REVIEW

Haemoglobin oxygen saturation as a biomarker: the problem and a solution

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Near-infrared spectroscopy measures of haemoglobin oxygen saturation are often used as an indicator of sufficient oxygen delivery to assess injury susceptibility and tissue damage. They have also often been used as a surrogate measure of oxygen metabolism. Unfortunately, these measures have generally failed to provide robust indicators of injury and metabolism. In this paper, we first review when haemoglobin oxygen saturation does work as a robust indicator, and then detail when and why it fails for assessing brain injury and breast cancer. Finally, we discuss the solution to obtain more robust measures of tissue injury and cancer by combining oxygen saturation measurements with measures of blood flow and volume to more accurately estimate oxygen metabolism.

Keywords: near-infrared spectroscopy; haemoglobin oxygen saturation; oxygen metabolism; muscle; breast; brain

1. Introduction

Oxidative metabolism provides more than 90 per cent of ATP production in aerobic organisms. A sensor of cellular oxygen and oxygen utilization has long been sought because of its importance in studying cellular energetics. A non-invasive oxygen sensor would have significant clinical utility for assessing tissue injury and disease that give rise to impaired oxygen delivery and/or utilization, and would be important for following tissue response to therapy. In 1977, Jobsis [1] first exploited the weak absorption of tissue in the near infrared to measure cytochrome c oxidase (CCO) in an intact organ, the feline brain, thus initiating the development of near-infrared spectroscopy (NIRS). Cytochrome oxidase is an important oxygen sensor because it reacts directly with oxygen at the terminus of the cellular respiratory chain. An insufficient supply of oxygen would lead to a...
build-up of reduced CCO. Oxidized CCO has an absorption band around 840 nm that decreases when CCO is reduced. Thus, it appeared that NIRS would provide the long-sought non-invasive oxygen sensor.

The technique for measuring CCO was quickly translated to the clinical environment to assess whether neonates in the intensive care unit had a sufficient supply of oxygen to the brain [2]. It was observed that hypoxia in these infants led to a more reduced state of CCO, as expected. Following this work, several other groups started using NIRS in the clinic [3,4]. In order to estimate the oxidized or reduced state of CCO, it was also necessary to untangle the contributing effects of oxygenated and deoxygenated haemoglobin (HbO and HbR, respectively) to the near-infrared absorption spectrum. This is a challenging task because of the low in vivo concentration of CCO relative to haemoglobin. But by measuring the light absorption at several wavelengths and correcting for the path length of light through the tissue at the different wavelengths, it is in principle possible to separate the spectroscopic signatures of the chromophores and estimate their in vivo concentrations [5]. By 1995, several different algorithms had been developed for estimating the concentrations with path length correction factors that varied with the particular application. This raised concerns that using the wrong algorithm could give rise to artefacts in the estimated CCO concentration. This was evidenced by Skov & Greisen [6], who found that the switch to cardiopulmonary bypass in hypoxaemic children with congenital heart disease led to a decrease in oxidized CCO, despite an improvement in cerebral oxygen delivery as indicated by an increase in the cerebral oxygenation index (i.e. HbO – HbR). They suggested that this non-physiological finding was probably a result of using an incorrect algorithm that did not accurately separate the confounding effects of HbO and HbR and total haemoglobin concentration (HbT = HbO + HbR) when estimating CCO. Matcher et al. [7] quantified the errors that arise from using the wrong algorithm, which supported the growing sense that in vivo quantification of CCO was not reliable.

While a few researchers continue the effort to improve the reliability of in vivo CCO quantification through multi-spectral measurements that robustly estimate and correct for wavelength-specific path length factors [8,9], NIRS use shifted to measuring haemoglobin oxygen saturation (SO2 = HbO/HbT) as a surrogate for tissue oxygenation and utilization for applications in the brain, muscle and breast. The rationale was clear. Oxygen utilization with insufficient oxygen delivery would certainly result in reduced oxygenation of haemoglobin. Further, since the haemoglobin chromophores dominated the absorption spectrum in the near infrared, the estimate of SO2 was less susceptible to algorithm errors, although partial volume effects [10], myoglobin [11] and the presence of other chromophores [12] can still confound the estimation of SO2. Finally, a decrease in SO2 was generally associated with an increase in the reduced form of CCO [1,2,4].

