Hemoglobin and Myoglobin Contribution to the NIRS Signal in Skeletal Muscle

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Contents

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6.1 Introduction

Oxidative ATP generation plays a central role in muscle contraction and implicates an essential role for oxygen supply and flux during exercise. Of the different methods to track the oxygen balance in skeletal muscle, optical methods present a noninvasive and simple approach. In 1937, Millikan introduced an optical method to assess oxygen levels in cat soleus muscle at rest and in response to electrical stimulation [1]. He used a point light source (a pointolite lamp) to introduce a beam of visible light through a heat filter and a condensing lens onto a muscle holder, where it made a right-angle reflection from a totally reflecting prism. The beam then passed through the muscle and onto a photocell colorimeter, which recorded the characteristic absorbance signals of oxygenated hemoglobin (Hb) and myoglobin (Mb). Given the *in vitro* binding constants of O_2 to Mb and Hb, a calculation leads to values for the partial pressure of O_2. In contrast to the standard gas-exchange technique at that time, which suffered from time delays, the optical method could follow in real time Mb and Hb saturation [1].

Unfortunately, visible light has very poor penetration depth, which limits the applicability of Millikan's approach. Forty years later, Jöbsis refined the optical approach by using near-infrared (NIR) light (650–1100 nm) [2]. At longer wavelengths, the infrared light can penetrate more deeply than visible light into biological tissues. He showed that near-infrared spectroscopy (NIRS) could interrogate noninvasively cerebral and myocardial oxidative metabolism as reflected by the signals of Mb, Hb, and cytochrome (Cyt). Measurements at two (or more) wavelengths helped to differentiate the relative contributions [3, 4]. Jöbsis chose to measure the signals near the isosbestic point of 810 nm, the wavelength where oxyhemoglobin (HbO_2), oxymyoglobin (MbO_2), deoxyhemoglobin (DHb), and deoxymyoglobin (DMb) have equal specific extinction coefficients. His work launched the NIRS method to measure tissue oxygenation *in vivo*.

6.2 Near-Infrared Spectroscopy

In contrast to the millimeter penetration of visible light, near-infrared radiation has a deeper penetration depth. Because scattering has wavelength dependence, the predominant water in tissue (70–80 % of the overall content) scatters near-infrared light less than visible light. Near-infrared radiation (750–1400 nm) penetrates up to 1.5 cm of tissue [5–7]. With a typical source–detector separation of 3 cm, NIRS will detect signals from a banana-shaped volume centered approximately 1.5 cm below the surface [8]. A larger spacing between the source and detector will sample signals from a deeper volume but with lower signal sensitivity.

HbO_2, MbO_2, DHb, and DMb absorb NIR light from 750 to 1400 nm. DHb and DMb have well-defined but overlapping absorbances at 760 nm. In contrast, MbO_2 and HbO_2 have overlapping broad absorbances extending from 750 to 1000 nm. Many NIRS instruments use the 850 nm signal in a relatively flat spectral region to index the MbO_2 and HbO_2 signals, partly because they skirt the lipid signal centered at 940 nm and the intense water signal at 1000 nm [9]. NIRS instruments also measure the 760 nm absorbance.

NIRS then assesses tissue oxygenation by quantifying the signal intensities of oxy- and deoxy-myoglobin (Mb) and hemoglobin (Hb) [2, 10–12]. The dissociation constants (K_d) of MbO_2 and HbO_2 in solution lead then to the determination of the tissue PO_2. Because the Mb and Hb signals overlap, the PO_2 reflects both the vascular and cellular oxygenation state in muscle. Most muscle studies assume a minor cytochrome (Cyt) contribution. Studies have used NIRS to investigate tissue oxygenation in different human organs *in vivo*, particularly brain and skeletal muscle [11, 13, 14].

Researchers have also used NIRS to delineate physiological and pathophysiological conditions, e.g., cerebrovascular disease, diabetes, or Becker muscular dystrophy [11, 15–17].

For brain studies, only Hb and Cyt contribute to the NIRS signal, since brain has no Mb. Spatial and time-resolved NIRS experiments have ascribed 55 and 69 % of the detected signal arising from cerebral tissue.

