

Normobaric hyperoxia increases cerebral aerobic metabolism after traumatic brain injury

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Abstract

Objects: Traumatic brain injury (TBI) is associated with depressed aerobic metabolism and mitochondrial dysfunction. Normobaric hyperoxia (NBH) has been suggested as a treatment for TBI but human studies have produced equivocal results. We used brain tissue oxygen tension (P_{brO_2}) measurement, cerebral microdialysis and near infrared spectroscopy to study the effects of NBH after TBI. We investigated the effects on cellular and mitochondrial redox state, measured by brain tissue lactate:pyruvate ratio (LPR) and change in oxidized cytochrome c oxidase concentration ($\Delta[oxCCO]$) respectively.

Methods: We studied eight adult human patients with TBI within the first 48 hours post injury. Inspired oxygen percentage (FiO_2), at normobaric pressure, was increased from baseline to 60% for 60 minutes and then to 100% for 60 minutes before being returned to baseline for 30 minutes. Results are presented as median (interquartile range). During the 100% FiO_2 phase, P_{brO_2} increased by 7.2 kPa (4.5-9.6) ($p < 0.0001$), microdialysate lactate concentration decreased by 0.26 mmol/l (0.0-0.45) ($p = 0.01$), microdialysate LPR decreased by 1.6 (1.0-2.3) ($p = 0.02$) and $\Delta[oxCCO]$ increased by 0.21 $\mu\text{mol/l}$ (0.13-0.38) ($p = 0.0003$). There were no significant changes in intracranial pressure, or arterial, or microdialysate, glucose concentration. $\Delta [oxCCO]$ correlated with changes in P_{brO_2} ($r_s = 0.57$ $p = 0.005$) and change in LPR ($r_s = -0.52$ $p = 0.006$).

Conclusions: We have demonstrated oxidation in cerebral cellular and mitochondrial redox state during NBH in adult patients with TBI. These findings

are consistent with increased aerobic metabolism and suggest that NBH has the potential to improve outcome after TBI. Further studies are warranted.

Introduction

Traumatic brain injury (TBI) is responsible for approximately 500,000 hospital admissions and 17,500 deaths in the United States per year and, as it predominantly affects young people, it results in a huge socioeconomic burden³⁰. TBI describes a heterogeneous set of injury mechanisms and pathologies but there are common metabolic pathways leading to depressed aerobic metabolism, reduced cellular adenosine triphosphate (ATP) production, inability to maintain ionic homeostasis and ultimately cell death^{1, 37, 46, 47}. The exact etiology of this cellular energy failure is poorly understood but both reduced substrate delivery and impaired mitochondrial substrate utilization appear to be implicated.

Using a cerebral fluid percussion insult in the cat, Alves *et al* demonstrated that TBI induces cerebral hypoxia despite unchanged arterial oxygen tension and arterial blood pressure¹ suggesting that reduced ATP production after TBI may be in part related to mitochondrial hypoxia. It is well established that hypotension and hypoxemia, are associated with poor functional outcome after TBI⁶ and so, within the context of attempting to minimize secondary injury after TBI, it appears vital to ensure adequate oxygen delivery to cerebral mitochondria, particularly in the early stages post TBI when reduced cerebral blood flow (CBF) increases the risk of cerebral hypoxia³.

Hyperoxia has been investigated as a potential treatment strategy for increasing aerobic metabolism after TBI and hyperbaric hyperoxia (HBH) in particular has

shown beneficial effects in both animals and humans^{9, 34, 35}. However chambers capable of delivering HBH to critically ill patients are expensive and availability is severely limited. Interest has therefore grown in the use of normobaric hyperoxia (NBH) which is cheap and simple to administer. Studies investigating the use of NBH in adults after TBI have consistently shown increases in brain oxygen tension (P_{brO_2}) and reductions in microdialysis measured brain tissue lactate concentration^{22, 28, 43} but interpretation of these findings is controversial. Some investigators conclude that they support a beneficial role for NBH⁴³ while others suggest that NBH may be detrimental²².

Cerebral microdialysis is an established technique that allows focal measurement of brain tissue biochemistry and is becoming incorporated into routine multimodality monitoring on the neurointensive care unit (NCU)⁴¹. Raised microdialysate lactate concentration is associated with tissue hypoxia and poor outcome after TBI¹⁴. However it reflects not only the degree of anaerobic metabolism but also the global glycolytic rate⁴¹. Lactate:pyruvate ratio (LPR) is considered a superior marker of anaerobic metabolism and is a measure of cellular redox state^{32, 41}. However, clinical TBI studies to date have shown no changes in LPR and this has contributed to the controversy surrounding interpretation of the resulting data.

