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Near-Infrared Spectroscopy: What Can It Tell Us about Oxygen Saturation in Skeletal Muscle?

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Near-infrared spectroscopy (NIRS) measures hemoglobin saturation in small vessels. A number of interesting studies have used this method. However, difficulties with signal quantification and studies in which NIRS oxygen saturation did not behave as expected raise concerns. NIRS remains promising for studies of skeletal muscle, but a better understanding of the method is needed. Keywords: near-infrared spectroscopy, optical spectroscopy, oxygen delivery, oxidative metabolism

INTRODUCTION

Oxygen plays a critical role in energy metabolism in mammalian skeletal muscle. Befitting this role, the delivery of oxygen and the potential role of insufficient oxygen delivery have been well-studied areas for a long time. It would be difficult to argue against the precept that almost any experiment that involves exercising muscle would be well served by measuring oxygen delivery and oxidative metabolism.

One of the important difficulties in measuring oxygen delivery is methodological. This is particularly true of human studies, in which there is (as there should be) an emphasis on noninvasive experiments. Two common noninvasive methods of measuring blood flow are plethysmography and Doppler imaging. These provide global measures of flow, with plethysmography measuring changes in limb volume corresponding to total arterial inflow, and Doppler imaging corresponding to the volume flow in a large artery. As useful as these measures are, they do not reflect the actual delivery and utilization of oxygen in skeletal muscle. Magnetic resonance spectroscopy (MRS) of high-energy phosphorus compounds can provide an accurate picture of energy metabolism (4), but this method cannot differentiate between limitations caused by oxygen delivery and oxygen utilization. MRS of muscle myoglobin has the potential to measure intracellular oxygen saturation. However, this methodological capability is still rare and all MRS experiments require large and expensive equipment.

In contrast, near-infrared spectroscopy (NIRS) has a number of significant advantages. It can be portable, and in its simplest forms, NIRS is relatively inexpensive ($4,000-$25,000). Signal detection is based on nonharmful levels of light, which are directed into the subject's muscles through the overlying skin. The resulting signal comes from hemoglobin (Hb) located in small vessels (or intracellular myoglobin) and thus indicates the balance between local oxygen delivery and oxygen utilization. Indeed, these very characteristics have stimulated a fairly rapid growth in the number of experimental studies that have used this technique (4).

HOW NIRS WORKS

A number of excellent reviews have focused on the principles of NIRS technology (3,4,12). This review will describe some of the essential aspects of continuous wavelength NIRS, which is the most commonly used and least expensive form of NIRS. Further, this review will focus only on interpretations of the oxygen saturation measurements, leaving blood volume measurements for another day. With continuous wavelength NIRS, the detector picks up a continuous stream of light from the light source, as opposed to time-resolved or phase-modulated forms of NIRS (12). Light in the range of 700–900 nm is used because a) these wavelengths show good penetration of biological tissues, b) the heme groups of Hb and myoglobin are among the primary absorbing compounds, and c) the absorption of light by the heme groups is altered by oxygen. The detectors pick up light after it has traveled through the tissue. In the simplest case, this light is from two different wavelengths, commonly 760 and 850 nm. At 760 nm, deoxygenated heme has a higher

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absorbency, and at 850 nm, oxygenated heme has a higher absorbency (Fig. 1). The difference signal (signal at 760 minus the signal at 850) will be sensitive to changes in heme-oxygen saturation and the sum signal to changes in overall heme concentration. Most continuous NIRS devices use white light with specific filters on the detectors (1,4,10), although some are the other way around, with specific wavelength light sources, and unfiltered light detectors (3,13,14). Reflected light is used to study skeletal muscles in humans as the muscles are too thick to allow light to be transmitted directly from the light source to the detector. This is different from pulse oximetry, in which the light is transmitted across the finger or earlobe (15). For obtaining a reflected light signal from skeletal muscle, the source and detector are usually separated by 3 cm. This results in the light traveling in a shallow arc with a penetration depth of approximately one half the separation distance, or 1.5 cm, into the tissue. Light is scattered in all directions, but is only picked up by the detectors in measurable amounts from the shallow arc (Fig. 1).

WHAT DOES NIRS MEASURE?

In Vitro Calibration Experiments

As mentioned above, the difference signal should reflect changes in heme-oxygen saturation. The initial experiments testing NIRS units used *in vitro* models. In these experiments, the light source and detectors were placed on solutions containing variable amounts of human blood (varying Hb concentrations). Yeast or sodium dithionite was used to alter oxygen saturation. These experiments found linear relationships between NIRS measured oxygen saturation and measurements with standard clinical laboratory equipment (5% or less deviation from the line of identity) (13). The difference signal not only tracked changes in oxygen concentration but also did so independently of changes in the total heme concentration.