It is becoming apparent that SO2 alone is not a robust indicator of sufficient oxygen delivery to the tissue [13]. In many cases, HbT and blood flow (BF) are proving to be better indicators of tissue status [14,15], and even better when combined with SO2 to estimate oxygen consumption (OC) [16–18]. Below, we review when SO2 works and when it should be replaced by more robust measures of OC. While, historically, the first and major focus of NIRS was to study the
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brain haemoglobin oxygen saturation (see [19] and [20] as recent reviews), we begin with the NIRS work in muscle, proceed to the application to breast cancer, and finish with efforts in the brain.

2. Muscle

Skeletal muscle is strongly dependent on oxidative metabolism. Muscle OC can increase 50-fold during exercise [21]. Any disruption of normal oxygen delivery or consumption is likely to adversely affect exercise tolerance. Training is known to improve muscle tolerance to increased energetic demands from exercise, and NIRS is being used to assess muscle ability to adequately support metabolic demands with the potential of using NIRS to guide and optimize individual training [22,23]. A number of chronic health conditions impair oxygen delivery to the muscle, including peripheral vascular disease [24] and chronic heart failure [25].

Resting SO₂ alone fails to detect disease-induced differences in oxygen delivery or utilization, probably owing to the large differences in fat and muscle distribution within and across individuals [26]. An advantage with peripheral muscle is the possibility of performing manipulations that allow a direct measure of OC. In fact, estimating OC from NIRS measurements of oxygenated haemoglobin is a common practice in muscle applications. Pressure cuff occlusion of BF in an arm or leg is an easy means of measuring tissue OC. The method is quite simple, as described in Cheatle et al. [27], De Blasi et al. [28] and Casavola et al. [29]. Rapidly increasing cuff pressure above arterial blood pressure will occlude BF in the extremity with no associated change in total haemoglobin concentration. A NIRS measurement downstream of the occlusion will reveal a resultant increase in HbR and a decrease in HbO owing to OC. If the pressure was increased sufficiently rapidly above arterial pressure, then there will be no change in total haemoglobin concentration

\[ HbT = HbO + HbR. \]

Using equation (2.1), the OC in the leg muscle was calculated to be

\[ OC = 3.2 \mu\text{mol} \text{ 100 ml}^{-1} \text{ min}^{-1}. \]

A partial pressure cuff occlusion will result in changes in HbT if the arteries are not fully occluded, thus allowing blood to flow in, but veins are occluded preventing blood from flowing out. In this case, De Blasi et al. [28] and Casavola et al. [29] have indicated that it is still possible to estimate OC provided that a correction is made for HbT changes. Specifically, any increase in HbT will result
Figure 1. Changes in the haemoglobin concentrations in leg muscle during an 8 min pressure cuff occlusion of arterial and venous blood flow. The occlusion occurred during the period indicated by the shaded box. During this period, deoxyhaemoglobin increased and oxy-haemoglobin decreased, while total haemoglobin remained constant. The slope of the changes indicates a muscle oxygen consumption (OC) of 3.2 µmol 100 ml$^{-1}$ min$^{-1}$. (Online version in colour.)

from inflowing arterial blood. Since this haemoglobin is not fully oxygenated, it will increase HbR. If we first subtract this contribution to the change in HbR, then OC can be accurately estimated

\[ OC = 4 \frac{\partial}{\partial t} \left( HbR - \frac{100 - SaO_2}{100} HbT \right), \]  

where SaO$_2$ is the arterial haemoglobin oxygen saturation.

Previous to this, De Blasi et al. [28] have tested the possibility of estimating OC during muscle exercise without using occlusion. The results are encouraging, showing similar results with and without occlusion. While there was no occlusion and thus generally the inflowing blood cannot be neglected, they still estimated OC using equation (2.1). We revisit this later in §5 below, but briefly this works because the increase in OC is sufficiently large that the effects of inflowing and outflowing blood can be neglected during the early transient.

3. Breast

Tumour physiology is marked by increased vascularization and hypoxia. Historically, blood volume (BV) by itself, as an indicator of increased vascularization, has not robustly detected and distinguished malignant and benign lesions [30,31]. Several papers suggest the importance of haemoglobin oxygenation for making the distinction, with the expectation that the hypoxic tissue environment of the tumour would be reflected by reduced haemoglobin
oxygen saturation [32–35]. One of the first papers to report tumour SO\textsubscript{2} versus healthy tissue found a reduction in SO\textsubscript{2} [32]. In a follow-up report of 58 patients, this reduction in SO\textsubscript{2} was not found to be significant [36].