Broadband near infrared spectroscopy (NIRS) is a non-invasive technique which measures the attenuation of light by tissue at multiple wavelengths. It exploits the

fact that biological tissue is relatively transparent to near infrared (NIR) light between 700-900 nm, allowing interrogation of the cerebral cortex by optodes placed on the scalp³¹. Biological tissue is a highly scattering medium and this complicates the calculation of chromophore concentration. However, if the average pathlength of light through tissue is known, the modified Beer-Lambert law, which assumes constant scattering losses, allows calculation of absolute changes in chromophore concentration. The *in vivo* use of NIR spectroscopy (NIRS) was first described by Jöbsis in 1977¹⁷, and has been used in animals and humans to measure change in concentration of oxy-hemoglobin ($\Delta[\text{HbO}_2]$), deoxy-hemoglobin ($\Delta[\text{HHb}]$), and oxidized cytochrome c oxidase ($\Delta[\text{oxCCO}]$)^{18, 25, 38, 40, 42}. Cytochrome c oxidase (CCO) is the terminal electron acceptor of the mitochondrial electron transfer chain. As electrons pass along the electron transfer chain, protons are pumped out of the mitochondrial matrix and into the intermembrane space against their concentration gradient, thus providing the driving force for ATP synthesis (figure 1). CCO therefore plays a crucial role in the dynamics of cellular oxygen utilization and energy production³³, with the movement of electrons down the mitochondrial respiratory chain (via redox reactions) to oxygen resulting in >95% of cellular oxygen utilization. CCO contains a unique Cu-Cu dimer (termed CuA) that is a strong NIR absorber at 830nm. If the total concentration of CCO remains constant during a study, changes in the NIRS CCO signal represent changes in the CCO redox state. This reflects the balance between electron donation from cytochrome c and oxygen reduction to water. In animals, broadband NIRS measured cerebral

$\Delta[\text{oxCCO}]$ has been validated as a marker of cellular energy status against magnetic resonance spectroscopy measured reduction in nucleoside triphosphate levels³⁹. It has also recently been shown to correlate with estimated change in cerebral oxygen delivery during hypoxemia in healthy adult humans⁴². Animal models indicate that CCO mediated oxidative metabolism is decreased after TBI and that this effect may last up to 10 days post injury¹⁵. Changes in CCO activity after TBI may therefore have an important effect on the ability of mitochondria to metabolize ATP aerobically and it is possible to investigate these changes non-invasively using NIRS.

We hypothesize that NBH will cause oxidation in cerebral cellular and mitochondrial redox state in adult patients in the early period post TBI.

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 and, as all patients were unconscious at the time of the study, written assent was obtained from their personal representatives. Inclusion criteria were a diagnosis of TBI requiring sedation and ventilation on the NCU and age >16 years. Exclusion criteria were the expectation of death or weaning of sedation within 24 hours of injury, a baseline $\text{FiO}_2 \geq 60\%$ or more than 48 hours elapsing between the time of injury and the start of the study.

Monitored Variables

Multimodality monitoring was instigated as part of routine care. Invasive intracranial pressure (ICP) (Microsensor, Codman, MA, USA), PbrO₂ (Licox PMO, Integra Neurosciences, NJ, USA) and cerebral microdialysis (CMA 70 or 71, CMA/Microdialysis, Solna, Sweden) catheters were inserted into the brain interstitium through a three divergent lumen skull bolt (Technicam Ltd, Newton Abbott, UK). Microdialysis catheters were targeted to the pericontusional white matter following the recommendations of the consensus meeting on microdialysis in neurointensive care². The microdialysis catheters were perfused with Perfusion Fluid CNS (CMA/Microdialysis), at a rate of 0.3 µl/min. No data were collected in the first 4 hours after catheter placement to avoid insertion artefacts. Catheter position was subsequently confirmed with radiological imaging. Microdialysate glucose, lactate and pyruvate concentrations were measured at the bedside using a CMA 600 analyzer (CMA/Microdialysis). Mean blood flow velocity in the basal middle cerebral artery (Vmca) ipsilateral to the NIRS optodes was measured using 2 MHz transcranial Doppler ultrasonography (Nicolet, UK), as a surrogate of CBF⁴⁴.

For the purposes of this study, broadband spectrometer optodes were placed 3.5 cm apart in a black plastic holder and fixed to the upper forehead in the midpupillary line ipsilateral to the invasive cerebral monitoring. Light from a stabilized tungsten halogen light source was filtered with 610nm long-pass and heat absorbing filters, and transmitted to the head via a 3.3mm diameter glass

optic fiber bundle⁴². Light incident on the detector optode was focused via an identical fiber bundle onto the 400 μm entrance slit of a 0.27m spectrograph (270M, Instruments SA, France) with a 300g/mm grating. NIR spectra between 650 and 980 nm were collected on a cooled charge coupled device detector (Wright Instruments, UK) giving a spectral resolution of $\sim 5\text{nm}$.