Although there is some controversy over how much of the NIRS signal might reflect myoglobin, studies by Seiyama et al. (11) clearly suggest that more than 90% of the signal comes from Hb. In these studies, NIRS measurements were made in rat hindlimb preparations in which the red blood cells were replaced with fluorocarbons. The addition of blood in either fully oxygenated or reduced conditions was faithfully tracked by the NIRS device (11). Species with higher myoglobin concentrations than rats will have a greater relative signal contribution from myoglobin, although it is still considered a minor contributor to the NIRS signal.

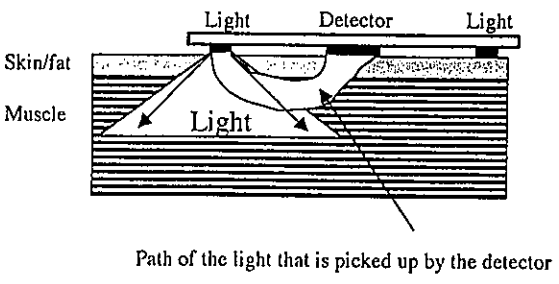
Most of the Hb signal is thought to come from small vessels, because large arteries and veins (>~1 mm diameter) have large heme concentrations that absorbs all the light. This means there is no detectable signal change in larger vessels with changes in oxygen saturation. The NIRS difference signal is thought to reflect the oxygen saturation in the small veins because most of the blood in skeletal muscle is located in the veins and because of the lower apparent hematocrit (Hct) in capillaries.

In Vivo Studies

A large number of studies have used the NIRS method to study oxygen saturation *in vivo*, but only a few will be mentioned here. NIRS-measured oxygen saturation has been shown to decline during incremental exercise (1,4,14). This decline has been correlated to decreases in directly measured venous oxygen saturation (14). Bhambhani et al. (1) found that at low levels of exercise the difference signal increased, indicating increased oxygenation. At higher work levels, the signal decreased progressively, indicating deoxygenation. The point at which the NIRS signal crossed the starting value correlated with the ventilatory threshold determined from expired gases (the traditional V-slope method) (1). Wilson et al. (14) found that patients with heart failure had lower levels of oxygen saturation at any given work level than age-matched control subjects. Interestingly, within the heart failure patients, there seemed to be an inverse correlation between peak oxygen consumption and the rate the patients desaturated. In contrast, dogs with phosphofructokinase deficiency have impaired oxidative metabolism and normal circulation. With electrical stimulation, dogs with phosphofructokinase deficiency had less oxygen desaturation than normal dogs (8). This study is among several that suggest that metabolic disorders can result in excess oxygen delivery.

Unsuccessful *In Vivo* Studies?

Not all published studies have found a good correlation between NIRS measurements and direct measurements of



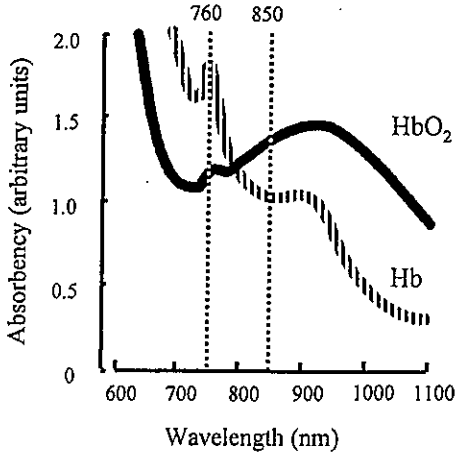


Figure 1 The left panel shows a typical arrangement of the NIRS device. The separation of the light sources and detectors allow the light to scatter throughout the tissue. Note that most of the light is scattered or absorbed and is not picked up by the detector. The detectors only pick up light that happens to follow a shallow arc between the source and the detector. The right panel shows the absorption curves for oxygenated and deoxygenated Hb. The careful choice of two wavelengths will result in a signal that is sensitive to changes in oxygen saturation.