Imaging studies reported by four other groups over the past 10 years have confirmed that SO\textsubscript{2} is not significantly different in malignant lesions versus healthy tissues or benign lesions. These results are summarized in figure 2. The results from 93 patients reported by Grosenick et al. [37] and from 49 patients reported by Intes [38], both using time-domain optical transmission imaging systems, are shown in figure 2\textit{a,b}. Figure 2\textit{c} summarizes the results from 14 patients reported by Ntziachristos et al. [39] using a time-domain optical transmission imaging measurement combined with magnetic resonance imaging. Figure 2\textit{d} presents the results from 116 patients reported by Chance et al. [40] using a continuous-wave optical imaging system in reflectance mode. From these plots, it is clear that there is significant overlap among healthy, benign and malignant lesions, when considering only SO\textsubscript{2}. Some of the studies reveal that this overlap is diminished when considering only HbT.

What is striking when examining these four different imaging studies is the significant separation of malignant lesions from benign lesions and healthy tissue when considering SO\textsubscript{2} and HbT simultaneously, a point also suggested by Cerussi et al. [36]. Note, in figure 2, the trend showing that tumour SO\textsubscript{2} is low when tumour HbT is comparable to non-malignant tissue and that tumour SO\textsubscript{2} is higher and can exceed non-malignant tissue SO\textsubscript{2} when tumour HbT is higher. This is a result of hyperperfusion, reflected by an increase in HbT, increasing oxygen delivery to the tissue beyond the increase in oxygen demand. This is an important result that we will return to in §5 that SO\textsubscript{2} can actually increase despite an increase in tissue oxygen metabolism and a possible decrease in tissue oxygenation. It is because of the confounding impact of BF on oxygen delivery that baseline SO\textsubscript{2} alone is not a robust measure of oxygen metabolism or sufficient oxygen delivery.

While baseline SO\textsubscript{2} alone does not differentiate breast disease, it may be possible to manipulate BF to induce changes in SO\textsubscript{2} that can reveal the metabolic needs of breast lesions. This manipulation would be analogous to the pressure cuff occlusion done in muscle studies. This possibility is suggested by Carp et al. [41], who reported that mild breast compression partially occludes BF and produces a transient decrease in SO\textsubscript{2}. By fitting the temporal decay of SO\textsubscript{2} with an oxygen mass balance equation, it is then possible to estimate tissue OC and BF. Future development of this approach in breast imaging will probably provide new biomarkers, OC and BF, for differentiating lesions.

4. Brain

A continuous supply of oxygen is critical to the health of the brain. As the brain has no store of oxygen, any disruption in supply would quickly result in tissue oxygen depletion and metabolic stress as the brain under anaerobic glycolysis would not be able to produce sufficient energy to maintain normal cell function. Thus, maintaining oxygen supply and utilization in the brain is a major goal when treating brain injury, during cardiac surgery and during general anaesthesia. The first attempt to use NIRS to obtain a more direct measure of brain oxygen

*Phil. Trans. R. Soc. A* (2011)
Figure 2. The results from four different optical breast imaging studies of malignant lesions compared with benign lesions and healthy tissue are presented in scattered plots of SO2 versus HbT. Details of the studies are provided in the text. While SO2 alone generally fails to distinguish malignant lesions from non-malignant tissue, we see a diagonal separation indicated by the grey lines that indicates that malignant lesions with HbT comparable to non-malignant tissue have a lower SO2, but that this SO2 increases as malignant lesion HbT increases. These results are adapted from the authors. (a) Grosenick et al. [37], (b) Intes [38], (c) Ntziachristos et al. [39] and Chance et al. [40]. Circles, malignant tumour; crosses, healthy or benign.

delivery and utilization, rather than rely on measurements of oxygen content at sites distant from the brain (e.g. pulse oximetry and blood gases), was in 1985 when Brazy et al. [2] used NIRS in the neonatal intensive care unit. They used a three-wavelength NIRS device with 775, 815 and 904 nm to achieve sensitivity to changes in HbO, HbR and CCO. In this way they could monitor changes in oxygen delivery as reflected by the oxygen saturation SO2 and total haemoglobin, as well as changes in oxygen utilization as indicated by the CCO concentration. Their results during brief apnoea in infants showed expected reductions in SO2 and CCO, with a prolonged recovery of CCO relative to SO2. As discussed in §1, subsequent studies revealed that the estimate of CCO was unreliable [7] and therefore clinical measures with NIRS focused on using only SO2.