The ratio of DHb/HbO_2 reflects the vascular PO_2, while DMb/MbO_2 reflects the cellular PO_2. Since the Hb concentration in erythrocyte doesn't change, any increase or decrease of the overall signal of Hb ($DHb + HbO_2$) must then correlate with changes in the blood volume. Given this viewpoint and the assumption of a predominant contribution of Hb to the NIRS signal, many studies have used the total Hb signal to estimate blood volume, O_2 extraction, and O_2 consumption ($\dot{V}O_2$) [18–20]. In skeletal muscle, using NIRS in a rest–exercise–recovery or ischemia–reperfusion paradigm casts insight into vascular response during exercise [11, 12, 19, 21, 22]. Several reviews have discussed NIRS signal quantification, limitations, and biomedical applications [10, 11].

6.3 Source of the NIRS Signal in Skeletal Muscle

In contrast to the consensus about the predominant contribution of Hb to the NIRS signal in brain, no such consensus exists for skeletal muscle [10]. The oxygenated and deoxygenated forms of Mb and Hb display almost identical absorbances [14]. Even though the relative contribution remains uncertain, many researchers have still presumed a dominant Hb contribution [11, 14].

Early theories posited that Mb cannot contribute significantly to the NIRS signal, because its high affinity for O_2 would preclude a rapid release during a surge in energy demand at the start of contraction [23, 24]. As such, Mb does not readily release its O_2 store. Hb must then supply all the O_2 from the very start of muscle contraction [23, 24]. However, NMR experiments have demonstrated that Mb does desaturate at the start of contraction [25, 26]. The cell can use the O_2 store of Mb to meet the sudden surge in O_2 demand [9]. Consequently, Mb can contribute to the NIRS signal. Nevertheless, many continue to assume that NIRS monitors predominantly Hb oxygen saturation and desaturation kinetics. The NIRS observed change (Δ[deoxy]) reflects only the capillary blood flow adjustment [12, 27]. Presupposing a dominant Hb contribution in the NIRS signal supports implicitly a school of thought that envisions the O_2 gradient from the capillary to the cell controlling O_2 consumption ($\dot{V}O_2$) [28–31].

6.4 Presuming Hb as the Predominant Source

On the basis of a comparative analysis of the NIRS signal with and without blood in isolated perfused rats hindlimb (a nonphysiological model), Seiyama *et al*. suggested that Mb contributed less than 10 % of the NIRS signal. Hb must then contribute predominantly [27]. In support of that, Wilson *et al*. showed in isolated perfused canine gracilis muscle that changes in Hb oxygen saturation were primarily responsible for the light absorption changes [24]. They actually compared the NIRS changes in muscle with and without ethyl hydrogen peroxide, which converts presumably the cellular Mb to the ferric state or metMb. Because metMb cannot bind O_2, it cannot undergo the transition from oxy to deoxy Mb. In their buffer perfused muscle, these authors did not observe any change in the 760–800-nm absorbance upon peroxide treatment. Since Mb oxidation doesn't alter the NIRS signals, only Hb must contribute to the NIRS signal in blood perfused tissue [12, 24]. However, these experiments never measured Mb saturation and used nonphysiological models. Moreover, they

presumed Mb oxidation with chemical manipulations without presenting any evidence to quantify the extent of metMb formation *in vivo*, especially given the experimentally observed and robust Mb and Hb reductase system that converts readily Fe^{3+} back to Fe^{2+} in *in vivo* muscle [25]. Moreover, rat hindlimb and canine gracilis muscle appear to have lower Mb concentration than human muscle, which would question the simple extrapolation of animal to human results [14, 32, 33].

6.5 Separating Mb and Hb Signals with a Second Derivative Approach

Even though the signals overlap, Mb and Hb do exhibit slight spectral differences. Under ideal conditions, a second derivative transformation reveals spectral differences and provides a basis for a wavelength shift analysis to distinguish Mb from Hb [34]. Given the slight difference between Mb and Hb in many species and under ideal conditions, a second derivative transformation can potentially separate the contribution from Mb and Hb. Some questions, however, remain about the accuracy of the approach in measuring Mb and Hb under physiological conditions. With the second derivative analysis, Hb contributes 21 % of the NIRS signal arising from the human first dorsal interosseous muscle. In contrast, Hb in mouse hindlimb contributes 86.5 %. The contrasting Hb contributions raise the puzzling question about species variation or measurement accuracy, which additional studies must clarify [34].