oxygen saturation. Several studies found that during exercise the NIRS signal initially desaturated and then showed significant resaturation (2,7) (Fig. 2). In these studies, direct measurements of venous oxygen saturation initially decreased but did not increase as the NIRS signals did. This represented a clear lack of correlation between the NIRS signal and direct measurements. Do these studies invalidate the NIRS method of measuring oxygen saturation? Based on the *in vitro* results and the very good correlation with the initial changes in oxygen saturation, one would suspect that the NIRS device is tracking oxygen saturation but that something else is happening during prolonged exercise that alters the NIRS signal independently of venous oxygen saturation. What could this 'something else' be? A number of possibilities are outlined in Figure 2. First, the deoxygenation signal calculated from the 760–850 nm signal is influenced by changes in blood volume. This is corrected by calibrating the signal, which is usually done at the start of the experiment. However, if blood volume changes occur during the course of an experiment, the calibration may need to be repeated throughout the experiment to avoid 'apparent' changes in oxygen saturation. A second possible explanation is that there could be changes in the anatomical location of the NIRS signal. The NIRS signal consists of a weighted average of the oxygen saturations of the heme groups in the vascular bed (arterioles, capillaries, and venules). If during exercise there was a change in relative volumes such that more blood or more red blood cells were located in the relatively more oxygenated capillaries relative to the veins, then the NIRS oxygen saturation would seem to rise. In support of this, several studies have shown that the magnitude of the 'rise' in NIRS measured oxygen saturation was reduced during hypoxic conditions (2,7). With hypoxia, overall oxygen saturation is reduced and the gradients along the vascular tree reduced. Thus, shifts in blood volume between compartments will have a lesser effect on NIRS measured oxygen saturation under hypoxic conditions. Although detecting shifts in the Hb content of the vascular bed is interesting in its own right, to what degree this confounds NIRS determinations of oxygen saturation needs to be carefully considered when designing and interpreting NIRS experiments.

Quantification of the NIRS Signal?

Quantification of the NIRS signal is one of the important issues concerning NIRS measurements. A number of NIRS studies have presented data in terms of absolute values (3,7,13), whereas other studies present relative changes or 'physiological' calibrations (4,8,14). To convert the absorption of light to absolute concentrations, the path-length of the light from source to detector must be known (12). A common approach is to use a differential path-length factor

