Broadband near-infrared spectroscopy can detect cyanide-induced cytochrome aa₃ inhibition in rats: a proof of concept study

La spectroscopie proche infrarouge à large bande peut détecter l'inhibition du cytochrome aa₃ induite par le cyanure chez le rat: une étude de démonstration de faisabilité

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Abstract

Purpose Clinically available near-infrared spectroscopy (NIRS) devices use two to five wavelengths of light to measure the relative amounts of oxyhemoglobin and deoxyhemoglobin in tissue to determine tissue hemoglobin oxygen saturation (StO₂). In addition to StO₂, broadband NIRS devices (using hundreds of wavelengths of light) may be able to measure the oxidation state of mitochondrial cytochrome aa₃ (Cyt_{ox}) which reflects the subcellular energetic state. We hypothesize that broadband NIRS devices can measure Cyt_{ox} independent of changes in hemoglobin saturation.

Methods In this prospective non-randomized study, 20 male Sprague-Dawley rats (300 g) were anesthetized with isoflurane, tracheally intubated, and ventilated with 100% O₂ containing 2% isoflurane. They were subsequently instrumented with a broadband NIRS device that used a halogen light source coupled to an emitting fiberoptic cable. Three receiving fiberoptic cables were utilized; one analyzed the light source and the other two were directed at the base of the skull. Each receiving fibre was connected to a spectrometer to measure light intensity. Sodium cyanide (NaCN) 5 mg·kg⁻¹ iv was injected in order to

produce cytochrome aa₃ reduction. Two to three minutes after injection, oxygen was eliminated and 100% nitrogen (i.e., anoxia) was used for ventilation in order to induce a reduction in both cytochrome aa₃ and hemoglobin desaturation. Changes in the cytochrome oxidation state and hemoglobin oxygenation were calculated using a broadband algorithm and compared before and after both the NaCN and anoxia interventions.

Results The NaCN injection resulted in a decrease in median [interquartile range (IQR)] deoxyhemoglobin (−0.014 [−0.29 to −0.005] arbitrary units [AU]; *P* < 0.001), an increase in oxyhemoglobin (0.013 [−0.011 to 0.031] AU; *P* < 0.001), and a reduction in cytochrome aa₃ (−0.015 [−0.020 to −0.011] AU; *P* < 0.001). Anoxia resulted in an increase in median [IQR] deoxyhemoglobin (0.13 [0.11 to 0.18] AU; *P* < 0.001), a decrease in oxyhemoglobin (−0.17 [−0.22 to −0.15] AU; *P* < 0.001), and a reduction in cytochrome aa₃ (−0.04 [−0.06 to −0.03] AU; *P* < 0.001).

Conclusion Broadband NIRS can effectively measure the directionality of changes in both Cyt_{ox} and StO₂ by uncoupling the cytochrome and hemoglobin signals through inhibition of the electron transport chain and anoxia.

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Résumé

Objectif Les dispositifs de spectroscopie proche infrarouge (ou NIRS pour near-infrared spectroscopy) disponibles dans la pratique clinique utilisent de deux à six longueurs d'onde de lumière pour mesurer les quantités relatives d'oxyhémoglobine et de déoxyhémoglobine dans les tissus, afin de déterminer la saturation en oxygène de l'hémoglobine dans les tissus (StO₂). Outre la StO₂, les

dispositifs de NIRS à large bande (qui utilisent des centaines de longueurs d'onde de lumière) pourraient être capables de mesurer l'état d'oxydation du cytochrome mitochondrial aa₃ (Cyt_{ox}), lequel reflète l'état énergétique sous-cellulaire. Nous avons posé l'hypothèse que les dispositifs de NIRS à large bande pourraient mesurer le Cyt_{ox} indépendamment des changements de la saturation d'hémoglobine.