Study Protocol

All patients received local protocolized ICP and cerebral perfusion pressure (CPP) therapy, based on the Joint Section of Neurotrauma and Critical Care of the American Association of Neurological Surgeons⁵ and the European Brain Injury Consortium guidelines²¹. After 30 minutes of baseline data collection, the inspired oxygen percentage (FiO_2) was increased to 60% for 60 minutes and then to 100% for 60 minutes before being returned to baseline for a further 30 minutes. Cerebral microdialysate specimens were collected and analyzed at intervals of 15 minutes and arterial blood gas (ABG) and glucose concentrations were measured at intervals of 30 minutes, commencing 15 minutes after the start of the baseline period. There is an inherent delay associated with cerebral microdialysis monitoring which relates to diffusion of metabolites into the extracellular space and across the catheter membrane, and the time taken to collect the microdialysate. Therefore, a final microdialysis sample was collected 75 minutes after the FiO_2 was returned to baseline. A schematic of the study protocol is shown in figure 2.

Data Analysis

All monitored variables were collected to a PC and synchronized. Absolute $\Delta[\text{oxCCO}]$, $\Delta[\text{HbO}_2]$ and $\Delta[\text{HHb}]$ were calculated from changes in light attenuation using a multiple regression technique termed the UCL n algorithm²⁴. Correction factors for the wavelength dependence of the optical pathlength were applied to the chromophore absorption coefficients. Individual optical pathlength was calculated continuously using second differential analysis of the 740 nm water feature of the spectral data^{23, 42}. Change in total hemoglobin concentration ($\Delta[\text{HbT}]$), which can be converted to changes in cerebral blood volume (CBV)¹¹, was defined as $\Delta[\text{HbO}_2] + \Delta[\text{HHb}]$ and change in hemoglobin difference concentration ($\Delta[\text{Hbdiff}]$), which represents changes in the balance of oxy- and deoxy-hemoglobin, was defined as $\Delta[\text{HbO}_2] - \Delta[\text{HHb}]$ ¹⁹.

Summary data were produced for the four phases of the study: baseline, 60%, 100% and return to baseline (baseline return) FiO_2 . For the initial and final baseline periods 15 minute means for each variable were calculated, centered on the time of ABG sampling within that phase. For the 60% and 100% FiO_2 phases, means of the data between the two ABG samples within that phase were calculated (figure 2). Where not otherwise stated, results are shown as median and inter-quartile range. Statistical analysis was carried out using SAS software (v9.1, SAS Institute, USA) and p values <0.05 were considered significant. Group changes were compared with baseline using non-parametric ANOVA and *post hoc* pairwise comparisons. Correlations between variables were assessed using

Spearman rank correlation analysis with Bonferoni corrected two-tailed tests of significance.

Results

Eight adult patients with median age 42 yrs (range 20-61) were recruited into the study. Patient demographics and details of presenting pathology are shown in table 1. PbrO₂ monitoring failed in one patient due to inadvertent catheter removal. All other monitoring modalities were successfully collected in all patients. The median time between the injury and the start of the study was 25 hours (range 22-47 hours).

Baseline values for the measured variables are shown in table 2 and summary changes from baseline in figure 2. Increase in FiO₂ to 60% and 100% was associated with an increase in PaO₂ of 16.5 (8.2-18.5) and 43.3 (33.8-45.5) kPa respectively (p<0.01), but there was no change in Vmca or [HbT]. There was also no change in ICP or CPP during NBH but a reduction in CPP from baseline of 2.9 (1.5-9.5) mmHg during the baseline return phase (p<0.05). PbrO₂ increased by 2.0 (0.9-2.4) kPa during the 60% FiO₂ phase and by 7.2 (4.5-9.6) kPa during the 100% FiO₂ phase (p<0.0001). Arterial and microdialysate glucose concentrations were unchanged during the study. Microdialysate lactate concentration decreased by 0.26 (0.0-0.45) mmol/l (p=0.01) during the 100% FiO₂ phase and was still significantly reduced (by 0.31 (0.17-0.71) mmol/l below baseline) during the baseline return phase (p<0.001). LPR was reduced by 1.6 (1.0-2.3) (p=0.02)

during the 100% FiO₂ phase but there was no significant change at FiO₂ 60%. The reduction in LPR was also maintained beyond the period of NBH, being 2.4 (0.2-3.4) below baseline ($p < 0.05$) during the baseline return phase. Lactate concentration and LPR were not significantly different from baseline by the time of the final microdialysate sampling 75 minutes after the end of NBH. $\Delta[\text{Hbdiff}]$ increased by 2.13 (1.92-4.25) mmol/l and 6.45 (3.98-9.36) during the 60% and 100% FiO₂ phases respectively ($p < 0.001$) and $\Delta[\text{oxCCO}]$ increased by 0.21 (0.13-0.3) during the 100% FiO₂ phase ($p = 0.0003$). Change in $[\text{oxCCO}]$ correlated with changes in PbrO_2 ($r_s = 0.57$ $p = 0.005$) and change in LPR ($r_s = 0.53$ $p = 0.006$).

