Estimated contribution of hemoglobin and myoglobin to near infrared spectroscopy

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ABSTRACT

We calculated the light absorbing potential (LAP) of hemoglobin (Hb) and myoglobin (Mb) in mammalian skeletal muscle at rest based on analysis of published chemical and morphometric data (Part 1), interpreted changes in total[Hb + Mb] from NIRS during exercise (Part 2), and estimated the potential contribution of Hb and Mb to changes in NIRS from rest to exercise (Part 3). Part 1: [Hb] in skeletal muscle was estimated from microvascular volume, systemic blood [Hb], and microvascular hematocrit and saturation at rest and during exercise. Part 2: Changes in total[Hb + Mb] (as t[Hb + Mb]) during cycling or knee extension exercise were interpreted using the results of Part 1. Part 3: Using estimates of mean microvascular PO_2, Hb and Mb contribution at peak exercise was estimated. Across several species, [Mb] contributed ∼50–70% of the total LAP to NIRS at rest in skeletal muscle. With exercise, increases in t[Hb + Mb] of up to 30% could be entirely explained by the predicted increase in microvascular hematocrit with exercise. Finally, Mb was estimated to contribute ∼70% of the changes in NIRS from rest to peak exercise.

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1. Introduction

NIRS is a non-invasive, optical technique that uses near infrared light to noninvasively estimate brain, blood and tissue oxygenation, making NIRS ideal for use in human studies. This technique is based on the fact that hemoglobin (Hb) and myoglobin (Mb) can exist in two forms, oxygenated (oxy-[Hb] and oxy-[Mb]) and deoxygenated (deoxy-[Hb] and deoxy-[Mb]), each with its own absorption spectra. However, the ability to quantify the contribution of Hb and Mb oxygenation to the NIRS signal has been a challenging and controversial task. On the one hand, it has often been assumed that both the NIRS signal at rest, and changes in the signal during exercise, are derived primarily from Hb (>90%), while Mb is thought to contribute minimally (<10%) to the signal (Mancini et al., 1994; Seiyama et al., 1988). In contrast, other studies suggest that Mb may be the predominant contributor to the NIRS signal (Tran et al., 1999; Richardson et al., 2001; Mole et al., 1999).

Several factors may affect tissue [Hb], and thus its relative contribution to NIRS, including muscle microvascular volume and the average hematocrit and saturation within that vascular volume. Skeletal muscle [Mb] likely remains constant from rest to exercise. However, whether or not microvascular volume changes, and to what extent, during exercise is controversial (Lai et al., 2009a). Microvascular hematocrit is less than that of the systemic circulation at rest (Kindig and Poole, 1998, 2002; Russell et al.,

2003; Barbee and Cokelet, 1971; Klitzman and Duling, 1979) and increases toward systemic hematocrit with increased flow, such as seen with muscle contractions (Kindig et al., 2002; Desjardins and Duling, 1990). Thus, these factors predict that the relative contributions of Mb and Hb to NIRS could change drastically from rest to exercise.

The increase in VO_2 at the onset of exercise relies on several factors, one of them being the ability to widen the O_2 gradient from microvessel to mitochondria (Tran et al., 1999; Richardson et al., 1995). It has been suggested that the myocyte can modulate its own PO_2 gradient with Mb facilitated diffusion contributing significantly to oxygen transport to the mitochondria (Gayeski and Honig, 1988; Wittenberg, 1970; Wittenberg and Wittenberg, 2003; Mole et al., 1999). However, assessing the microvessel to mitochondria PO_2 gradient is exceptionally challenging in humans. NIRS in combination with 1H MRS has been used to noninvasively assess changes in tissue (i.e., Hb and Mb) saturation, in an attempt to distinguish Hb from Mb desaturation under ischemic and exercise conditions, with conflicting results (Mole et al., 1999; Tran et al., 1999; Jue et al., 1999). In other applications (e.g., estimation of microvascular blood flow (Q_{cap}) (Ferreira et al., 2006)), the assumption is made that the NIRS signal reflects primarily or exclusively Hb saturation status. If the NIRS signal contains significant contribution from Mb, the appropriateness of this methodology may need to be reevaluated.

Recently, Koga et al. (2012) have shown that vascular Hb begins to desaturate before intracellular Mb following the onset of contractions in the rat gastrocnemius. The fact that Hb and Mb may desaturate with different time courses during exercise challenges

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our current understanding of microvascular gas exchange, and reinforces the need to quantify the relative contributions of Hb and Mb to the NIRS signals at rest and during exercise.

Therefore, the purposes of this study were to (a) estimate the potential contributions of Hb and Mb to the total NIRS signal at rest (expressed as light absorbing potential (LAP)), using the relative concentrations of [Mb] and [Hb] in mammalian skeletal muscle based on published chemical and morphometric data (Part 1), (b) use the information in a) to interpret changes in total[Hb + Mb] by NIRS during exercise (Part 2), and (c) estimate the relative contribution of changes in Hb and Mb oxygenation to the NIRS signals from rest to maximal exercise (Part 3). Using appropriate assumptions regarding muscle microvascular density, systemic [Hb], relative contribution of Hb and Mb molecules to the LAP based on heme groups, average skeletal muscle [Mb] and microvascular hematocrit at rest, the contribution of Hb and Mb to the total NIRS signal can be calculated. We predicted that Mb will contribute significantly more to the NIRS signal in human skeletal muscle than the often assumed value of $\leq 10\%$ (Seiyama et al., 1988), perhaps contributing closer to 50% of the NIRS signal.