Phil. Trans. R. Soc. A (2011)
One example of the usefulness of NIRS measures of cerebral SO₂ is in assessing collateral perfusion through the circle of Willis during carotid endarterectomy [42–44] or carotid balloon occlusion test [45–48]. This is an important test before carotid surgery since insufficient collateral perfusion during the surgery would result in ischaemic damage to the brain. Immediately following carotid occlusion, NIRS reveals a decrease in SO₂ in the ipsilateral cortex. In patients with sufficient collateral perfusion, SO₂ generally returns to the baseline value within a minute during the occlusion. In patients with insufficient collateral perfusion, the SO₂ remains below the baseline value for the duration of the occlusion. An experimental example is shown in figure 3. Changes in HbT and HbR are shown for 1 min before and 2 min after total balloon occlusion of the patient’s right internal carotid artery. At the onset of the occlusion, we observe a dramatic drop in HbT with little change in HbR. As the occlusion reduces the perfusion pressure, the compliant vessels contract reducing HbT. The corresponding little change in HbR indicates that SO₂ has dropped as expected owing to an increased extraction fraction to maintain a constant oxygen delivery. In this patient, however, collateral perfusion through the circle of Willis responds within 30 s, producing a hyperaemic overshoot in HbT with a corresponding decrease in HbR, with everything returning to baseline levels within 2 min, although the right internal carotid is still occluded.
NIRS measures of cerebral SO$_2$ have also demonstrated the ability to assess delivery of oxygen to the brain during cardiac surgery [49–53]. During cardiopulmonary bypass surgery, drops in SO$_2$ serve as an indicator of reduced BF to the brain that can be corrected by the surgical team. Following bypass surgery, the SO$_2$ then provides assurance of the success of the surgery to restore and maintain sufficient oxygen delivery to the brain. While the changes in SO$_2$ clearly correspond with cardiac surgery, there is no clear conclusion as to the utility of using these measures to predict the outcome of the patient. In infants, the outcome of the patient probably depends more on the cerebral damage acquired from insufficient cardiac output before the surgery than on maintaining cerebral perfusion during the surgery [49]. In adults, a recent randomized, prospective study has demonstrated that patients with a lower mean SO$_2$ during the surgery had a longer length of stay in the hospital after the surgery owing to a greater incidence of major organ dysfunction [51]. For adults, further study is needed to optimize neuro-monitoring feedback during cardiac surgery, particularly to better understand the importance of decreased cerebral oxygenation versus cerebral emboli as the cause of neurological injury [50], the latter of which requires transcranial ultrasound to detect.

Neonates in the intensive care unit receive numerous interventions that can have an impact on oxygen delivery to the brain, such as manipulation of haematocrit and blood pressure, yet the impact on the brain is not directly measured nor is it understood. Thus, the possibility exists that such interventions could adversely affect the patient. Wardle et al. [54] measured cerebral SO$_2$ in anaemic and hypotensive neonates and found no significant difference with respect to normal controls, suggesting that cerebral autoregulation maintained oxygen delivery. When infants are asphyxiated at birth, an increase in cerebral SO$_2$ relative to healthy controls has been observed to correlate with abnormal outcome [55]. In a different study, a reduction in SO$_2$ relative to healthy controls on the first day of life correlated with severity of a subsequently developed intraventricular haemorrhage [56]. While these results indicate the possibility of using SO$_2$ to identify those patients who need aggressive treatment, there was significant overlap in the SO$_2$ values with infants that had normal outcome. To prevent over-treatment, it has been suggested that NIRS instruments need to be improved to provide a more precise measure of SO$_2$ in order to distinguish subtle differences with more power [57]. An important relevant observation is that the measured SO$_2$ can vary by more than 10 per cent with repeated placement of the optodes, presumably because of subtle differences in underlying tissue heterogeneity [57,58], thus indicating the need to reduce this variation through repeated probe placement or spatially resolved oximetry [56].

