Integrative Computational Analysis of Muscle Near-Infrared Spectroscopy Signals: Effects of Oxygen Delivery and Blood Volume

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INTEGRATIVE COMPUTATIONAL ANALYSIS OF MUSCLE NEAR-INFRARED SPECTROSCOPY SIGNALS: EFFECTS OF OXYGEN DELIVERY AND BLOOD VOLUME

by

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Approved by:

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ABSTRACT

INTEGRATIVE COMPUTATIONAL ANALYSIS OF MUSCLE NEAR-INFRARED SPECTROSCOPY SIGNALS: EFFECTS OF OXYGEN DELIVERY AND BLOOD VOLUME

Bhabuk Koirala
Old Dominion University, 2021
Co-Directors: Dr. Michel Audette
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Near-infrared spectroscopy (NIRS) is a non-invasive technology to evaluate skeletal muscle oxidative metabolism in healthy and disease states. This technology allows us to measure the dynamic response of oxygenated (ΔHbMbO₂) and deoxygenated (ΔHHbMb) heme group concentrations during muscle contraction. These O₂ kinetics are valuable for inferring the interplay between muscle oxygen delivery and utilization. However, the semi-quantitative nature of the NIRS signal limits its clinical application. Some of the challenges in interpreting the NIRS signal are related to the difficulties in quantifying the: 1) contribution of blood volume changes to the ΔHbMbO₂ and ΔHHbMb; 2) contribution of Hb and Mb to both NIRS signals; 3) relationship between the NIRS signals and the heterogeneous O₂ distribution in the muscle region investigated. Computational models of O₂ transport and metabolism in skeletal muscle can be used to analyze these limitations and suggest strategies to overcome these limitations.

A computational model of O₂ transport and utilization is used to analyze the NIRS data obtained from an animal model of muscle oxidative metabolism under different experimental conditions previously investigated. The aims of this analysis are to study: 1) the effects of blood flow on hemoglobin (Hb) and myoglobin (Mb) oxygenation kinetics in contracting muscles; 2) the effects of O₂ delivery and blood volume changes on the NIRS signals; 3) the relationship between venous and NIRS tissue oxygenation; 4) the Hb and Mb contribution to the absolute and relative
changes of heme group concentrations; and 5) the relationship between NIRS signal and oxygen distribution in the microvascular and extravascular volumes in contracting muscle.

The simulation results show that the mathematical model has the capability to simulate and predict the O₂ changes measured in the blood and tissue domains with invasive and non-invasive methods (NIRS) to evaluate metabolic function in contracting muscle under different experimental conditions (blood flow, metabolic rate). The main findings of the computational analysis are summarized as follows.

1) Faster O₂ delivery is responsible for slower ΔHbMbO₂ and ΔHHbMb kinetics. Hb and Mb contributions to the oxygenated heme groups differ from those to the deoxygenated heme groups.

2) Both ΔHbMbO₂ and ΔHHbMb are affected by the blood volume changes. The analysis indicates that microvascular O₂ saturation is a key factor in determining the sensitivity of ΔHbMbO₂ and ΔHHbMb to blood volume changes.

3) At low O₂ delivery, the Mb contribution to NIRS signal is responsible for the non-linearity in the relationship between venous and NIRS oxygenation.

4) For an increase of O₂ delivery, Hb contribution to the absolute oxygenated heme group concentrations decreases, whereas the same Hb contribution to the relative oxygenated heme group concentration increases.

5) Differential pathlength factor (DPF) for constant wave NIRS can be estimated by an integrative approach combining simulated and experimental data for NIRS kinetics. The estimated DPF can be used with the simulation of the microvascular and tissue oxygenation to predict the changes of oxygenated and deoxygenated heme group concentrations measured by NIRS.
The analysis indicates that the computational model can overcome some of the NIRS limitations in providing a quantitative relationship between NIRS signals and the oxygenation distribution in the main compartments of the muscle region investigated.
To my mother: Kalyani Koirala,

To my father: Madhav Prasad Koirala,

To my brother: Mohak Koirala.
I would like to express my sincere thanks to my advisor, Dr. Nicola Lai, for his guidance and supervision in this journey.

I am very thankful to all my dissertation committee members: Dr. Michel Audette, Dr. Christian Zemlin, Dr. Barbara Hargrave, and Dr. Stephen Beebe. I am also very thankful to the chair of my department, Dr. Oscar Gonzalez.

Special thank goes to Dr. L. Bruce Gladden of the University of Alabama for his generous contributions, assistance, and criticism.