Méthode Dans cette étude prospective non randomisée, 20 rats Sprague-Dawley mâles (300 g) ont été anesthésiés avec de l'isoflurane, reçu une intubation trachéale, et été ventilés à l'aide d'O₂ à 100 % contenant 2 % d'isoflurane. Par la suite, on les a examinés avec un dispositif de NIRS à large bande qui utilisait une source de lumière halogène couplé à un câble fibroscopique émetteur. Trois câbles fibroscopiques récepteurs ont été utilisés; l'un analysait la source de lumière et les deux autres étaient dirigés vers la base du crâne. Chaque fibre réceptrice était connectée à un spectromètre afin de mesurer l'intensité de la lumière. On a injecté 5 mg·kg⁻¹ de cyanure de sodium (NaCN) iv afin d'entraîner une réduction du cytochrome aa₃. Deux à trois minutes après l'injection, l'oxygène a été éliminé et on a utilisé 100 % de nitrogène (c.-à-d. une anoxie) pour ventiler les animaux afin d'induire une réduction du cytochrome aa₃ et de l'hémoglobine. Les modifications de l'état d'oxydation du cytochrome et de l'oxygénation de l'hémoglobine ont été calculées à l'aide d'un algorithme à large bande et comparées avant et après les interventions de NaCN et d'anoxie.

Résultats L'injection de NaCN a entraîné une diminution de la déoxyhémoglobine médiane [écart interquartile (ÉIQ)] (−0,014 [−0,29 à −0,005] unités arbitraires [UA]; $P < 0,001$), une augmentation de l'oxyhémoglobine (0,013 [−0,011 à 0,031] UA; $P < 0,001$), et une réduction du cytochrome aa₃ (−0,015 [−0,020 à −0,011] UA; $P < 0,001$). L'anoxie a entraîné une augmentation de la déoxyhémoglobine médiane [ÉIQ] (0,13 [0,11 à 0,18] UA; $P < 0,001$), une diminution de l'oxyhémoglobine (−0,17 [−0,22 à −0,15] UA; $P < 0,001$), et une réduction du cytochrome aa₃ (−0,04 [−0,06 à −0,03] UA; $P < 0,001$).

Conclusion La spectroscopie proche infrarouge à large bande peut mesurer efficacement l'orientation des changements du Cyt_{ox} et de la StO₂ en séparant les signaux du cytochrome et de l'hémoglobine via l'inhibition de la chaîne de transport des électrons et l'anoxie.

Hemoglobin and oxyhemoglobin are chromophores, and their absorption of different amounts of red and infrared light allows for the measurement of the oxygen saturation of pulsatile arterial blood with a conventional two-wavelength pulse oximeter.¹ Similarly, near-infrared spectroscopy

(NIRS) devices are designed to measure the hemoglobin oxygen saturation in tissue (StO₂)—i.e., the amount of oxygenated hemoglobin relative to total hemoglobin in a mixture of arterial, venous, and capillary blood. Unfortunately, clinical studies utilizing NIRS to guide physiologic interventions aimed at optimizing clinical outcomes have been equivocal. In particular, there is a lack of prospective studies showing an improvement in perioperative mortality when using NIRS. While some retrospective studies have shown a relationship between StO₂ desaturations and various indices of organ dysfunction, methodological shortcomings and highly variant results make the NIRS literature difficult to interpret.^{2,3}

Other biological compounds besides hemoglobin are involved in oxygen transport, cellular respiration, metabolism, and regulation of oxidative state (e.g., the cytochromes). They also have distinct light absorption spectra and can theoretically be measured using additional chromophore-specific wavelengths of light. Of particular interest is cytochrome aa₃, the terminal component of the electron transport chain (ETC) (Fig. 1).^{4,5} Of note, cytochrome aa₃ is sometimes designated as complex IV and is also functionally known as cytochrome c oxidase. Like hemoglobin, cytochrome aa₃ absorbs near-infrared radiation (NIR), with an absorption peak around 820 nm. Crucially, the absorption spectrum of cytochrome aa₃ changes when it transitions from an oxidized to a reduced state. When sufficient molecular oxygen is supplied to the mitochondria of an active cell and electrons pass down the ETC, cytochrome aa₃ exists in an oxidized state. When one of these critical steps does not occur, or oxygen demand exceeds supply, cytochrome aa₃ is reduced, and this is theoretically detectable. Unlike StO₂, which analyzes oxygen binding to hemoglobin, the oxidation state of cytochrome aa₃ (Cyt_{ox}) provides information about the energetic state of cells and can detect cellular ischemia directly.⁶ The inability of clinically available NIRS devices to measure the subcellular energetic state directly may partly explain why the use of tissue hemoglobin saturation monitors to guide perioperative management has not consistently been found to be organ protective.²

