurement algorithms.[10,14] However, mitochondrial inhibitor and perfluorocarbon-blood exchange studies in animals have recently shown[15,16] that Δ[oxCCO] measurements are stable during large contemporaneous Δ[HbO_2] and Δ[HHb]. Furthermore, data from human visual stimulation studies suggest that cerebral Δ[oxCCO] is not merely crosstalk artifact.[17]

Importantly, Δ[oxCCO] has been validated, in animals, as a marker of cellular energy status against magnetic resonance spectroscopy measured reduction in phosphocreatine and nucleoside triphosphates levels.[18,19] Although cerebral Δ[oxCCO] has been measured in humans in clinical situations associated with reduced cerebral oxygen delivery, namely, cardiac surgery[8] and obstructive sleep apnea,[20] these studies are hard to standardize and controversy remains regarding the relationship between Δ[oxCCO] and oxygen delivery.

This paper aims to quantify broadband NIRS measured cerebral Δ[oxCCO] during hypoxemia in healthy human volunteers and examine its relationship to cerebral oxygen delivery and NIRS hemoglobin measurements.

2 Materials and Methods

This study was approved by the Joint Research Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology. We studied eight healthy volunteers (seven male and one female, with a median age 31.5 years, and range of 30 to 36). Broadband spectrometer (BBS) optodes were placed 3.5 cm apart in a black plastic holder and fixed to the right side of the forehead in the midpupilary line. Light from a stabilized tungsten halogen light source was filtered with 610-nm long-pass and heat-absorbing filters, and transmitted to the head via a 3.3-mm-diam glass optic fiber bundle. Light incident on the detector optode was focused via an identical fiber bundle onto the 400-μm entrance slit of a 0.27-m spectrograph (270 M, Instruments SA, France) with a 300-g/mm grating. NIR spectra between 650 and 980 nm were collected at 1 Hz on a cooled-charge coupled device detector (Wright Instruments, United Kingdom) giving a spectral resolution of ~5 nm. An oximeter probe (Novametrix Medical Systems Inc., USA) measured arterial oxygen saturation (SaO_2), and a Portapres finger cuff (Biomedical Instrumentation, TNO Institute of Applied Physics, Belgium) measured mean blood pressure (MBP) and heart rate (HR). Blood flow velocity in the basal right middle cerebral artery (vMCA) was collected using 2-MHz transcranial Doppler ultrasonography (Nicolet, United Kingdom). A modified anesthetic machine delivered gas to the subject via a mouthpiece. Inspired oxygen concentration (FiO_2) and end tidal partial pressure of carbon dioxide ($EtCO_2$) were measured using an inline gas analyzer (Hewlett Packard, United Kingdom) and a CO_2SMO optical sensor (Novametrix Medical Systems Inc.), respectively. The study commenced with 5 min of monitoring at normoxia and normocapnea. Nitrogen was then added to the inspired gases, to induce a gradual fall in SaO_2 to 80%, and immediately after this was achieved, the FiO_2 was returned to normoxia for 5 min. This cycle was repeated three times. $EtCO_2$ was continuously fed back to subjects and they adjusted their minute ventilation to maintain normocapnea throughout the study.

Absolute Δ[oxCCO], Δ[HbO_2], and Δ[HHb] were calculated from changes in light attenuation using a multiple regression technique termed the UCL*n* algorithm.[21] Correction factors for the wavelength dependence of the optical path length were applied to the chromophore absorption coefficients. Individual baseline optical path length was calculated using second differential analysis of the 740-nm water feature[22] of the initial 60 s of spectral data. Change in total hemoglobin concentration (Δ[HbT]) was defined as Δ[HbO_2]+Δ[HHb] and change in hemoglobin difference concentration (Δ[Hbdiff]) as Δ[HbO_2]−Δ[HHb].[23] Cerebral oxygen delivery (cDO_2) in milliliters O_2/100 g tissue/min is defined as

$$cDO_2 = CBF(1.39 \times Hb \times SaO_2 + 0.003 \times PaO_2), \quad (1)$$

where CBF is cerebral blood flow (ml/100 g tissue/min), 1.39 is the oxygen carrying capacity of hemoglobin (in milliliters per gram Hb), Hb is arterial hemoglobin saturation (in grams per deciliter), 0.003=solubility of oxygen in blood (ml/mmHg PaO_2/dL) and PaO_2 is arterial partial pressure of oxygen (in millimeters of Hg).