6.6 Modeling Mb and Hb Contribution

Considering the respective concentrations of Hb and Mb, Davis and Barstow investigated Mb and Hb contribution to the NIRS signal in human resting muscles [14]. With this in mind, authors calculated the relative contribution of [Hb] and [Mb] to the total heme concentration at different microvascular densities [14]. For [Mb], authors used values measured by van Beek-Harmsen *et al*. in human muscles [35]. Regarding Hb, muscle microvascular [Hb] (Total $[Hb]_{cap}$) was calculated using the following equation:

$$\text{Total}[\text{Hb}]_{\text{cap}} = 10\,\text{mmol/L of blood} \times \text{microvascular volume} \times 0.5\text{Hct}_{\text{cap}}$$

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 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 % was used and an average resting microvascular tube hematocrit of 22 % was assumed. The microvasculature volume was calculated as previously described by Richardson *et al*. [36].

From these [Hb] and [Mb], authors deduced contribution to Hb and Mb to the total light absorbing potential, knowing that Hb is composed of four heme units whereas Mb contains only one and so that four times as much light can be absorbed per Hb molecule compared to Mb [14]. These calculations allowed authors to conclude that, whatever the microvascular density, Mb contributes much more than Hb to the NIRS signal in human resting muscles [14].

Authors also examined Mb and Hb contribution to NIRS signal during muscle contraction. To this end, they have taken into account the increase in total [Hb + Mb] during exercise and approximated the oxygenated Hb portion of the NIRS signals by the weighted average oxygen microvascular saturation of the microvascular network at rest and peak exercise. This study concluded that Mb could contribute to ~70 % of the changes in NIRS signal from rest to peak exercise [14].

Using computer simulation, Nioka et al. also examined the relative contribution of Hb and Mb to the NIRS signal [37]. They used the following equation to predict Hb saturation ($S_{Hb}O_2$):

$$S_{Hb}O_2 = (1/(4f_{Hb}) + 3/4)S_{NIR}O_2 - (1/(4f_{Hb}) - 1/4)S_{Mb}O_2$$

where $S_{NIR}O_2$ and $S_{Mb}O_2$ are saturations obtained from NIRS and NMR experimentally, respectively. f_{Hb} and f_{Mb} are fractions of Hb and Mb assumed in the NIRS signal, respectively.

These authors conclude that Hb contributes about 50 % to the NIRS signal. Other simulation studies also indicate that Mb contributes significantly to the NIRS signal.

6.7 NIRS Signal in Blood Free Isolated Heart

A spectroscopic imaging investigation of isolated-perfused pig hearts further supports the non-negligible contribution of Mb to the NIRS signal. Isolated hearts were perfused either with a Hb-free blood substitute (Krebs–Henseleit buffer) or blood mixture to assess the Mb–Hb contribution [38]. The corresponding investigations disclosed that Mb contributed about 50 % of the composite (Mb + Hb) NIRS signal. The results further confirm a substantial Mb contribution to the near-infrared absorbance spectra of blood perfused cardiac tissue [38].

6.8 Delineating Mb and Hb Contribution with ^{1}H-NMR Spectroscopy

Because NMR can observe the distinct deoxy Mb and deoxy Hb signals, it can determine the relative change in intracellular and extracellular O_2, as reflected in the respective Mb and Hb signals. Several studies have used ^{1}H-magnetic resonance spectroscopy (MRS) to determine the Mb contribution to the NIRS signal in skeletal muscle [26, 39–41]. ^{1}H-MRS can detect the His-F8 N_δ proton from the deoxy-Mb and also the His-F8 N_δ proton of the deoxy-Hb in red blood cells [40, 41]. During ischemia and contraction, ^{1}H-MRS detects a predominant Mb desaturation. As blood flow decreases, both the deoxy Mb and deoxy Hb signals rise with the release of O_2 from the heme protein store. However, in all experiments, the deoxy Mb signal dominates [41]. Contracting muscle exhibits no DHb signal. During ischemia, DHb comprises only a small fraction of the overall DMb + DHb signal.