Both pulse oximetry and NIRS are based on the modified Beer-Lambert law for a scattering medium, which is stated in equation 1^{7,8}:

$$OD_{\lambda} = \epsilon_{\lambda} \cdot c \cdot L \cdot DPF + OD_{R,\lambda}$$

where, OD_λ is the optical density of a medium; ε_λ represents the extinction coefficient of a chromophore (e.g., oxyhemoglobin); c represents the concentration of the chromophore; L represents the path length of electromagnetic radiation; DPF (differential pathlength factor) represents a correction factor for scattering; OD_{R,λ} represents light losses due to scattering.

Fig. 1 Depiction of the electron transport chain (ETC) as it relates to cellular and subcellular energetics. Notice that cytochrome aa₃ (also known as complex IV or cytochrome c oxidase), the final component of the ETC, can exist in either an oxidized or a reduced state, depending on the availability of electron donors NADH and FADH₂.

NO = nitric oxide; mtNOS = mitochondrial nitric oxide synthase; ADP = adenosine diphosphate; ATP = adenosine triphosphate; Cyt = cytochrome; Arg = arginine; CoA = coenzyme A; TCA = tricyclic acid

This equation can be simplified to $\Delta c = \Delta OD_{\lambda} / (e_{\lambda} \cdot L \cdot DPF)$ for one chromophore as OD_R is a constant and drops out. For multiple chromophores, multiple equations must be developed and solved simultaneously using linear algebraic techniques. One wavelength is required for each chromophore.

Because hemoglobin absorbs approximately 25 times as much near-infrared light as cytochrome aa₃ on a per weight basis⁷ and the concentration of cytochrome aa₃ in tissue is approximately 10% that of hemoglobin,⁸ the total contribution of cytochrome aa₃ to NIRS is relatively small, resulting in a poor signal-to-noise ratio (SNR). Measurement of Cyt_{ox} using NIRS is therefore technically challenging. Early investigators added additional wavelengths to their instruments, which increased the SNR. To improve the accuracy of cytochrome aa₃ measurements, several investigators developed broadband techniques in experimental animal models (i.e., pig, rabbit) which utilize over 100 wavelengths of light.^{2,9-11}

It is unknown whether results derived from experiments in pigs and rabbits are generalizable to other animals.^{6,12} Additionally, previous work indicated that broadband NIRS used to measure Cyt_{ox} relied on sophisticated equipment,^{6,12} and it is not known whether commercial off-the-shelf equipment could be used to make these measurements. Accordingly, the purpose of this study was to test the ability of a broadband NIRS device (constructed of commercial off-the-shelf equipment) to measure Cyt_{ox} independent of StO₂ changes in rats.

Methods

This prospective non-randomized animal study was approved on December 5, 2014 by the Institutional

Animal Care and Use Committee at the University of Virginia. In reporting the enclosed work, we adhered to the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines, with the exception of housing and husbandry (which were not relevant and thus omitted).¹³

Animal model

Twenty two-month-old male Sprague-Dawley rats weighing approximately 300 g were housed in individual cages under natural light and dark cycles at a mean (SD) temperature of 28 (4)°C. The rats were given a standard pellet diet and water *ad libitum* and were acclimatized for three days before the beginning of the experiments. All experiments were conducted from 9:00 AM to 5:00 PM.