The mean vMCA measured using transcranial Doppler ultrasonography correlates with cerebral blood flow.[24] Ignoring the small dissolved oxygen component, we define estimated cerebral oxygen delivery ($ecDO_2$) as

$$ecDO_2 = k \times vMCA(1.39 \times Hb \times SaO_2), \quad (2)$$

where k is an individual specific constant.

Assuming constant arterial haemoglobin concentration during the study, percentage change in $ecDO_2$ ($\Delta ecDO_2$) is calculated as percentage change from baseline of $SaO_2 \times$ vMCA.

The start and end of each hypoxemic period was identified from the SaO_2 data. Individual subjects desaturate at different rates and, to enable description of the group data, each individual hypoxemia was divided into equal time periods, with each time point representing an eighth of the total time course of the hypoxemia. This produced nine time points with point 1 representing the point just prior to the start of hypoxemia and point 9 the nadir of hypoxemia. The same technique was applied separately to the recovery period, producing points 9 (just prior to start of recovery) to 17 (end of recovery period). At each time point, the mean of the preceding 10 s seconds of data was calculated. Data from the three experimental cycles were averaged to give a single course of hypoxemia and recovery for each subject. Group median changes from baseline at each time point were produced.

Statistical analysis was carried out using SAS software (v8.2, SAS Institute, USA) and p values <0.05 were considered significant. Group changes were compared with baseline using nonparametric analysis of variance (ANOVA) and *post hoc* pairwise comparisons.

Correlations between variables were assessed by applying Spearman rank correlation to data from the 17 time points, with Bonferroni-corrected two-tailed tests of significance.

Table 1 Median and interquartile range (IQR) ($n=8$) for baseline FiO_2, SaO_2, $EtCO_2$, HR, MBP, and vMCA.

	Median	IQR
FiO_2 (%)	21.0	21.0 to 21.0
SaO_2 (%)	98.6	98.2 to 99.2
$EtCO_2$ (kPa)	5.7	4.9 to 5.8
HR (min^{-1})	61.1	56.7 to 70.4
MBP (mm Hg)	74.4	67.7 to 79.8
vMCA ($cm\ s^{-1}$)	43.2	37.9 to 49.7

A multiple linear regression model was produced from the hypoxemic period group data (time points 1 to 9) with $\Delta[\text{oxCCO}]$ as the dependent variable, and $\Delta[\text{HbO}_2]$ and $\Delta[\text{HHb}]$ as the independent variables. To assess whether the measured $\Delta[\text{oxCCO}]$ ($\Delta[\text{oxCCO}]_{\text{meas}}$) was crosstalk artifact, a predicted $\Delta[\text{oxCCO}]$ ($\Delta[\text{oxCCO}]_{\text{pred}}$) for the recovery period was derived from the recovery period $\Delta[\text{HbO}_2]$ and $\Delta[\text{HHb}]$ using the multiple linear regression model. $\Delta[\text{oxCCO}]_{\text{pred}}$ and $\Delta[\text{oxCCO}]_{\text{meas}}$ for the recovery period were compared using a mixed model analysis.

3 Results

Table 1 shows baseline systemic data for the subject group. The median time of hypoxia required to achieve arterial oxygen saturation of 80% was 4.7 min (range 3 to 12 min). The length of each recovery period was fixed at 5 min for all subjects.