By introducing a blood tracer with optical contrast, NIRS measurements can be made to estimate BF and BV. Oxygen is an ideal tracer to use with NIRS to estimate BF and BV, as detailed by Reynolds et al. [59] and Wyatt et al. [60], respectively. An early demonstration of the predictive power of BF and BV from Meek et al. [14] showed that increases in BF and BV above normal levels in infants with perinatal asphyxia correlated with adverse outcome of the infants. They suggest that acute hypoxia–ischaemia releases several vasoactive agents, resulting in the most severely affected infants having the greatest cerebral hyperaemia. While these results demonstrate the promising potential of NIRS for assessing brain injury in newborns, they state that BV measurements as an
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early marker of the severity of perinatal asphyxia are of limited value because of a poor specificity of 38 per cent (sensitivity of 85%) and the poor reproducibility in making such oxygen manipulations. They suggest that upcoming advances in NIRS technology may make it possible in the future to improve specificity by obtaining absolute haemoglobin concentrations and obtaining more direct measures of oxygen extraction fraction and oxygen metabolism [61,62] without the need for introducing a blood tracer.

Multi-distance frequency-domain technology provides a means to estimate absolute absorption and scattering properties of the tissue, and thus estimate absolute haemoglobin concentrations without the need for vascular tracers [63]. This technology offers a robust approach to estimate cerebral BV and oxygenation in infants, as demonstrated by measures of the twofold increase in cerebral BV in the first year of life by Franceschini et al. [64]. Grant et al. [15] used this approach to measure alterations in cerebral BV and SO2 in newborns with a variety of brain injuries compared with infants without brain injury. Consistent with the earlier work of others, they found no significant difference in SO2 between the infants with brain injury and the stable control infants without brain injury. Similar to Meek et al. [14], they also found that cerebral BV was significantly elevated in the brain-injured infants, but, because no vascular tracers are needed, it is now possible to apply this approach to all patients.

5. More robust measures of oxygen consumption

From the above examples, we have seen that, when there are transient changes in BF or OC, changes in haemoglobin oxygenation can reveal information about oxygen delivery and consumption. It is important to emphasize that, here, transient refers to the SO2 changes that occur during the first minutes of the oxygen delivery or consumption change.

Generally, OC can be estimated by the difference between the quantity of oxygen being delivered by the arteries and that drained by the veins in a tissue of interest. In a steady state, OC can be calculated from the flow of blood multiplied by the difference between the arterial oxygen saturation (SaO2) and the venous oxygen saturation (SvO2), where the saturations are given as a fraction. That is,

$$OC = \frac{HGB}{MW_{Hb}} \frac{BF}{4} (SaO2 - SvO2), \quad (5.1)$$

where HGB is the haemoglobin concentration in the blood (g l$$^{-1}$$) and MW_{Hb} is the molecular weight of haemoglobin (g mol$$^{-1}$$) and the 4 accounts for the haemoglobin to oxygen molar ratio. An abrupt change in BF (1100 g$$^{-1}$$ min$$^{-1}$$) or OC (mol O2 100 g$$^{-1}$$ min$$^{-1}$$), as can result from a pressure cuff occlusion or exercise, will result in an imbalance of the left- and right-hand side of equation (5.1), thus giving rise to temporal changes in HbO and HbR. The temporal changes are described by Carp et al. [41]

$$\frac{dHbO}{dt} = \frac{HGB}{MW_{Hb}} BF(SaO2 - SvO2) - \frac{1}{4} OC. \quad (5.2)$$

Subject to the conditions that BF and/or OC change abruptly at $t = 0$ and then remain constant, we obtain the solution linearized for small changes in HbO and
SvO$_2$, as occurs at time scales that are short compared with oxygen delivery and consumption time scales,

\[
\Delta \text{HbO}(t) = \frac{1}{\text{VF}_{V}} \frac{\text{HGB}}{\text{MW}_{\text{Hb}}} \text{BF} (\text{SaO}_2 - \text{SO}_{2o}) t - \frac{1}{4} \text{OC} t. \tag{5.3}
\]