Discussion

This is the first report of combined microdialysis and broadband NIRS monitoring of changes in cerebral redox state in patients with TBI. Our results demonstrate oxidation in cerebral cellular and mitochondrial redox state during NBH in the first 48 hours post injury. This finding is consistent with the significant regional ischemia that occurs in the acute phase post TBI⁷ and suggests that the patients in this study were suffering a degree of mitochondrial hypoxia at baseline. The oxidation in cerebral redox state that we observed is likely to be associated with increased aerobic metabolism and NBH therefore has the potential to increase cell survival after TBI.

There are several mechanisms whereby NBH might improve brain cellular metabolism in patients with severe TBI. An increase in arterial oxygenation will improve oxygen delivery as measured by an increase in P_{brO_2} . However, oxygen delivery is determined by both arterial oxygen content (CaO_2) and CBF. CaO_2 is determined mainly by hemoglobin saturation and, if hemoglobin is already well saturated, the small contribution from the additional amount of dissolved oxygen during NBH is unlikely to have a significant impact on overall oxygen delivery. Alternatively, NBH might improve cerebral metabolic consumption of oxygen ($CMRO_2$) by improving the brain's ability to utilize the delivered oxygen. This might be because impaired mitochondria require a higher PO_2 to function or alternatively because an increased oxygen tension gradient is required to drive oxygen across edematous tissue to reach the mitochondria²⁷. Oxygen tension has both direct and indirect modulatory effects on oxidative metabolism and a variety of pathways may be implicated. Intriguingly, an increasing body of literature has demonstrated the ability of nitric oxide (NO), which is implicated in the pathobiology of TBI, to inhibit oxygen binding to CCO, and hence its subsequent reduction, by competing for the oxygen binding site in a reversible manner²⁹. Raised cerebral NO levels are present after TBI and might therefore contribute to mitochondrial dysfunction⁴⁷. It is possible that elevated mitochondrial oxygen tension might antagonize the effects of NO and therefore favor the binding of oxygen and its subsequent reduction, but this mechanism remains hypothetical at present.

PbrO₂ reflects the balance between *tissue* oxygen delivery and utilisation¹. In agreement with several other investigators we found that PbrO₂ increased during NBH, indicating increased cerebral oxygen availability^{26, 28, 45}. Δ[Hbdiff] represents changes in the balance between arterial oxygen delivery and oxygen offloading to tissue in the context of stable CBF and CBV. As we observed no changes in Vmca or [HbT] during NBH, it can be assumed that cerebral hemodynamics were stable during the study period. The increase in Δ[Hbdiff] during NBH therefore suggests improvement in the balance between oxygen delivery and demand and this is further supported by the associated increase in PbrO₂.

The decrease in microdialysate lactate concentration that we observed is similar in direction and magnitude to that found by other workers and is likely to represent improvement in tissue hypoxia during NBH^{22, 28, 43}. Microdialysate LPR is a marker of cellular redox state and reflects the nicotinamide adenine dinucleotide (NAD) to reduced-NAD ratio, and the degree of aerobic metabolism³⁷. In contrast to previous studies, we observed a significant reduction in microdialysate LPR during NBH, suggesting an increase in aerobic metabolism. The changes in microdialysate variables that we report are small and it is not possible to ascertain their clinical significance from these data. Nevertheless this pilot study protocol utilized a relatively short (2 hour) period of NBH and similar changes in microdialysate lactate concentration during NBH have been associated with improved outcome after TBI⁴³. There was no change

in arterial or microdialysate glucose concentrations during the study. However, in contrast to the arterial measurements, there was a trend toward reduced microdialysate glucose concentration during NBH and this might represent increased cerebral glucose utilization.

We observed an increase in $\Delta[\text{oxCCO}]$ during NBH that returned to baseline by the end of the study. There was a positive correlation between $\Delta[\text{oxCCO}]$ and change in PbrO_2 and a negative correlation between $\Delta[\text{oxCCO}]$ and change in LPR. These changes in cellular and mitochondrial redox state during NBH indicate an increase in electron transfer from CCO to oxygen, thus favoring increased flux through the mitochondrial electron transfer chain and increased aerobic metabolism. In combination with the $\Delta[\text{Hbdiff}]$, these data suggest that increased arterial and tissue oxygen delivery is driving an increase in oxygen utilization and ATP production. However, simultaneous measurements of CMRO_2 and ATP concentration are needed to confirm this hypothesis.