Figure 1 shows data for a single subject, demonstrating the experimental time course. Group changes from baseline during hypoxemia and recovery for FiO_2, HR, MBP, and vMCA are shown in Fig. 2 and for SaO_2, $EtCO_2$, $\Delta ecDO_2$, $\Delta[\text{HbT}]$, $\Delta[\text{Hbdiff}]$, and $\Delta[\text{oxCCO}]$ in Fig. 3. There were no significant changes in the measured optical pathlength during the study ($p>0.05$). Table 2 shows changes in variables from baseline to the nadir of hypoxemia and from baseline to the end of the normoxic recovery period.

Assessment of the data during both hypoxemia and recovery revealed a significant correlation between $\Delta ecDO_2$ and $\Delta[\text{oxCCO}]$ ($r=0.78$, $p<0.001$), but no correlation between $\Delta ecDO_2$ and $\Delta[\text{Hbdiff}]$ ($r=0.49$, $p=0.145$) or between $\Delta ecDO_2$ and $\Delta[\text{HbT}]$ ($r=-0.33$, $p=0.584$).

Multiple linear regression of the group data from the hypoxemic period revealed:

$$\Delta[\text{oxCCO}] = 0.04220 \times \Delta[\text{HbO}_2] + 0.00652 \times \Delta[\text{HHb}] - 0.01730,$$

$$p < 0.0001, \quad r = 0.51. \tag{3}$$

To check for crosstalk between the hemoglobin and CCO signals, Eq. (3) was used to derive $\Delta[\text{oxCCO}]_{\text{pred}}$ for the recovery period. $\Delta[\text{oxCCO}]_{\text{pred}}$ and $\Delta[\text{oxCCO}]_{\text{meas}}$ were different ($p=0.01$) (Fig. 4).

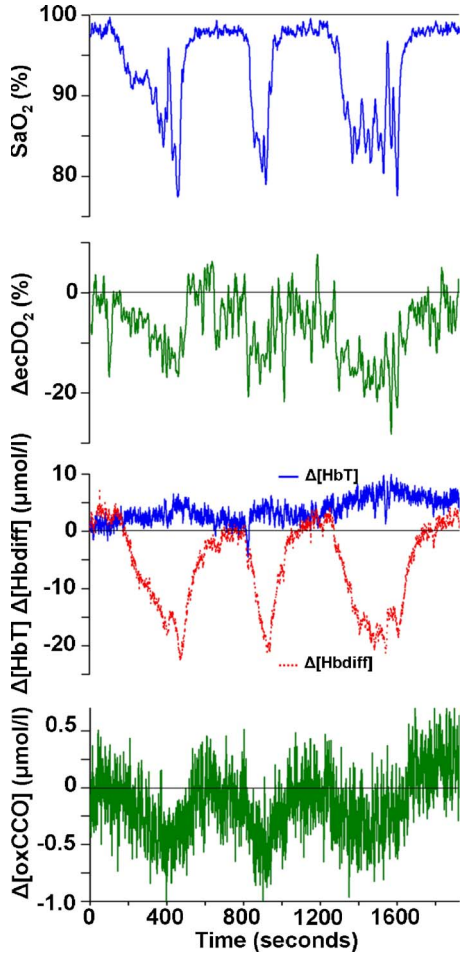

Fig. 1 SaO_2, $\Delta ecDO_2$, $\Delta[\text{Hbdiff}]$, $\Delta[\text{HbT}]$, and $\Delta[\text{oxCCO}]$ for a single subject during three cycles of hypoxemia.