Here, for simplicity, we assume that the haemoglobin saturation measured by NIRS is the volume fraction weighted average of SaO$_2$ and SvO$_2$, i.e. SO$_2 = \text{VF}_A \text{SaO}_2 + \text{VF}_V \text{SvO}_2$, where \( \text{VF}_V (\text{VF}_A) \) is the venous (arteriole) volume fraction and \( \text{VF}_V + \text{VF}_A = 1 \). The subscript ‘o’ refers to the initial value. During a pressure cuff arterial occlusion, BF = 0 and we arrive at the same equation relating OC to \( \Delta \text{HbO}(t) \) as used in muscle studies (equation (2.1) above). We still arrive at the same equation, if the rate of OC, i.e. \( 1/4 \) OC, is much greater than the rate of oxygen delivery,

\[
2 \frac{\text{HGB}}{\text{MW}_{\text{Hb}}} \text{BF} (\text{SaO}_2 - \text{SO}_{2o}),
\]

as demonstrated by De Blasi et al. [28], when estimating OC during exercise with and without cuff occlusion. These equations demonstrate formally when we can use transient changes in SO$_2$ (or HbR) to estimate OC.

For measurements of the brain or breast, it is generally not feasible to introduce an abrupt change in BF or OC. As a result, we must rely on equation (5.1) and use measurements of SO$_2$ combined with measurements of BF to estimate OC. The need for combining these measurements is nicely illustrated by the breast imaging studies depicted in figure 2. While it was reasonable to expect to observe a decreased SO$_2$ because of increased metabolic activity in the tumour, this was not observed because of the confounding effect of BF. Higher BF, as suggested by an increased HbT, increases oxygen delivery and thus SO$_2$. By considering both BF and SO$_2$, as we did with the diagonal line separating malignant breast lesions from the healthy tissue and benign lesions in figure 2, we see that malignant lesions are generally consuming more oxygen.

Recognizing the confounding effect of BF on estimating OC from SO$_2$, Grant et al. [15] applied equation (5.1), using BV as a surrogate measure of BF, to estimate changes in OC in brain-injured infants relative to healthy controls and found that OC was significantly elevated. The summary of results from 14 brain-injured infants compared with 29 infants without brain disorders is shown in figure 4. These infants were in the acute stage of brain injury and so the observation of elevated BV and OC suggests that the brain disorders measured shared a common mechanism of hyperperfusion, possibly arising from increased neuronal activity owing to excitotoxic processes evolving in the tissue over days. In the chronic stage after approximately 7 days, they have observed that OC drops to zero, thus providing a time scale for cell death in these injuries.

As discussed by Leung et al. [65] and Boas & Payne [66], care must be taken in estimating BF from BV. It is common to use the Grubb relation [65,67], but this relation may not be valid under all conditions [68], particularly pathophysiological conditions. Thus, it is preferable to obtain independent measures of BF as offered by diffuse correlation spectroscopy (DCS) when estimating OC [18]. A recent example by Roche-Labarbe et al. [17] in neonates during the first six weeks of life nicely demonstrates first the uncoupling that can arise between BF and BV, and second the improved estimates of OC that can be
Figure 4. Measurements of cerebral OC versus blood volume (BV) are shown for acutely brain-injured newborns compared with newborns without brain injury. OC is reported relative to healthy age-matched controls. In general, acutely brain-injured newborns have increased OC and increased BV with respect to non-brain-injured newborns. This is indicated by the diagonal black line separating brain-injured from non-brain-injured infants with a sensitivity of 78.6% and a specificity of 96.6%. Filled circles, brain injured; triangles, unstable; diamonds, stable; open circles, healthy.

obtained with independent measures of BF, HbT and SO2. Fifty-six premature infants were measured longitudinally during their hospital stay for a total of 230 measurements. Figure 5 reports average results as a function of corrected gestational age (cGA) for BV measured with the frequency-domain multi-distance method, an index of blood flow (BFi) measured with DCS, and the relative OC calculated with respect to infants born at 35 weeks GA. OC was calculated using either BFi measured by DCS or using Grubb’s relation to estimate BF from the measured BV. Figure 5a,b shows the uncoupling between BV and BF, with BV more or less constant with cGA and BF increasing with age. This uncoupling is probably due to the large decreases in haematocrit during the first two months of life, which alter haemoglobin content and blood viscosity. As a result, in the newborn population, quantifying OC using BV as a surrogate for BF not only gives an incorrect result, but also produces a larger variance in OC as shown in figure 5c,d.