Anesthesia was induced by placing rats in a chamber containing 3% isoflurane in oxygen. The rats then received tracheal intubation, with a 16G catheter, and mechanical ventilation. Anesthesia was maintained by isoflurane carried by 100% O₂ to maintain the end-tidal isoflurane concentration at 2.0%. The ventilation was adjusted to maintain end-tidal CO₂ around 32 mmHg. The inhaled and exhaled gas concentrations were monitored continuously with a DatexTM infrared analyzer (Capnomac, Helsinki, Finland). Rectal temperature was maintained at 37°C with the aid of a servo-controlled warming blanket (TCAT-2LV, Physitemp Instruments Inc., Clifton, NJ, USA). Heart rate and SpO₂ were measured continuously during anesthesia with a MouseOxTM Pulse Oximeter (Harvard Apparatus, Holliston, MA, USA). After tracheal intubation, the right or left lateral tail vein was cannulated with a 24G intravenous catheter for drug injection, and NIRS instrumentation was then applied.

After approximately one minute of NIRS recordings, sodium cyanide (NaCN) 5 mg·kg⁻¹ *iv* was injected in order

to produce cytochrome aa₃ reduction independent of any change in StO₂.⁶ Two to three minutes after the NaCN injection, the oxygen supply was eliminated and 100% nitrogen (i.e., anoxia) was used for ventilation to induce cytochrome aa₃ reduction and hemoglobin desaturation. After completion of monitoring, the rats were sacrificed with an isoflurane anesthesia overdose.

Device design and construction

In order to transmit near-infrared radiation to the rat via its mouth (Fig. 2), as previously described,¹⁴ we used a single halogen light source (Pentax LH-150, Tokyo, Japan) coupled to a single emitting fibreoptic cable (Gulf Fiberoptics, 3-mm diameter bundled borosilicate fibre, Oldsmar, FL, USA) with an attached prism (Gulf Fiberoptics, aluminum, Oldsmar, FL, USA) at the end. Three receiving fibreoptic cables (Thorlabs FG550LEC, 550 µm, 0.22 NA, Newton, NJ, USA) were utilized - one was directed at the light source to measure the intensity of light continuously at all wavelengths (I_0), and the other two were directed at the base of the skull. Each receiving fibre was connected to an independent charge-coupled device (CCD)-based spectrometer (Ocean Optics USB2000 VIS-NIR, Dunedin FL; grating #H4, slit 50 µm) which decomposed the returning signal into a broadband spectrum with < 1 nm resolution. The USB2000 VIS-NIR utilizes a 25 µm entrance slit, a grating with 600 lines·mm⁻¹ density, and a 2,048-element linear silicon CCD array (pixel size: 14 µm × 200 µm [14 µm pitch]),

resulting in a signal-to-noise ratio of 250:1. Sampling time was selected individually to maximize signal strength without causing washout. Intensity data from the spectrometers was exported into MATLAB® (Mathworks, Natick, MA, USA) for analysis.

Broadband algorithm

We based our algorithm on the University College London algorithm (UCL n ; where the “ n ” represents the number of wavelengths utilized) which employs 112 wavelengths from 770–900 nm.⁹ Chromophore absorption data for the oxidized and reduced forms of hemoglobin, cytochrome b, cytochrome c, and cytochrome aa₃ were obtained from the Biomedical Optics Research Laboratory (BORL) at UCL. Absorbance of all eight chromophores from 600–900 nm (1-nm intervals) was inserted into a table. The table was then pseudoinverted to provide a series of eight equations describing the concentration of each chromophore as a function of 301 absorption measurements.

Signal intensity (I) was converted to absorbance (A) using equation 2:

$$A = -\log_{10}(I/I_0)$$

where I_0 represents the intensity of the incident light. Absorption values were entered into the pseudoinverted matrix (as described above) in order to calculate *relative* chromophore concentrations. Because ϵ and L (see equation 1) are constant, *relative* changes in concentration can be estimated from relative changes in

Fig. 2 Schematic of data acquisition. Near-infrared radiation from a halogen lamp is emitted into the rat via the mouth (aluminum-coated fibreoptic cable bundle), and the resulting transmitted signals are detected bilaterally at the skull base (fibreoptic cable bundle housed in blue plastic). The emission signal is measured simultaneously. All three signals are exported to MATLAB for analysis and calculation of relative chromophore concentrations

1. Broadband light (VIS-NIR) is produced by a halogen light source

2A. Fiberoptic cables transmit light directly to a spectrometer, in order to measure the intensity of emitted light