4 Discussion

We described significant cerebral $\Delta[\text{oxCCO}]$ measured using NIRS during hypoxemia to an SaO_2 of 80% in healthy adult humans. We found distinct differences between the measured CCO and hemoglobin signals. Figure 3 shows $\Delta[\text{HbT}]$ rising during the hypoxemic challenge before gradually returning toward, but not reaching, baseline values after 5 min of normoxic recovery. This infers an increase in cerebral blood volume during hypoxemia, probably as a result of hypoxemic vasodilatation. $\Delta[\text{oxCCO}]$, which provides an assessment of changes in the balance of $\Delta[\text{HbO}_2]$ and $\Delta[\text{HHb}]$,[23] shows the opposite pattern, decreasing during hypoxemia and returning toward, but not reaching, baseline values after 5 min of normoxic recovery. $\Delta[\text{oxCCO}]$ decreases during hypoxemia and returns to baseline before $\Delta[\text{Hbdiff}]$ with a subsequent increase above baseline during the normoxic recovery period. Increased cerebral $\Delta[\text{oxCCO}]$ during the recovery period after hypoxemia has been demonstrated in animal models[11] and has not been fully explained.

Calculation of the correlation between $\Delta ecDO_2$ and $\Delta[\text{Hbdiff}]$, $\Delta[\text{HbT}]$, and $\Delta[\text{oxCCO}]$ was performed on the data from both the hypoxemic and recovery phases of the study to assess the ability of the three measures to detect both decreased and increased $ecDO_2$. Both $\Delta[\text{Hbdiff}]$ and $\Delta[\text{HbT}]$

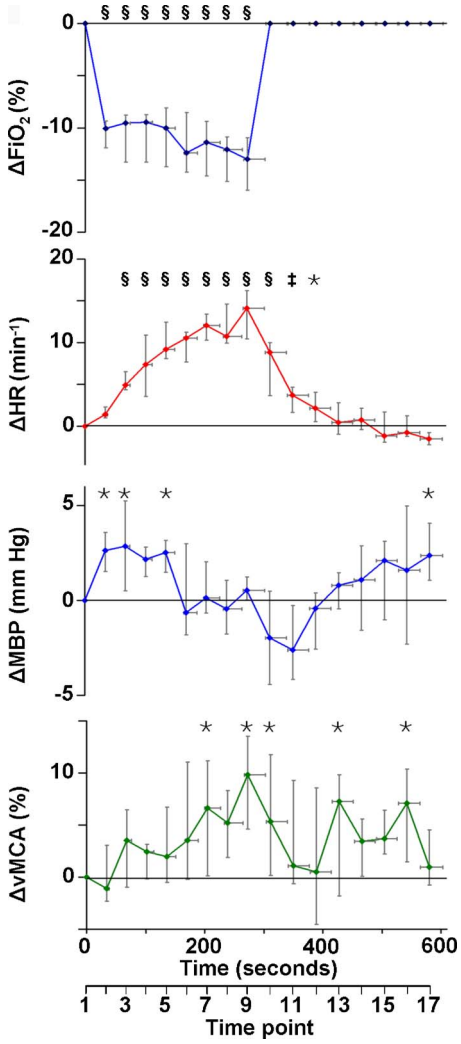

Fig. 2 Group median and interquartile range ($n=8$) for changes from ΔFiO_2, ΔHR, ΔMBP, and ΔvMCA; ⋆, $p<0.05$; †, $p<0.01$; ‡, $p<0.001$; and §, $p<0.0001$ for change from baseline.