$\Delta[\text{oxCCO}]$ in the human brain has not previously been compared with other markers of cellular redox state. The correlation between non-invasive regional (NIRS), and invasive focal (cerebral microdialysis), measures of changes in cerebral redox state suggest that the NIRS changes that we are recording are related to cellular metabolism. The time course of the microdialysate data appears to differ from that of the PbrO_2 and NIRS data. This may contribute to the correlation between change in PbrO_2 and change in LPR failing to reach

significance after the Bonferoni correction and influence the strength of the relationship between changes in PbrO_2 and $[\text{oxCCO}]$.

HBH has been shown to improve cerebral metabolism and outcome, but studies of NBH have produced variable results. In a fluid percussion injury model in rats, HBH alleviated injury-induced reduction in mitochondrial redox and increased cerebral oxygen consumption⁹. In a randomized controlled clinical trial, Rockswold *et al* found that HBH reduced mortality after TBI without increasing the number of patients with favorable outcome³⁴. In a further study by the same group, HBH reduced CSF lactate concentrations and this effect lasted for six hours after the end of the treatment period³⁵. However, CSF pyruvate was not measured in this study so LPR could not be calculated. In the clinical situation of TBI, NBH has been more widely investigated. Menzel *et al* reported reduced microdialysate lactate levels in TBI patients treated with NBH²⁸. In a later study, the same group confirmed reduced microdialysate lactate concentrations but found no significant change in LPR during a 24 hour period of 100% FiO_2 commenced within the first 6 hours post injury, with patients acting as their own controls⁴³. In this study NBH resulted in decreased mortality compared to historic controls. Magnoni *et al* also reported reduced microdialysate lactate and no significant changes in LPR after NBH in patients with TBI, but interestingly found no change in cerebral arterio-venous oxygen difference²². These findings were interpreted by the authors as indicating no change in oxidative glucose metabolism during NBH.

Our study differs from others in several respects. Firstly we positioned our microdialysis catheters in the more affected cerebral hemisphere, and targeted the pericontusional brain tissue², using post procedural imaging to ensure that the catheter tip was not placed within a contusion itself. In the study by Tolias *et al* microdialysis catheters were placed in the least affected hemisphere⁴³ whereas in the study by Magnoni *et al* the catheter positioning is unclear²². Oxidative depression after TBI is primarily restricted to the ipsilateral cerebral cortex¹⁵ and positioning of microdialysis catheters has a critical effect on metabolite microdialysate concentrations¹². Secondly there are differences in the timing of the studies and the duration of NBH treatments. In particular, the study by Magnoni *et al* enrolled patients up to 79 hours post injury and studied them for several subsequent days²². The potential for cerebral hypoxia is most likely early after TBI³ and there may be a critical time window for NBH treatment. In our patient group all studies commenced less than 48 hours post injury.

If hyperoxia improves mitochondrial function and cerebral aerobic metabolism, CMRO₂ will increase. We did not measure CMRO₂ so are unable to comment further on this issue in relation to our study. However, in a previous study investigating the impact of HBH (100% oxygen at 1.5 atm) on cerebral metabolism, there was a modest improvement in global CMRO₂ in the 15% of patients with reduced CBF prior to treatment³⁵. In contrast to findings in HBH, current evidence reporting the effect of NBH on CMRO₂ after TBI is inconclusive.

In their study, Magnoni *et al*/reported a non-significant reduction in arterio-venous oxygen content difference during NBH²², but interpretation of these findings is difficult because CBF was not measured and venous oxygen content was assessed using jugular bulb venous oximetry, which provides a global measure and may miss important regional effects. Conversely animal studies reveal increased CMRO₂ in response to NBH after TBI²⁰. More recently, Diring *et al* examined the direct effect of NBH on cerebral metabolism, assessed using positron emission tomography, in five patients with severe TBI¹⁰. This study is the first to present data directly measuring CMRO₂ during NBH and, on the face of it, seems to indicate that there is no role for this treatment. However, this study measured global CMRO₂ and regional changes might have been missed. It is also possible that NBH might improve outcome in severe TBI through mechanisms that are not reflected in a measurable increase in CMRO₂¹³. Further clinical research is therefore needed in larger numbers of patients to establish whether NBH is of therapeutic value after TBI.

In the normal brain, at least, hyperoxia is generally believed to cause vasoconstriction but we found no changes in Vmca, CBV or ICP during NBH in our study. Although there was a statistically significant reduction in CPP during the baseline return phase, we do not believe that this was clinically significant and, in any case, the lowest CPP lay within the range allowed in our management protocols. Simple explanations for the lack of evidence of cerebral vasoconstriction in our study are the placement of the microdialysis and NIRS

monitors to target the more injured areas of brain, with our findings being consistent with impaired autoregulation within the regions of interest. Alternatively the slight increase in PaCO₂ that we recorded, which in isolation would tend to cause vasodilatation and increase in ICP, might have counteracted any effects of hyperoxic vasoconstriction. However, the absence of evidence of vasoconstriction in our study is also likely to be related to the complex response of the injured brain to NBH¹. Tolias *et al* reported a reduction in ICP during NBH in patients with TBI⁴³ but Rockswold *et al* found that CBF and ICP were only decreased during NBH in those with elevated baseline CBF and that CBF increased during HBH in patients in whom CBF was reduced or normal prior to treatment³⁵. Once again, when interpreting these data, it is important to bear in mind the significant metabolic heterogeneity that exists after TBI.