In one human gastrocnemius muscle study, a combined NIRS and ^{1}H-MRS study has shown that submaximal plantar flexion induces a large change in the NIRS signal, but the ^{1}H-NMR detects no Mb desaturation. By inference, the authors conclude that Mb does not contribute significantly to the NIRS signal in exercising muscle [39]. However, in another comparable study, ^{1}H-NMR detects an obvious Mb desaturation even during moderate exercise and contradicts the initial finding by Mancini *et al*. [26, 39, 42]. The contrasting results suggest that the Mancini experiments did not have sufficient sensitivity to detect Mb in exercising gastrocnemius muscle, which led to its faulty inference. In fact, ^{1}H-MRS and NIRS exhibit similar kinetic profiles, while Hb desaturation (measured by ^{1}H-MRS) has no significant contribution [40, 41].

6.9 Combined ^{1}H-NMR, ^{31}P-NMR, and NIRS Approach

Combined and simultaneous NMR/NIRS investigations can then serve as a valuable tool to better understand the regulation of oxidative metabolism in muscle during contraction. Moreover, interleaved ^{1}H/^{31}P-NMR signal acquisition combined with simultaneous NIRS measurement can record simultaneously NIRS signals, Mb (^{1}H-MRS) saturation, high energy phosphate level and pH

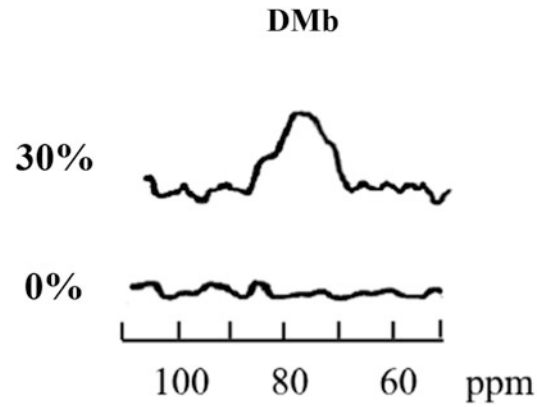

Fig. 6.1 ^{1}H nuclear magnetic resonance (NMR) spectra at rest (0 % MVC) and during exercise at 30 % MVC. The signal originates from the proximal histidyl-$N_{\delta}H$ of deoxymyoglobin (DMb), and its intensity reflects the level of cellular deoxygenation

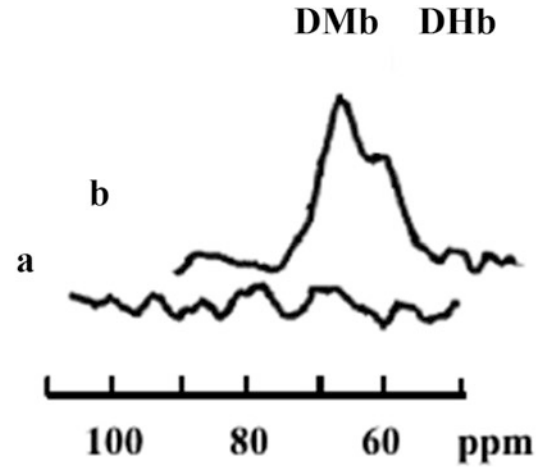

Fig. 6.2 ^{1}H nuclear magnetic resonance (NMR) spectra (*a*) at rest and (*b*) 6-min ischemia. Upon reperfusion the signals from the proximal histidyl-$N_{\delta}H$ of deoxymyoglobin (DMb) and deoxyhemoglobin (DHb) disappear (data not shown). Both the DMb and DHb signal intensities reflect increasing cellular deoxygenation

changes during superficial finger flexor muscles contractions at 10, 20, and 30 % of maximal voluntary contraction (MVC). These experiments confirm that Mb desaturation predominates, and Mb desaturation contributes much more prominently than Hb desaturation to the NIRS signal (Figs. 6.1 and 6.2) [43]. Moreover, the pH changes derived from the ^{31}P-MRS Pi signal, which reflect the lactate production, indicate that muscle produces lactate even at low MVC well above any hypoxia limit. The results support the idea that muscle does not need to reach any hypoxic or ischemic threshold, before it begins to produce lactate [44–46].