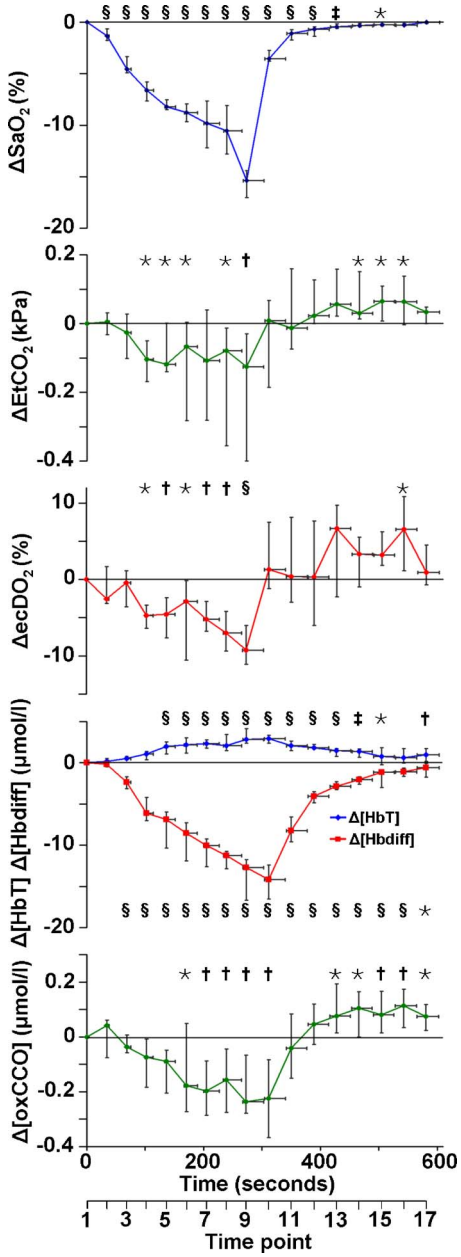

Fig. 3 Group median and interquartile range ($n=8$) for changes from baseline of ΔSaO_2, $\Delta EtCO_2$, $\Delta ecDO_2$, Δ[Hbdiff], Δ[HbT], and Δ[oxCCO]; ⋆, $p<0.05$; †, $p<0.01$; ‡, $p<0.001$; §, and $p<0.0001$ for change from baseline.

did not rise above, or drop below, baseline, respectively, in response to the increase in $ecDO_2$ during recovery and this results in the lack of significant correlations. There was a significant linear correlation between $\Delta ecDO_2$ and Δ[oxCCO], inferring that this NIRS measurement has clinical relevance as a measure of changes in cerebral oxygen delivery. We therefore suggest that Δ[oxCCO] provides a more reliable assessment of changes in cerebral oxygen delivery than either Δ[Hbdiff] or Δ[HbT].

Although NIRS-measured hemoglobin concentrations reflect intravascular oxygenation, the CCO signal indicates changes in *mitochondrial* oxygen delivery and utilization. In health, there is likely to be a close relationship between intravascular and mitochondrial oxygen delivery. However, in pathological situations, this relationship may be altered by tissue edema, which reduces oxygen diffusion from capillary to mitochondrion. In addition, mitochondrial dysfunction, which reduces the ability to metabolise oxygen, may occur. It is anticipated that in these situations, the mitochondrial CCO signal will yield different information to the intravascular hemoglobin signal, and will provide clinicians with a bedside tool with which to ensure adequate mitochondrial oxygen delivery and thus potentially preserve cell function.

NIRS monitoring of cerebral hemoglobin changes is liable to "contamination" of the cerebral signal by hemoglobin in the skin vasculature. The CCO signal is less prone to extracerebral contamination, since CCO is present in low concentrations in skin compared to brain and zero concentration in red blood cells.[25]

Edwards et al.[7] studied neonates using a commercial six-wavelength NIRO 1000 spectrometer. They found no Δ[oxCCO] during alterations in SaO_2 between 85 and 99%.

Table 2 Median and IQR (n=8) for changes from baseline to nadir of hypoxemia, and end of recovery period for ΔFiO_2, ΔSaO_2, $\Delta EtCO_2$, ΔHR, ΔMBP, ΔvMCA, $\Delta ecDO_2$, Δ[Hbdiff], Δ[HbT], and Δ[oxCCO].