2B. Fiberoptic cables transmit light to the animal's brain via the mouth

3. Fiberoptic cables receive light from the animal's skull and transmit it to two spectrometers (L and R)

4. Spectroscopic data is recorded by a laptop computer for later processing (see text for algorithm)

Table Differences between traditional (both laser and light-emitting diode [LED]) and broadband near-infrared spectroscopy

Feature	Traditional NIRS (laser)	Traditional NIRS (LED)	Broadband NIRS
Light Source	Laser	LED	Halogen bulb
Detector	Photodiode	Photodiode	Spectrometer
No. of Wavelengths	2 to 5	2 to 5	> 100
Validated Chromophores	Hemoglobin/myoglobin	Hemoglobin/myoglobin	Hemoglobin/myoglobin, cytochrome aa ₃
Additional Chromophores (unvalidated)	none	none	Cytochrome b, cytochrome c, water content
Clinically Available Devices	CASMED FORE-SIGHT® (CAS Medical Systems; Branford, CT)	CASMED FORESIGHT ELITE (CAS Medical Systems; Branford, CT), EQUANOX™ (Nonin; Plymouth, MN), INVOS™ (Covidien; Dublin, Ireland)	None

Importantly, all devices are capable of measuring the oxygenation state of hemoglobin and myoglobin, but they are unable to differentiate between the two (because their absorption spectra are almost identical). NIRS = near infrared spectroscopy

Fig. 3 Relative changes in deoxy-Hgb (blue), Hgb-O₂ (red), and the cytochrome difference (black) for all 20 rats after injection of sodium cyanide 5 mg·kg⁻¹ *iv* (t = 0). Median data are presented in bold

A (or I, since I_0 is known). Absolute measures of chromophore concentration require additional techniques (e.g., phase modulation spectroscopy, second differential spectroscopy) to measure L as well as knowledge of DPF (which is dependent on species and tissue). Thus, chromophore concentrations are described in arbitrary units (AU), which relate the concentration of measured chromophores to each other. Because total cytochrome concentrations do not change over short periods of time, this technique permits trend monitoring without knowledge of absolute chromophore concentrations. Lastly, for each animal, data from the left and right optode were averaged.

Statistical analysis

Unlike hemoglobin concentrations, which change over time, the total amount of cerebral cytochrome aa₃ is fixed over short time periods. Therefore, it is conventional to report Cyt_{ox} as the “cytochrome difference” (CytoD), defined as the difference between oxidized and reduced forms of cytochrome aa₃.^{7,9} We therefore compared changes in deoxygenated hemoglobin (deoxy-Hgb), oxygenated hemoglobin (Hgb-O₂), and CytoD that occurred following each intervention (NaCN injection, anoxia). Based on previous animal experiments, we would expect NaCN injection to lead to a reduction in cytochrome

Fig. 4 Relative changes in deoxy-Hgb (blue), Hgb-O₂ (red), and the cytochrome difference (black) for all 20 rats after initiation of anoxia ($t = 0$). Median data are presented in bold

aa₃ accompanied by an increase in StO₂, and subsequent anoxia to lead to a reduction in cytochrome aa₃ accompanied by an decrease in StO₂.

With a lack of published reports of NaCN injection in rodents, we conservatively estimated that 20 animals would be needed to ensure that our study would be adequately powered to detect the cytochrome aa₃ reduction that accompanies injection of NaCN in this proof of concept study. This sample size is comparable with that of previous authors who utilized five to six animals in pig and rabbit models, respectively.^{6,12} Because of the small size of this study, non-parametric statistical techniques were used to compare animals pre-post intervention. Specifically, the two-sample Kolmogorov-Smirnov test was used to test the null hypothesis that data in two vectors (pre-event and 90-sec post-event) are from the same continuous distribution. Because multiple comparisons (three chromophores, two events) were performed, a Bonferroni correction was utilized and a $P < 0.00833$ (i.e., $0.05/6$) was considered statistically significant. A significant reduction in cytochrome aa₃ and an increase in StO₂ after NaCN injection, followed by a significant reduction in cytochrome aa₃ and a decrease in StO₂ after anoxia, was established *a priori* as an indication that the device could independently measure changes in Cyt_{ox} and StO₂.