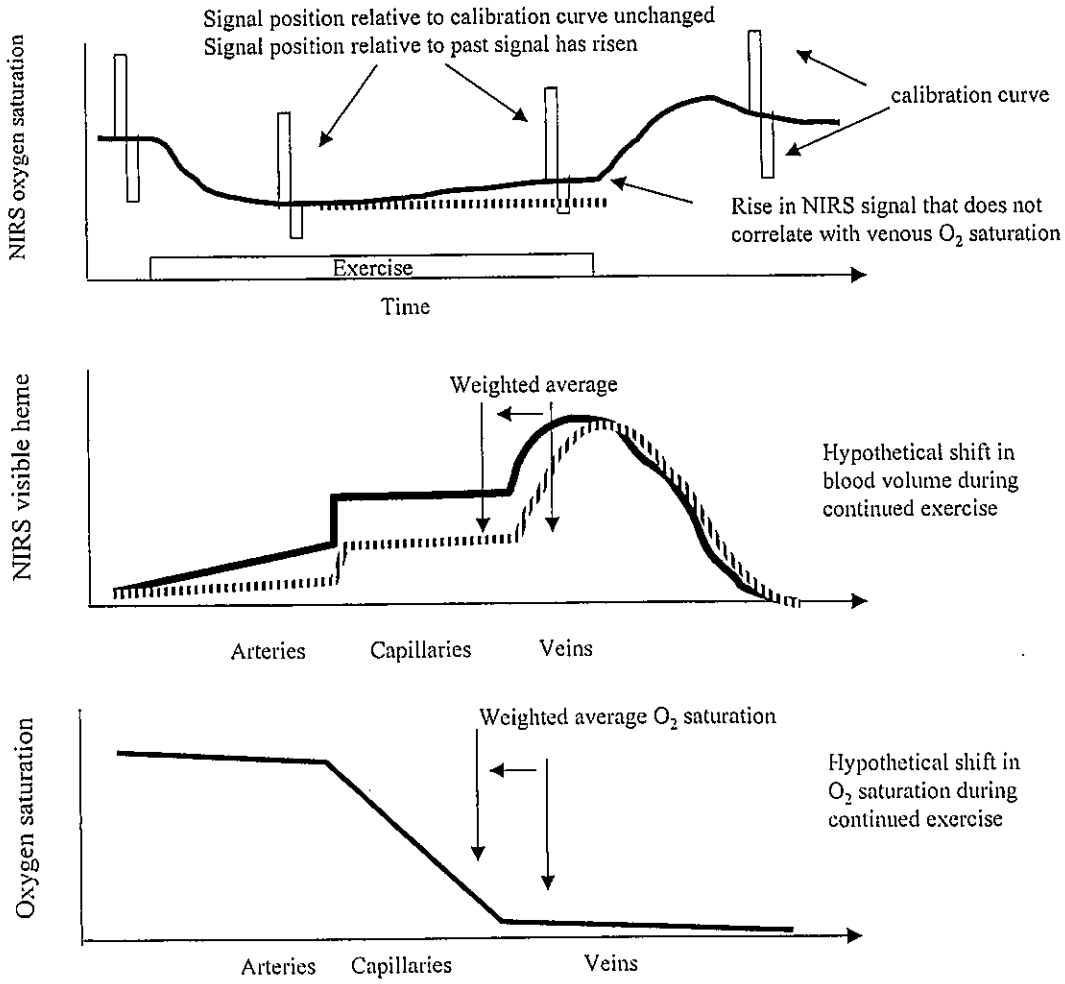


Figure 2 Several possible explanations for NIRS-measured oxygen saturation rising with continued exercise (top). Top, a change in path-length could result in a change in the calibration curve, producing an 'apparent' rise in oxygen saturation. The middle panel shows oxygen saturation along the vascular tree and shows how an increase in relative blood volume/hematocrit in the capillaries could shift the weighted average of the NIRS signal toward the capillaries. Bottom, how a shift in the signal could result in an increase oxygen saturation of the NIRS signal.

determined from other measurements, such as phase-modulated optical systems (3). This approach is facilitated by using three or four wavelengths (13). The main problem with this approach is that the differential path-length factor(s) are based on a homogeneous absorbing medium. However, skin and subcutaneous fat have very different absorption properties than muscle. Not only do different people have different proportions of subcutaneous fat and muscle, but also the absorption characteristics of the two regions might change relative to each other during the course of an experiment. Calibrations of absolute values often work well for carefully chosen populations, but this does not mean they will work well on specific persons who differ from the 'average' condition.

Subcutaneous fat has a large impact on the NIRS signal. Figure 3 illustrates the impact of variable amounts of subcutaneous fat on the relative oxygen saturation. Whereas more fat will mean less light absorption and thus a stronger signal, the metabolically inactive fat with lower Hb levels will result in less signal change with the experimental condition. Most NIRS studies have examined carefully selected subjects who were relatively thin. However, to be useful in a clinical setting, NIRS measurements need to be made on 'real world' subjects. In a study of older subjects with suspected vascular disease, 21% were too obese to obtain reliable NIRS measurements (9). In this study, the cut-off for a successful NIRS signal was a body mass index [height (m)/weight (kg^2)] less than 32.

Instead of calibrations based on differential path-lengths and multiple wavelengths, some studies have used 'physiological calibrations' (14). Wilson et al. (14) normalized the difference signal to the maximally observed physiological range. The physiological range was determined by making the muscle ischemic to obtain the lowest value (0%) and then releasing the cuff and using the highest value during the subsequent hyperemia as 100%. This result is certainly practical, but results expressed in percentage of oxygen saturation can be confusing because they don't correspond to directly measured Hb oxygen saturation (which for the veins typically ranges from 40% at rest to ~20% during heavy exercise).

Studies That Do Not Quantify Oxygen Saturation

An approach to using NIRS that does not depend on absolute measurements is to measure the recovery kinetics of the difference signal (9,10,14). The calculation of a time constant of recovery or a half-time of recovery does not require knowing absolute signal intensity. This approach assumes that recovery kinetics are not linear, but exponential in nature. Although the recovery of the NIRS difference signal after exercise or ischemia does not always fit an exponential shape (9), it does seem to have the qualities of an exponential curve. The rate of recovery of oxygen saturation after exercise is a function of oxygen delivery and oxygen utilization. Thus, it is not surprising that NIRS-measured recovery rates are similar to those for phosphocreatine (PCr) recovery measured with MRS. A number of studies have used this approach to show that patients with impaired peripheral circulation have slowed recovery rates (9). Interestingly, patients with chronic heart failure were shown to have slower recovery rates for PCr than for oxygen saturation (5). This suggests these patients have greater reductions in muscle mitochondrial function than in oxygen delivery. Patients with chronic fatigue syndrome have been shown to have just the opposite result: they have slower rates of oxygen delivery than PCr recovery (10). This supports the idea that these patients have abnormal vascular control. A variation of this approach is to measure the recovery of oxygen saturation after 4 min of cuff ischemia. After 4 min, PCr depletion has not occurred, and there should be no significant oxygen consumption after release of the ischemia. The recovery of oxygen saturation should only be a function of oxygen delivery (10). Using this approach, patients with chronic fatigue syndrome had slower rates of recovery than normal subjects, supporting the idea that they have abnormal vascular control (10).