	Hypoxemia		Recovery	
	Median	IQR	Median	IQR
ΔFiO_2 (%)	−13.0[d]	−10.8 to −16.1	0	0 to 0
ΔSaO_2 (%)	−15.4[d]	−14.3 to −17.5	0	−0.2 to 0
$\Delta EtCO_2$ (kPa)	−0.1[b]	0 to −0.4	0	0 to 0.1
ΔHR (min^{-1})	14.1[d]	10.3 to 17.2	−1.5	−0.1 to 2.4
ΔMBP (mm Hg)	0.5	−0.2 to 1.4	2.4[a]	0.9 to 5.2
ΔvMCA (%)	9.9[b]	4.1 to 13.7	0	0 to 0
$\Delta ecDO_2$ (%)	−9.2[d]	−5.4 to −12.1	0	0 to 0
Δ[Hbdiff] (μmol/l)	−12.7[d]	−11.4 to −16.9	−0.6[c]	−0.1 to −1.8
Δ[HbT] (μmol/l)	2.8[d]	2.3 to 4.5	1.0[b]	0 to 1.8
Δ[oxCCO] (μmol/l)	−0.24[b]	−0.06 to −0.28	0.01[a]	0 to 0.12

[a] $p<0.05$.
[b] $p<0.01$.
[c] $p<0.001$.
[d] $p<0.0001$ for change from baseline.

Our previous work in patients with obstructive sleep apnea demonstrated a reduction in Δ[oxCCO] during severe desaturation,[20] but this clinical paradigm did not allow for controlled SaO_2 manipulation and cellular and cerebrovascular responses in this patient group, who are exposed to repeated severe hypoxic episodes, may not reflect those of healthy individuals. The clinical relevance of cerebral Δ[oxCCO] has been demonstrated by NIRS measurements in adult patients undergoing cardiac surgery, where cerebral Δ[oxCCO] correlates with neurological outcome.[8,9]

Changes in arterial carbon dioxide tension ($PaCO_2$) have been shown to effect NIRS measured Δ[oxCCO] in both neonatal humans[7] (increase in $PaCO_2$ of 1.1 kPa) and piglets[11,16] (increase in $PaCO_2$ of 2.8 to 3.8 kPa), and to isolate the effect of hypoxemia, we used an $EtCO_2$ feedback loop to minimize changes in $PaCO_2$. Despite this, we found a small but significant median reduction in $EtCO_2$ of 0.1 kPa at the nadir of hypoxemia. We do not believe this magnitude of change in $EtCO_2$ will affect the CCO signal, although we are carrying out further studies to test this hypothesis.

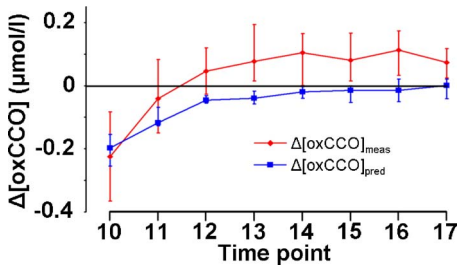

Fig. 4 Group median and interquartile range (n=8) for changes from baseline of $\Delta[oxCCO]_{meas}$ (◆), and $\Delta[oxCCO]_{pred}$ (■) during recovery period (time points 10 to 17). Predicted and measured results were different (p=0.01).

Controversy exists over how readily CCO becomes reduced following reduced oxygen tension. Several different algorithms exist for the conversion of light attenuation to chromophore concentration changes and the choice of algorithm can affect the results.[21] Some animal studies suggest that CCO reduction only occurs during extreme reduction in cerebral oxygen delivery,[6,11,16] while others have found a gradual reduction in CCO during hypoxemia.[26] These variations may relate to the experimental challenges, which comprised graded hypoxia,[26] anoxia,[11,16] or induced hypotension.[6] Evidence for "late" reduction in CCO in some animal studies following anoxia is a 20 to 25-s delay between changes in hemoglobin concentrations and CCO redox state,[11,16] and we also show a temporal delay between the first significant drops in Δ[Hbdiff] and Δ[oxCCO]. These animal data have been interpreted as suggesting that CCO reduction does not occur at moderate hypoxemia, but the instigation of anoxia may be too swift a challenge to enable full investigation of the effects of moderate hypoxemia. In addition, these studies[6,11,16,26] used animals initially ventilated with supranormal concentrations of oxygen, resulting in baseline arterial oxygen tensions between 14.7 and 65 kPa. Elevated baseline values might further delay the onset of changes in CCO redox during hypoxemia, leading to the conclusion that CCO reduction only occurs after severe reduction in oxygen delivery. These comparisons are further complicated by the fact that some studies have been performed in perfluorocarbon exchanged animals[26] with resultant greatly decreased tissue oxygen delivery compared to the blooded animal for a given arterial oxygen ten-

sion. We show that in the healthy human brain, gradual CCO reduction takes place during moderate hypoxemia (Fig. 3)—an essential prerequisite for a useful clinical marker of dysoxia.