As another example of combining measures of BF with SO2 to estimate OC, Zhou et al. [69] combined DCS with frequency-domain NIRS to measure breast tumour response to chemotherapy. The results from a single patient are shown in figure 6 following the tumour response from baseline on day 0 to 7 days after chemotherapy. A significant decrease in HbT and SO2 is observed during the first 7 days following chemotherapy. Interestingly, BF first significantly increased on day 3 and then decreased below baseline on days 4–7, revealing an uncoupling between HbT and BF that could be a result of a reduced haematocrit following...
Figure 5. Plotted are average results of (a) BV and (b) blood flow index (BFi) measured on 56 newborn premature infants and measured longitudinally over several weeks on each infant. Standard errors are indicated except for the last 2 points, which are from single measurements. After 28 weeks, corrected gestational age (cGA) and BV remain constants while BFi continually increases. If we estimate OC assuming Grubb’s relation to approximate BF from BV, then OC is seen to remain constant from 28 to 40 weeks cGA as shown in (c). Instead, using the experimentally measured BF, we see that OC actually increases significantly over this period of time as shown in (d). Further, we see that the variation across the population is greatly reduced when using BFi.

chemotherapy [70], changes in vascular morphology and resistance, changes in intra-tumour pressure or a complex combination of these factors. The estimate of OC revealed that it first increased on day 3 and then decreased below baseline, suggesting a complex metabolic response to the therapy that is interesting for better understanding tumour response to therapy and will potentially help to better identify therapy responders from non-responders in the first week of therapy.

As suggested by the above-mentioned studies, multi-modal estimates of OC will enable new physiological studies in health and disease leading to new diagnostic and therapeutic approaches. One interesting physiological finding is the constancy of SO2 in developing human infants over the first year of life despite a doubling of BV [64] and a presumed twofold to fourfold increase in BF. This observed tight coupling of oxygen demand and oxygen supply to maintain a constant SO2 suggests that SO2 is an important factor in the regulation of BF over a long-term time scale [71]. This tight coupling has recently been observed at the level of the microvasculature by Yaseen et al. [72], in which, in individual rats, BF in distinct ascending venules draining the cortex can vary by 20-fold over a distance of 500 μm, yet SO2 within those individual draining venules is constant. These results indicate that oxygen demand in a volume of tissue drained by
6. Concluding remarks

NIRS has tantalized us with the prospect of obtaining non-invasive measurements of an important oxygen sensor, CCO. Work continues to realize a robust measure of CCO that can be used routinely in humans. Until that is achieved, NIRS efforts have instead used the far more robust measures of haemoglobin SO$_2$ as a surrogate tissue oxygen sensor. As we have discussed in this paper, when there is an abrupt change in BF or OC, transient changes in SO$_2$ can reveal information about tissue OC. However, when abrupt changes in BF or OC are not feasible, or the measurement cannot be made in the first minute, then inference of tissue OC or sufficient oxygen delivery from SO$_2$ measures alone is confounded by BF. This is nicely illustrated by studies of SO$_2$ in asphyxiated newborn infants revealing a slight increase in SO$_2$ relative to healthy controls, which has been interpreted as a reduction in oxygen demand from cerebral injury leading to cellular energy failure. Alternatively, the injury could elicit a strong BF response to overcompensate for an increased metabolic demand. Thus, generally speaking, in order to robustly assess tissue OC and sufficient oxygen delivery, it is necessary to measure both SO$_2$ and BF. More and more groups are now exploiting all
optical methods, and optical methods combined with other imaging modalities, to obtain this important physiological measurement and use it in a broad range of applications.

Britton Chance’s leadership in biomedical optics and mitochondrial respiration has inspired us in our endeavour to translate optical methods to the clinic and to unravel the dynamic energetic processes in health and disease. We will miss him but are comforted by the opportunity to follow in his giant footsteps. In preparing this presentation and manuscript, we are grateful for the assistance of and discussions from Juliette Selb, Nadege Roche-Labarbe, Ellen Grant, Abbas Yaseen, Bruce Tromberg, Arjun Yodh and Marco Ferrari. We acknowledge support from the National Institute of Health under R01-EB06385, P41-14075, R01-HD042908, R01-NS057476 and P01-NS055104.

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