6.10 Summary

Near-infrared spectroscopy (NIRS) technique investigates tissue oxygenation *in vivo* by measuring the signals of oxy- and deoxy-myoglobin (Mb) and hemoglobin (Hb). Because the Mb and Hb signals overlap, the NIRS signal reflects both the vascular and cellular oxygenation state in muscle. Even though the precise relative contribution of Hb and Mb remains moot, many researchers have simply presumed a dominant Hb contribution to the NIRS signal based on conclusions arising from nonphysiological models and contentious simulation studies. The assumption has provided support

for the supposition that only the O_2 gradient from capillary to cell controls O_2 consumption. However, recent studies have shown that based on a second derivative analysis that Mb contributes significantly to the Mb/Hb NIRS signal. Recent computer simulations have also ascribed a substantial Mb contribution. Indeed, ^{1}H-NMR measurements detect only Mb desaturation. Hb contributes insignificantly. Combined and simultaneous ^{31}P-NMR, ^{1}H-NMR, and NIRS investigations appear to support a dominant Mb contribution and to present a valuable tool to better understand oxidative metabolism in muscle.

Problems

6.1. Mild muscle activity has been associated with pH changes, which reflects an increase in lactate concentration, suggesting that muscle produces lactate even during low-intensity exercises and under well-oxygenated condition. Discuss the underlying mechanisms and the implications in associating lactate production with a hypoxia or ischemia threshold.

6.2. If Mb has a dominant contribution to the NIRS signal, the sudden deoxygenation of Mb (as observed by NIRS) at the initiation of muscle contraction implies a rapid rise in oxygen demand or consumption. What then is the role of glycogenolysis? Does compartmentalizing rigidly energy metabolism into an independent nonoxidative vs. oxidative pathway have any physiological validity?

Solutions

6.1. Brooks, G.A.: Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. Fed. Proc. **45**(13), 2924–2929 (1986).
Brooks, G.A.: Intra- and extra-cellular lactate shuttles. Med. Sci. Sports Exerc. **32**(4), 790–799 (2000)
Brooks, G.A.: Cell-cell and intracellular lactate shuttles. J. Physiol. **587**(Pt 23), 5591–5600 (2009)

6.2. Chung, Y., *et al.*: Control of respiration and bioenergetics during muscle contraction. Am. J. Physiol. Cell. Physiol. **288**(3), C730–C738 (2005)

Further Study

Ferrari, M., Muthalib, M., Quaresima, V.: The use of near-infrared spectroscopy in understanding skeletal muscle physiology: recent developments. Philos. Trans. R. Soc. A **369,** 1–14 (2011)

Gros, G., Wittenberg, B.A., Jue, T.: Myogloblin's old and new clothes: from molecular structure to function in living cells. J. Exp. Biol. **213,** 2713–2725 (2010)

Masuda, K., Jue, T.: Application of near infrared spectroscopy in biomedicine. In: Jue, T. (series editor) Handbook of Modern Biophysics, vol. 4. Humana Press, New York (2013)