The challenge we utilize in this study is obviously far less severe than that used in many animal studies, and we demonstrate only modest reductions in $\Delta[\mathrm{oxCCO}]$. We suggest that CCO redox may show a biphasic response to hypoxemia. Our finding of an early modest reduction in $\Delta[\mathrm{oxCCO}]$ may be followed by a threshold (below the extent of our challenge) beyond which a steeper reduction occurs. Our further work investigating CCO redox changes in brain-injured patients who occasionally suffer more severe hypoxemia may address this point.

The SNR for the calculation of optical path length using second differential spectroscopy was estimated using Monte Carlo simulation.[22] From these data, we would estimate the predicted accuracy of our pathlength calculation to be in the region of 5.2%.

We found no significant change in mean optical pathlength during the study. Therefore, if $\Delta[\mathrm{oxCCO}]_{\mathrm{meas}}$ was an artifact of $\Delta[\mathrm{HbO_2}]$ and $\Delta[\mathrm{HHb}]$, then a relationship between $\Delta[\mathrm{oxCCO}]_{\mathrm{meas}}$, $\Delta[\mathrm{HbO_2}]$, and $\Delta[\mathrm{HHb}]$ derived from the hypoxemia part of the study should also apply during the recovery period. If this were the case, $\Delta[\mathrm{oxCCO}]_{\mathrm{pred}}$ for the recovery period derived using Eq. (3) would not differ from $\Delta[\mathrm{oxCCO}]_{\mathrm{meas}}$ during recovery. $\Delta[\mathrm{oxCCO}]_{\mathrm{pred}}$ and $\Delta[\mathrm{oxCCO}]_{\mathrm{meas}}$ were different (Fig. 4), suggesting that $\Delta[\mathrm{oxCCO}]_{\mathrm{meas}}$ is not merely a crosstalk artifact resulting from the large changes in $\Delta[\mathrm{HbO_2}]$ and $\Delta[\mathrm{HHb}]$. However, additional modelling and experimental studies are required to further investigate the use of the UCL*n* algorithm to detect changes in the CCO signal in a multilayer system, and we are addressing this issue using a combination of continuous wave and phase-resolved spectroscopy together with knowledge of the CCO concentrations in the various cranial layers and their respective optical characteristics.

We are currently studying patients with traumatic brain injury to investigate the response of the NIRS CCO signal to periods of intracranial perturbation. NIRS provides the opportunity to make regional measurements of brain metabolism, making probe positioning less critical than in hyperfocal measurements made by invasive techniques, such as cerebral microdialysis, while still retaining the ability to target the tissue at greatest risk of secondary injury: a feature lost when using global measures such as jugular venous oximetry. We aim to show that NIRS measurement of CCO redox changes in patients with brain injury is a useful, noninvasive real-time marker of alterations in mitochondrial oxygen availability. Identification of failing mitochondrial metabolism might then enable NIRS measurement of $\Delta[\mathrm{oxCCO}]$ to guide neuroprotective treatment strategies. The work described in this paper is an essential step toward understanding CCO signal changes in the injured brain.

5 Conclusion

We described, for the first time, the quantification of cerebral $\Delta[\mathrm{oxCCO}]$ during hypoxemia in healthy adults and showed that this measurement provides a marker of reduced cellular oxygen availability in healthy humans. We demonstrated a protocol that produces $\Delta[\mathrm{oxCCO}]$ and provides an ideal paradigm for the *in vivo* development of NIRS algorithms and instrumentation.

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