Results

All 20 rats completed the study. Ninety seconds after injection of NaCN, median [interquartile range (IQR)]

deoxy-Hgb decreased from pre-NaCN levels in 80% of the animals (-0.014 [-0.29 to -0.005] AU; $P < 0.001$); Hgb-O₂ increased in 75% of the animals (0.013 [-0.011 to 0.031] AU; $P < 0.001$); and a reduction in cytochrome aa₃ occurred in 100% of the animals (-0.015 [-0.020 to -0.011] AU; $P < 0.001$) (Fig. 3). These data confirmed that broadband NIRS was able to detect the expected decrease in Cyt_{ox} and increase in StO₂ following NaCN administration. Confirmation of these results required analysis of the anoxia data, in which we expected both Cyt_{ox} and StO₂ to decrease.

Ninety seconds after initiation of anoxia, median [IQR] deoxy-Hgb increased from pre-anoxia levels in 95% of the animals (0.13 [0.11 to 0.18] AU; $P < 0.001$); Hgb-O₂ decreased in 95% of the animals (-0.17 [-0.22 to -0.15] AU; $P < 0.001$); and a reduction in cytochrome aa₃ occurred in 100% of the animals (-0.04 [-0.055 to -0.031] AU; $P < 0.001$) (Fig. 4).

Discussion

Using a NaCN validation model^{6,14} in which ETC inhibition and anoxia are induced independently, we have shown that broadband NIRS (constructed of commercial off-the-shelf equipment) is capable of detecting a reduction in Cyt_{ox} independent of changes in StO₂. Early Cyt_{ox} measurements were tested in a fluorochemical exchange model, in which hemoglobin was completely removed from the animal, using discrete wavelength devices.¹⁵ Some investigators have questioned whether these early measurements were accurate

Fig. 5 Schematic depicting the advantage of cytochrome measurements over measuring StO_2 . StO_2 is only capable of measuring the oxygen content of arterial and venous hemoglobin in tissue—it provides no information about the energetic state of the cell itself. In contrast, Cyt_{ox} measures the oxidation state of the electron transport chain, which can be affected by alterations in StO_2 as well as by inhibition from various toxins, lipopolysaccharide (LPS), and reactive oxygen species (ROS), the latter of which are implicated in ischemia-reperfusion and sepsis, respectively

(as opposed to being simply an artifact of the deconvolution algorithms designed to measure the concentration of individual chromophores) and whether or not they were clinically relevant, as removal of the dominant chromophore (hemoglobin) greatly simplifies the measurement of Cyt_{ox} .^{2,6} This novel study represents a significant attempt to validate the ability of broadband NIRS to measure changes in Cyt_{ox} and StO_2 independently, and it adds to a growing body of literature which suggests that noninvasive measurement of Cyt_{ox} is possible in real time.^{6,12} Importantly, our use of commercial off-the-shelf equipment suggests that this field of research is accessible to an increasing number of investigators.

Cooper *et al.* developed the NaCN model, in which cyanide inhibits the ETC but also leads to a compensatory increase in StO_2 , in order to validate the ability of broadband NIRS to decouple cytochrome aa_3 from StO_2 measurements.⁶ By applying broadband NIRS to a two-stage piglet model of cerebral ETC dysfunction (NaCN followed by anoxia), Cooper *et al.* convincingly showed that the UCLn algorithm could measure changes in cytochrome aa_3 and StO_2 in five pigs.⁶ Eight years later, Lee *et al.* performed a similar experiment on six rabbits. They used a combination of multifrequency frequency-domain photon migration (FDPM) with time-independent NIRS to measure changes in deoxy-Hgb, Hgb- O_2 , and

Cyt_{ox} in rabbits subjected to NaCN infusions designed to elicit cerebral ETC dysfunction. Lee *et al.* also showed an increase in StO₂ following an NaCN injection, as well as a reduction in Cyt_{ox}, but they did not induce anoxia after completion of the NaCN injection.¹²