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References

1. Millikan, G.A.: Experiments on muscle haemoglobin *in vivo*; the instantaneous measurement of muscle metabolism. Proc. R. Soc. Lond. B: Biol. Sci. **123**(831), 218–241 (1937)
2. Jobsis, F.F.: Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. Science **198**(4323), 1264–1267 (1977)
3. Klungsoyr, L., Stoa, K.F.: Spectrophotometric determination of hemoglobin oxygen saturation: the method of Drabkin & Schmidt as modified for its use in clinical routine analysis. Scand. J. Clin. Lab. Invest. **6**(4), 270–276 (1954)
4. Refsum, H.E.: Spectrophotometric determination of hemoglobin oxygen saturation in hemolyzed whole blood by means of various wavelength combinations. Scand. J. Clin. Lab. Invest. **9**(2), 190–193 (1957)
5. van Staveren, H.J., *et al*.: Light scattering in Intralipid-10% in the wavelength range of 400-1100 nm. Appl. Opt. **30** (31), 4507–4514 (1991)
6. Mourant, J.R., *et al*.: Predictions and measurements of scattering and absorption over broad wavelength ranges in tissue phantoms. Appl. Opt. **36**(4), 949–957 (1997)
7. Pogue, B.W., Patterson, M.S.: Review of tissue simulating phantoms for optical spectroscopy, imaging and dosimetry. J. Biomed. Opt. **11**(4), 041102 (2006)
8. Strangman, G., Boas, D.A., Sutton, J.P.: Non-invasive neuroimaging using near-infrared light. Biol. Psychiatry **52** (7), 679–693 (2002)
9. Chung, Y., Jue, T.: Noninvasive NMR and NIRS measurement of vascular and intracellular oxygenation in vivo. In: Jue, T., Masuda, K. (eds.) Application of Near Infrared Spectroscopy in Biomedicine, pp. 123–137. Springer, Boston (2013)
10. Colier, W.N.J.M.: Near infrared spectroscopy: toy or tool? An investigation on the clinical applicability of near infrared spectroscopy. 1995
11. Ferrari, M., Muthalib, M., Quaresima, V.: The use of near-infrared spectroscopy in understanding skeletal muscle physiology: recent developments. Philos. Trans. A Math. Phys. Eng. Sci. **369**(1955), 4577–4590 (2011)
12. Ferrari, M., Binzoni, T., Quaresima, V.: Oxidative metabolism in muscle. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci **352**(1354), 677–683 (1997)
13. Nielsen, H.B.: Systematic review of near-infrared spectroscopy determined cerebral oxygenation during non-cardiac surgery. Front. Physiol. **5**, 93 (2014)
14. Davis, M.L., Barstow, T.J.: Estimated contribution of hemoglobin and myoglobin to near infrared spectroscopy. Respir. Physiol. Neurobiol. **186**(2), 180–187 (2013)
15. Molinari, F., *et al*.: Empirical mode decomposition analysis of near-infrared spectroscopy muscular signals to assess the effect of physical activity in type 2 diabetic patients. Comput. Biol. Med. **59**, 1–9 (2015)
16. Allart, E., *et al*.: Evaluation of muscle oxygenation by near-infrared spectroscopy in patients with Becker muscular dystrophy. Neuromuscul. Disord. **22**(8), 720–727 (2012)
17. Obrig, H.: NIRS in clinical neurology—a 'promising' tool? Neuroimage **85**(Pt 1), 535–546 (2014)
18. Cope, M., Delpy, D.T.: System for long-term measurement of cerebral blood and tissue oxygenation on newborn infants by near infra-red transillumination. Med. Biol. Eng. Comput. **26**(3), 289–294 (1988)
19. De Blasi, R.A., *et al*.: Noninvasive measurement of forearm blood flow and oxygen consumption by near-infrared spectroscopy. J. Appl. Physiol. **76**(3), 1388–1393 (1994)
20. De Blasi, R.A., *et al*.: Noninvasive measurement of human forearm oxygen consumption by near infrared spectroscopy. Eur. J. Appl. Physiol. Occup. Physiol. **67**(1), 20–25 (1993)
21. Praagman, M., *et al*.: Muscle oxygen consumption, determined by NIRS, in relation to external force and EMG. J. Biomech. **36**(7), 905–912 (2003)
22. Van Beekvelt, M.C., *et al*.: Performance of near-infrared spectroscopy in measuring local O(2) consumption and blood flow in skeletal muscle. J. Appl. Physiol. **90**(2), 511–519 (2001)
23. Costes, F., *et al*.: *Comparison of muscle near-infrared spectroscopy and femoral blood gases during steady-state exercise in humans*. J. Appl. Physiol. (1985). **80**(4), 1345–1350 (1996)
24. Wilson, J.R., *et al*.: Noninvasive detection of skeletal muscle underperfusion with near-infrared spectroscopy in patients with heart failure. Circulation **80**(6), 1668–1674 (1989)
25. Chung, Y., *et al*.: Control of respiration and bioenergetics during muscle contraction. Am. J. Physiol. Cell Physiol. **288**(3), C730–C738 (2005)
26. Mole, P.A., *et al*.: Myoglobin desaturation with exercise intensity in human gastrocnemius muscle. Am. J. Physiol. **277**(1 Pt 2), R173–R180 (1999)
27. Seiyama, A., Hazeki, O., Tamura, M.: Noninvasive quantitative analysis of blood oxygenation in rat skeletal muscle. J. Biochem. **103**(3), 419–424 (1988)
28. Bank, W., Chance, B.: An oxidative defect in metabolic myopathies: diagnosis by noninvasive tissue oximetry. Ann. Neurol. **36**(6), 830–837 (1994)