NEW DEVELOPMENTS IN NIRS TECHNOLOGY

An interesting development with NIRS is the combining of multiple sources and detectors and producing an NIRS imaging device (6). Images have been produced in this man-

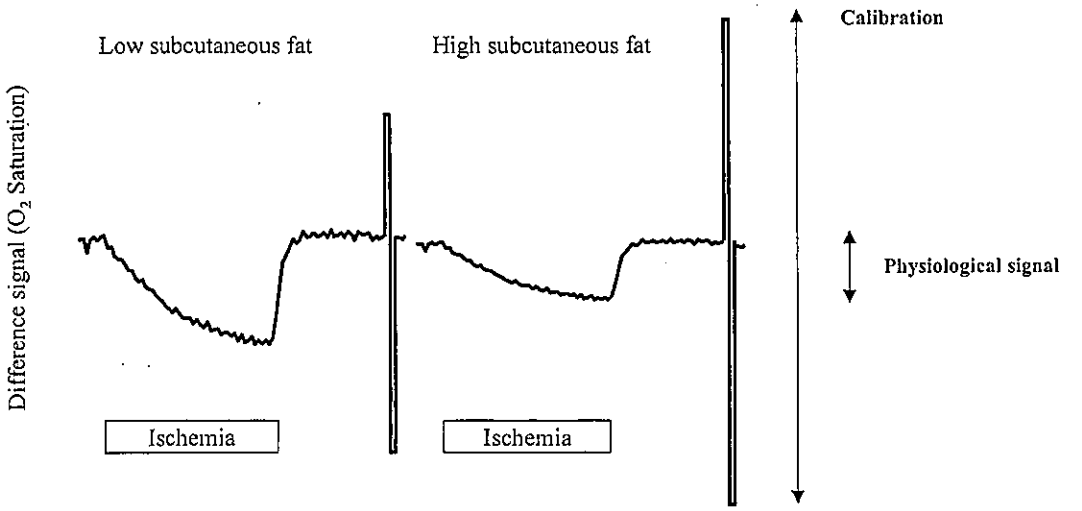


Figure 3 This figure shows expected results for a subject with low subcutaneous fat (left) and high subcutaneous fat (right). The calibration signal is larger in the high fat subject, indicating less absorption of light. However, the change in signal with ischemia is greater in the subject with low subcutaneous fat, demonstrating why more reliable NIRS signals are obtained from thin subjects.

ner of brain, breast, and skeletal muscle. The goal with this approach is to identify tumors and ischemic areas in brain and breast tissue and to measure heterogeneity in oxygen delivery in skeletal muscle. For skeletal muscle, the problems have been that the light sources do not cycle fast enough, so time resolution is not as good as it could be (8 s, for example). Another problem has been making the imaging surface flexible enough to fit on the surface of exercising muscle. Any subjects thin enough to get adequate signal, will have contours in their muscles with exercise that will move the source or detectors off the skin. The current 4- × 12-cm imaging arrays also can be too large for single muscles in many human subjects.

SUMMARY: WHAT CAN NIRS TELL US ABOUT OXYGEN DELIVERY?

A large number of studies have made use of NIRS in a variety of ways. This review covers only the use of continuous wavelength devices to study oxygen saturation. Early studies have shown that careful use of the NIRS device can provide interesting information about oxygen delivery and oxidative metabolism. The NIRS device is not without its inherent weaknesses, however. Despite the continued use of quantification with NIRS, it remains very difficult to measure absolute oxygen saturation levels because of the difficulties in determining optical path-length. Other aspects of the device also pose questions, such as: Why, under some conditions, does the NIRS-measured oxygen saturation increase at a time when directly measured venous oxygen saturation remains constant? Further studies into correcting the NIRS signal for variations in fat thickness, development of more robust signal quantification, and correcting for shifts in vascular blood volume are needed. Newer uses of the NIRS technology, such as imaging, remain in the developmental stage. Therefore, although useful experiments have been performed, care must be taken when designing studies with the current continuous wavelength NIRS devices.

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