In our study, NaCN 5 mg·kg⁻¹ did not induce complete cytochrome aa₃ reduction. The dose effect of NaCN on animals may be species specific. For example, Cooper *et al.* administered NaCN 10 mg·kg⁻¹ to some pigs and Lee *et al.* utilized an escalating dose via infusion in rabbits.^{6,12} Since we did not correlate NIRS changes with histopathologic changes in cerebral tissue, we cannot comment regarding the relative sensitivity or specificity of Cyt_{ox} and StO₂ for the detection of specific cerebral ischemia/hypoxia. This is the subject of current ongoing studies. While our device and algorithm do not calculate absolute chromophore concentrations, these features could be added with additional hardware and software modifications.

Our study, in combination with Cooper and Lee's work,^{6,12} is important because, while NIRS for the measurement of StO₂ has been commercially available for over a decade, the outcome data are not strong to support using these devices, even in patients undergoing cardiac surgery.¹⁶ This may be due to a variety of factors, including extracranial contamination, changes in arterial and venous compliance, or the assumption that the arterial:venous blood volume ratio is the same between individuals.¹⁷⁻²¹ Interestingly, three recent multicentre randomized-controlled trials with a total of 4,192 subjects failed to show a mortality benefit when hemodynamics were guided by ScvO₂ in patients with septic shock.²²⁻²⁴ Combined, these data suggest that hemoglobin saturation, either in blood or in tissue, may not be as useful a measure of end-organ health as once thought.

Cytochrome aa₃, which reflects the state of subcellular energetics directly, as opposed to oxygen availability, may be a more physiologically relevant chromophore than hemoglobin (Fig. 5). Lipopolysaccharide, for instance, has been shown to uncouple oxidative phosphorylation, leading to mitochondrial respiratory dysfunction and swelling in animal models of sepsis.²⁵ In experimental models of sepsis, mitochondrial dysfunction due to nitric oxide overproduction and complex I inhibition leads to adenosine triphosphate depletion and is related to sepsis severity.²⁶ The term "cytopathic hypoxia" was recently developed to describe the phenomenon of defective cellular respiration leading to decreased oxygen consumption.²⁷

A comparison between StO₂ and Cyt_{ox} in pigs subjected to cardiopulmonary bypass and deep hypothermic circulatory arrest showed significant differences between the two measures during cooling.²⁸ A comparison between StO₂ and Cyt_{ox} in piglets subjected to hypoxic-ischemic insult designed to replicate neonatal hypoxic injury showed a

steady reduction in Cyt_{ox} ten hours after the insult, despite the fact that StO₂ remained normal.²⁹ Magnetic resonance spectroscopy measurements of the phosphocreatine:inorganic phosphate (PCr:P_i) ratio have shown that brain injury occurs several hours after a hypoxic-ischemic insult, suggesting that these Cyt_{ox} measurements capture the progression of brain injury in real time.³⁰

It is worth mentioning that current NIRS devices utilize two to five semidiscrete bands of light ranging from 690–880 nm and are incapable of measuring cytochrome concentrations, which seems to require broadband instrumentation focused around 820 nm.³¹ Light-emitting diodes in most commercially available oximeters produce a band of light, and because photodiodes are utilized to detect light intensity (as opposed to spectrometers), these devices cannot accurately measure the intensity of specific wavelengths of transmitted light.

Our data show that broadband NIRS can effectively measure the directionality of changes in both Cyt_{ox} and StO₂ using readily available equipment. This could potentially lower the barrier to further development of devices designed to measure ETC dysfunction / subcellular energy derangements in real time, both for research purposes and clinical use.

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Conflicts of interest None declared.

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Author contributions Robert H. Thiele and Zhiyi Zuo contributed to the study design. Robert H. Thiele, Keita Ikeda, Raquel R. Bartz, and Zhiyi Zuo contributed to the analysis and interpretation of data and manuscript preparation. Robert H. Thiele and Keita Ikeda attest to the integrity of the original data and the analysis reported in this manuscript. Robert H. Thiele is the archival author. Keita Ikeda and Yunzhen Wang contributed to the conduct of study.

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