Microdialysis provides a hyperfocal measurement of brain tissue biochemistry but does not identify metabolic changes in tissue distant from the catheter. As the perfusate is not static there is insufficient time for equilibrium to occur across the membrane and the concentration of metabolites in the microdialysate therefore only represents a fraction of the true brain tissue concentration. This fraction is termed the relative recovery. Relative recovery has been calculated for the metabolites analyzed in our study and has been shown to be equivalent for the CMA 70 and 71 catheters used in this study¹⁶. As lactate and pyruvate have similar molecular weights, LPR is not affected by changes in relative recovery and this is one advantage of this measurement over other microdialysis

variables. In common with other investigators we demonstrated a prolonged effect of NBH on microdialysate variables, lasting beyond the period of NBH. There is an inherent delay involved in microdialysis monitoring of cellular redox state and although we applied a timing correction to account for the time taken for perfusate to pass along the catheter tubing the metabolite concentrations measured represent an average over the time of sampling. A delay may also exist related to the diffusion distance between the cellular and extracellular spaces. However, in our study microdialysate lactate concentration and LPR were not significantly different from baseline values by 75 minutes after the end of the hyperoxygenation period.

Historically, the algorithms used to separate the *in vivo* CCO and hemoglobin signals have been the source of some controversy³⁶. Although COO is a strong NIR absorber it is present in much lower concentrations in the tissue (~5.5 μ mol/l measured in the adult rat brain) than those of oxy- and deoxy-hemoglobin⁴. This can result in difficulty in separating the CCO and hemoglobin signals, a phenomenon known as crosstalk. Commercially available NIRS systems use only a small number of wavelengths and this makes cross talk more likely. The broadband instrumentation that we used in this study uses 120 wavelengths and this approach, in conjunction with the UCLn algorithm, has been shown to produce minimal crosstalk in modeling studies²⁴. Furthermore animal studies using mitochondrial inhibitors show that NIRS Δ [oxCCO] measurements are stable during large contemporaneous Δ [HbO₂] and Δ [HHb]⁸. Data from studies in

humans during hypoxemia and severe orthostatic hypotension also suggest that the NIRS measured cerebral $\Delta[\text{oxCCO}]$ signal changes independently of $\Delta[\text{HbO}_2]$ and $\Delta[\text{HHb}]$ ^{40, 42}. We continuously measured optical pathlength during this study and, although there was a small change during the 100% FiO_2 phase of the study, all data were corrected for pathlength changes. The potential clinical application of NIRS measurement of $\Delta[\text{oxCCO}]$ has been identified by studies correlating $\Delta[\text{oxCCO}]$ with post-operative neurological dysfunction in patients undergoing cardiac surgery¹⁸ and during severe reductions in SaO_2 associated with sleep apnea²⁵. We have demonstrated oxidation in CCO during NBH and this implies that CCO is not fully oxidized in the early stages after severe TBI. Similarly oxidation in CCO above the resting state has been shown in healthy animals³⁸ and humans⁴² in the recovery period following hypoxemia. NIRS therefore provides the opportunity to make continuous, non-invasive, multi-site measurements of changes in regional oxygenation and mitochondrial redox state at the bedside.

NBH has potentially toxic effects on the lungs, eyes and central nervous system and, although the doses and duration of treatment required to produce these effects are not clearly defined and are likely to vary between individuals, oxygen toxicity is unlikely to occur when breathing 100% FiO_2 for less than 24 hours¹. There is evidence to suggest increased free radical production when breathing air at high pressure (3 atmosphere) but none showing increased free radical levels at 1.5 atmosphere or less⁹. Whilst hyperoxygenation has the theoretical

capacity to increase free radical production, it is also possible that by promoting electron flux through the electron transfer chain, it might prevent the formation of free radicals produced by build up of reducing equivalents. Oxygen toxicity is extremely unlikely following the regime applied during this study but further work is required to investigate the potential risks of longer periods of NBH after TBI.