29. Harper, A.J., *et al*.: Human femoral artery and estimated muscle capillary blood flow kinetics following the onset of exercise. Exp. Physiol. **91**(4), 661–671 (2006)
30. Kindig, C.A., Richardson, T.E., Poole, D.C. *Skeletal muscle capillary hemodynamics from rest to contractions: implications for oxygen transfer*. J. Appl. Physiol. (1985). **92**(6):2513–2520 (2002).
31. McCully, K.K., Hamaoka, T.: Near-infrared spectroscopy: what can it tell us about oxygen saturation in skeletal muscle? Exerc. Sport Sci. Rev. **28**(3), 123–127 (2000)
32. Hickson, R.C.: Skeletal muscle cytochrome c and myoglobin, endurance, and frequency of training. J. Appl. Physiol. Respir. Environ. Exerc. Physiol. **51**(3), 746–749 (1981)
33. Kagen, L.J.: Myoglobin; Biochemical, Physiological, and Clinical Aspects. Columbia University Press, New York (1973)
34. Marcinek, D.J., *et al*.: Wavelength shift analysis: a simple method to determine the contribution of hemoglobin and myoglobin to in vivo optical spectra. Appl. Spectrosc. **61**(6), 665–669 (2007)
35. van Beek-Harmsen, B.J., *et al*.: Determination of myoglobin concentration and oxidative capacity in cryostat sections of human and rat skeletal muscle fibres and rat cardiomyocytes. Histochem. Cell Biol. **121**(4), 335–342 (2004)
36. Richardson, R.S., *et al*.: Red blood cell transit time in man: theoretical effects of capillary density. Adv. Exp. Med. Biol. **361**, 521–532 (1994)
37. Nioka, S., *et al*.: Simulation of Mb/Hb in NIRS and oxygen gradient in the human and canine skeletal muscles using H-NMR and NIRS. Adv. Exp. Med. Biol. **578**, 223–228 (2006)
38. Nighswander-Rempel, S.P., Kupriyanov, V.V., Shaw, R.A.: Relative contributions of hemoglobin and myoglobin to near-infrared spectroscopic images of cardiac tissue. Appl. Spectrosc. **59**(2), 190–193 (2005)
39. Mancini, D.M., *et al*.: Validation of near-infrared spectroscopy in humans. J. Appl. Physiol. **77**(6), 2740–2747 (1994)
40. Jue, T., *et al*.: Myoglobin and O2 consumption in exercising human gastrocnemius muscle. Adv. Exp. Med. Biol. **471**, 289–294 (1999)
41. Tran, T.K., *et al*.: Comparative analysis of NMR and NIRS measurements of intracellular PO2 in human skeletal muscle. Am. J. Physiol. **276**(6 Pt 2), R1682–R1690 (1999)
42. Richardson, R.S., *et al*.: Myoglobin O2 desaturation during exercise. Evidence of limited O2 transport. J. Clin. Invest. **96**(4), 1916–1926 (1995)
43. Bendahan, D., Chatel, B., Jue, T.: Myoglobin contribution to the near infrared signal in exercising skeletal muscle. In: Proceedings of the International Society of Magnetic Resonance in Medicine, vol. 23, p. 4234 (2015).
44. Brooks, G.A.: Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. Fed. Proc. **45**(13), 2924–2929 (1986)
45. Brooks, G.A.: Intra- and extra-cellular lactate shuttles. Med. Sci. Sports Exerc. **32**(4), 790–799 (2000)
46. Brooks, G.A.: Cell-cell and intracellular lactate shuttles. J. Physiol. **587**(Pt 23), 5591–5600 (2009)