2. Methods

The present study was divided into three parts. In Part 1, we utilized values for microvascular densities and tissue [Mb] from several published studies to calculate the relative contribution of [Hb] and [Mb] to NIRS in mammalian skeletal muscle. Recognizing that the vascular component to near infrared signals arise exclusively from hemoglobin in blood vessels less than 1 mm in diameter (Mancini et al., 1994), which includes small arterioles, capillaries and venules, but that within skeletal muscle tissue capillaries contribute the vast majority to vascular volume (Poole and Mathieu-Costello, 1989), we will refer to the anatomical site for both gas exchange and source of the NIR signals as the microvasculature. An equation was developed to calculate muscle microvascular [Hb] and compared to reported tissue [Mb] values in the same muscles, whenever possible. In Part 2, the calculated contribution of [Hb] and [Mb] to the total NIRS signal from the present study was compared to NIRS measurements of total[Hb + Mb] from previous studies from this lab (Ferreira et al., 2006; Lutjemeier et al., 2008). Both of these published human protocols had been previously approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University. In Part 3, we utilized information on the mean capillary $PmvO_2$ and intramyocyte PiO_2 (Richardson et al., 2001, 2006) and resting microvascular Hct to estimate the relative contribution of changes in Hb and Mb oxygenation to the various components of the NIRS signal.

2.1. Part 1 – Estimation of tissue [Mb] + [Hb]

The first project utilized values of tissue [Mb] and microvascular density from the literature for a variety of mammalian skeletal muscles, including human, in order to estimate the concentration of tissue [Mb] and microvascular [Hb], which permitted calculation of the relative contribution of Hb and Mb to the total NIRS signal (expressed as light absorbing potential (LAP)). Microvascular volume in human skeletal muscle was estimated using the equations and values from Richardson et al. (1994):

$$\text{Cap Vol} = \frac{(28.3\ \text{mm}^2)(\text{number of cap} \times \text{mm}^2)}{1\ \text{mm}^2} \times 1.2 \qquad (1)$$

where $28.3\,\text{mm}^2$ represents the average microvascular area in human skeletal muscle. For the number of capillaries $\times\,\text{mm}^{-2}$, a range of $200–500\,\text{cap} \times \text{mm}^{-2}$ was used (Richardson et al., 1994), which allowed for multiple theoretical totals of microvascular volume to be calculated and compared.

The following equation was developed to obtain muscle microvascular [Hb] concentrations, from which LAP was estimated:

$$\text{Total[Hb]}_{cap} = 10\ \text{mmol/L of blood}$$
$$\times \text{Microvascular volume} \times 0.5\ \text{Hct}_{cap} \qquad (2)$$

where 10 mmol of hemoglobin per liter of blood was an average value which lay in the normal span of values for both men and women. The $0.5\,\text{Hct}_{cap}$ was used to reflect the reduction in microvascular tube hematocrit at rest, relative to that for systemic hematocrit. An average systemic hematocrit value of 45% (Kindig et al., 2002) was used and an average resting microvascular tube hematocrit of 22% was assumed (Kindig and Poole, 1998, 2002; Russell et al., 2003). In order to calculate LAP, 4 heme units per hemoglobin molecule and one heme per myoglobin molecule were assumed. Thus, 4 times as much light can be absorbed per Hb molecule compared to Mb, which will affect the contribution of each to the NIRS signal.

The calculated [Hb]$_{cap}$ values were compared to [Mb] values that were obtained from the same mammalian muscles when possible. For human muscle, the values of [Mb] came from van Beek-Harmsen et al. (2004), who measured myoglobin levels in skeletal muscles from male and female humans with and without disease. From their data an average of 0.5 mM [Mb] was used as the default value to compare to the estimated [Hb]. See original report for more information.

2.2. Part 2 – Exercise studies

2.2.1. Protocol I

The first protocol was an incremental ramp cycling exercise test (Ferreira et al., 2006), performed by nine healthy, physically active subjects, seven males and two females (24.7 ± 6.3 yrs, 67.9 ± 12.2 kg). Incremental cycling exercise was performed on an electronically braked cycle ergometer (Corival 400, Lode, The Netherlands). The exercise protocol began with 4 min of baseline cycling at 20 W at a cadence of 60 rpm. This was followed by a ramp increase in exercise intensity of 15–30 W/min until volitional fatigue was reached or cadence fell below 55 rpm for 5–10 s. Throughout the test, total[Hb + Mb] levels in the right vastus lateralis were measured by NIRS. See original report (Ferreira et al., 2006) for more details.

2.2.2. Protocol II

The second protocol (Lutjemeier et al., 2008) examined responses in eight subjects; seven men and one women volunteer (23 ± 2 yrs, 175 ± 9 cm and 71 ± 13 kg), during one leg, upright knee extension exercise on a specially built leg ergometer. A strap was placed around the subject's ankle and attached to a pneumatic cylinder through a cable pulley system. Each subject completed two work bouts at a contraction frequency of 40 contractions per minute. A moderate work bout was performed at 30% of peak work rate and a heavy work bout was performed at 80% of peak work rate. There was at least 20 min of rest between the two work bouts. Tissue oxygenation by NIRS was measured on the right rectus femoris throughout each test.

2.2.3. Procedures

In both studies, skeletal muscle oxygenation was estimated by a frequency domain multidistance (FDMD) NIRS system (OxiplexTS, ISS, Champaign, IL). The FDMD NIRS has 8 light emitting diodes using wavelengths of 692 and 834 nm and one detector fiber bundle. The source-detector separations were 2.0, 2.5, 3.0 and 3.5 cm. The probe was positioned longitudinally on the belly of the vastus lateralis (Protocol I) or the rectus femoris (Protocol II) for each