This pilot study has several limitations. We studied only a small number of TBI patients and further data collection in a large cohort of patients is required to validate these findings. Vmca is a surrogate marker of CBF and relies on there being no significant changes in middle cerebral artery caliber during the study. Continuous bedside measurement of absolute CBF, in conjunction with measurements of arterial and venous oxygen content difference and calculation of CMRO₂, would aid further investigation of NBH. In this study patients acted as their own controls and it is possible that the changes that we observed might have been influenced by the natural course of TBI. However, we do not believe this is the case as all measured variables returned towards, or reached, baseline values by the end of the study. Furthermore, changes in cerebral metabolism unrelated to our interventions are likely to have been minimal as systemic variables were stable over the time course of this study. The significant disease heterogeneity that exists within the diagnosis of TBI makes it extremely difficult, if not impossible, to identify control cohorts adequately matched for disease type and severity within a dataset of the size that we present. There was a relatively high mortality rate in our study population and we believe that this is likely to be

related to our selection of patients for cerebral microdialysis monitoring. In our centre, patients in whom sedation is stopped shortly after admission following either conservative or surgical management are not monitored using cerebral microdialysis and patients included in this study therefore represent the more severely injured end of the spectrum of our TBI admissions.

Conclusions

We have demonstrated oxidation in cerebral cellular and mitochondrial compartments during NBH in patients with TBI using two independent monitoring techniques. Cerebral microdialysis and NIRS monitoring provide complementary information which can further our understanding of TBI pathophysiology. It might also be possible to use these techniques to guide targeted treatment strategies. Our results suggest that NBH has the potential to improve outcome after TBI and further investigation is warranted.

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Legends to Figures

Figure 1: A simplified scheme of the electron transfer chain (modified from⁴⁷). cyt c - cytochrome c.

Figure 2: Study and analysis protocol for 8 patients with traumatic brain injury during normobaric hyperoxia. ABG - arterial blood gas sampling, MD - microdialysate sampling, FiO₂ - inspired oxygen percentage, BL - baseline, NIRS - near infrared spectroscopy.

Figure 3: Group median and interquartile range for absolute values of FiO₂ and changes from the initial baseline for all other variables in seven patients with traumatic brain injury during normobaric hyperoxia. FiO₂ - inspired oxygen percentage, Δ SaO₂ - change in arterial oxyhemoglobin saturation, Δ PaO₂ - change in arterial oxygen tension, Δ PbrO₂ - change in brain tissue oxygen tension, Δ PaCO₂ - change in arterial carbon dioxide tension, Δ ICP - change in intracranial pressure, Δ CPP - change in cerebral perfusion pressure, Δ A[Gluc] - change in arterial glucose concentration, Δ MD[Gluc] - change in microdialysate glucose concentration, Δ MD[Lac] - change in microdialysate lactate concentration, Δ MD LPR - change in microdialysate lactate pyruvate ratio, Δ [Hbdiff] - change in hemoglobin difference concentration, Δ [HbT] - change in total hemoglobin concentration, Δ [oxCCO] - change in oxidized cytochrome c oxidase concentration.

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Table 1

Demographic data and details of presenting pathology

<i>Patient</i>	<i>Age</i>	<i>Sex</i>	<i>Injury Mechanism</i>	<i>Pathology</i>	<i>GCS</i>	<i>Outcome</i>	<i>Injury to Study Time (hrs)</i>
1	42	Male	Assault	EDH	7	Survived	25
2	61	Male	Fall	Contusions	8	Died	29
3	45	Male	Fall	ASDH	7	Died	47
4	22	Male	Assault	ASDH	11	Survived	22
5	44	Male	Fall	Contusions	7	Died	25
6	27	Male	Unknown	EDH	8	Died	24
7	20	Male	RTA	EDH	7	Survived	25
8	34	Male	Fall	ASDH	4	Survived	43

Table 2

Baseline values for measured variables in patients with traumatic brain injury (n=8) prior to institution of normobaric hyperoxia

	<i>FiO₂</i>	<i>SaO₂</i>	<i>PaO₂</i>	<i>PbrO₂</i>	<i>PaCO₂</i>	<i>ICP</i>	<i>CPP</i>	<i>A[Gluc]</i>	<i>MD[Gluc]</i>	<i>MD[Lac]</i>	<i>MD</i>
	(%)	(%)	(kPa)	(kPa)	(kPa)	(mmHg)	(mmHg)	(mmol/l)	(mmol/l)	(mmol/l)	LPR
Median	28.3	99.0	14.40	1.82	4.46	18.8	67.0	5.80	2.49	3.36	22.7
Q1	25.4	97.8	12.0	1.16	4.35	11.9	63.5	5.58	1.55	2.61	16.5
Q3	31.0	99.3	15.4	3.03	4.52	24.4	69.8	6.75	3.37	4.34	26.6

FiO₂ - inspired oxygen percentage, SaO₂ - arterial oxyhemoglobin saturation, PaO₂ - arterial oxygen tension, ΔPbrO₂ - change in brain tissue oxygen tension, PaCO₂ - arterial carbon dioxide tension, ICP - intracranial pressure, CPP - cerebral perfusion pressure, A[Gluc] - arterial glucose concentration, MD[Gluc] - microdialysate glucose concentration, MD[Lac] - microdialysate lactate concentration, MD LPR - microdialysate lactate pyruvate ratio

Figure 1

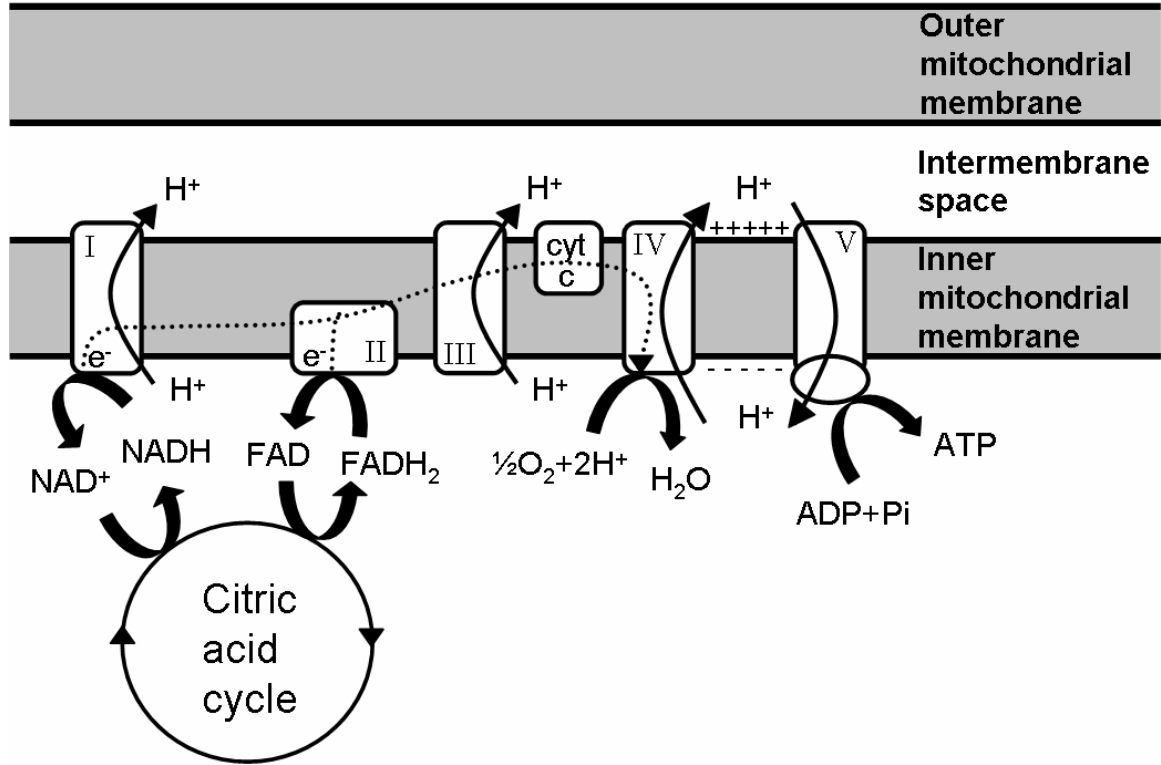


Figure 2

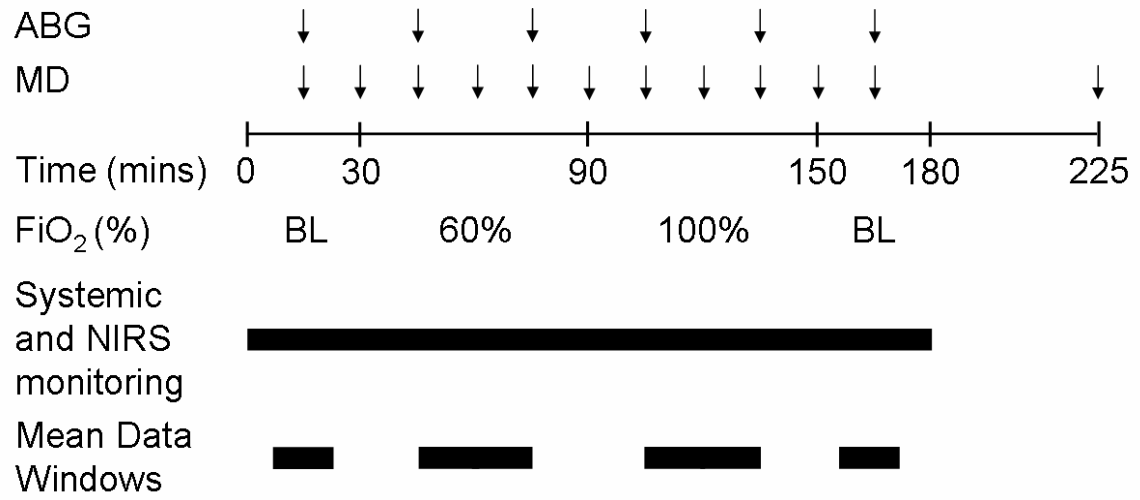


Figure 3