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**NOMENCLATURE**

- $C_{ATP}$: Concentration of ATP in tissue (mM)
- $C_{b,Hb}$: Concentration of Hb in the blood (mM)
- $C_{t,Mb}$: Concentration of Mb in tissue (mM)
- $C_{O_2}^B$: Bound oxygen concentration in blood and tissue (mM)
- $C_{O_2}^F$: Free oxygen concentration in blood and tissue (mM)
- $C_{O_2}^T$: Total oxygen concentration in blood and tissue (mM)
- $D_b, D_c$: Effective dispersion coefficient in blood and tissue (L$^2$ min$^{-1}$)
- $f_b$: Blood volume fraction in muscle (-)
- $f_{b,m}$: Microvascular volume fraction in muscle (-)
- $f_t$: Extra-vascular muscle tissue volume fraction in muscle (-)
- $f_m$: Microvascular volume fraction in blood (-)
- $HbO_2, MbO_2$: Concentration of oxygenated heme of Hb and Mb ($\mu$M)
- $HHb, HMb$: Concentration of deoxygenated heme of Hb and Mb ($\mu$M)
- $k_{ATPase}$: ATPase rate constant (min$^{-1}$)
- $K_{Hb}$: Hill constant at which Hb is 50% saturated by O$_2$ (mM$^n$)
- $K_{Mb}$: Hill constant at which Mb is 50% saturated by O$_2$ (mM$^{-1}$)
- $n$: Hill coefficient (-)
- $P_{O_2}$: Oxygen partial pressure (mmHg)
- $PS$: Permeability surface area product (L L$^{-1}$ min$^{-1}$)
- $SO_2$: Oxygen saturation (-)
- $Q$: Muscle blood flow (L min$^{-1}$, L L$^{-1}$ min$^{-1}$)
- $V_{art}, V_{cap}, V_{ven}$: Anatomical volume of arteriole, capillary, venule (L)
- $V_{mus}$: Muscle volume (g)
- $V\dot{O}_2$: Muscle oxygen uptake (mLO$_2$ 100g$^{-1}$ min$^{-1}$)
**Greek letters**

- \( \alpha_{o_2} \): Oxygen solubility in blood (mM mmHg\(^{-1}\))
- \( \gamma_b \): Derivative term (-)
- \( \gamma_c \): Derivative term (-)
- \( v \): Volume coordinate (L)
- \( \omega_{art}, \omega_{cap}, \omega_{ven} \): Arteriole, capillary, venule volume fractions in microvascular (-)

**Superscript**

- \( B \): Bound oxygen concentration
- \( exp \): Experimental data
- \( F \): Free oxygen concentration
- \( O \): Occlusion
- \( mod \): Model simulation
- \( R \): Resting condition
- \( T \): Total oxygen concentration

**Subscript**

- \( art \): Artery
- \( b \): Blood
- \( c \): Cell
- \( cap \): Capillary
- \( mus \): Muscle
- \( t \): Tissue
- \( ven \): Venous
- \( A-V \): Arterio-venous difference
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CHAPTER 1

INTRODUCTION

1.1 Exercise as a diagnostic tool

Exercise can be used to evaluate skeletal muscle function. Under exercise stress it is possible to identify abnormalities in cardiovascular and metabolic function, which might not be identified when an individual is at rest (Ashish et al., 2015). Exercise testing is emerging as a potential tool to evaluate health and disease, assessing the progression of therapeutic interventions (Mezzani et al., 2013) and as a complementary tool for drug development (Ashish et al. 2015). Although exercise is recognized as a biomarker of health and disease, challenges remain to translate this potential into clinical practice.

The increase of oxygen utilization during exercise is measured to evaluate impairments in oxidative metabolism that affect exercise tolerance and performance (Bauer et al., 2004; Grassi & Quaresima, 2016). Alterations of oxidative metabolism arise from an imbalance between oxygen delivery and utilization, which is observed in several diseases such as peripheral arterial disease (PAD), type 2 diabetes (T2D), metabolic myopathies, McArdle disease, spinal cord injury, multiple sclerosis, heart failure, cystic fibrosis, chronic obstructive pulmonary disease, Friedreich's ataxia, etc. (Bauer et al., 2004; Bauer et al., 2007; Grassi & Quaresima, 2016). Thus, noninvasive tools to quantify oxygen delivery and oxidative metabolism are of utmost necessity for clinical and sports medicine applications.

To evaluate metabolic dysfunction during muscle contraction, a temporal profile of the oxygen content in the blood and tissue domains as well as oxygen uptake ($\dot{V}O_2$) at pulmonary and
skeletal muscle levels are characterized. However, there exist challenges with the methods used to measure these variables.

Mitochondrial performance in contracting muscle can be studied by evaluating oxygen kinetics during exercise. Invasive technique that utilizes the blood samples and non-invasive technique such as indirect calorimetry can be used to study mitochondrial function to measure $\dot{V}O_2$. $\dot{V}O_2$ kinetics measured at the mouth (pulmonary) can be obtained by a metabolic cart whereas those measured at the muscle level require blood samples to determine the oxygen content in artery and venous. Pulmonary $\dot{V}O_2$ kinetics provides only indirect information about the oxygen utilization in exercising muscle whereas muscle oxygen uptake represents the gold standard, but its invasive measure limits its utilization. Indeed, muscle oxygen uptake kinetics requires not only the measure of blood flow but also that of venous oxygenation during contraction (Costes et al., 1996; Grassi et al., 1996; Poole et al., 1991). As an alternative to this invasive measure, Near-Infrared Spectroscopy (NIRS) can be used to measure the tissue oxygenation in exercising muscle non-invasively. The time profile of tissue oxygenation from NIRS (Grassi et al., 2003; Grassi et al., 2019) is used to quantify the imbalance between $O_2$ delivery and utilization (Barstow, 2019; Boushel & Piantadosi, 2000; Ferrari et al., 2004; Goodwin et al., 2011; Quaresima et al., 2001). Thus, this technology represents a promising substitute for the invasive method to evaluate mitochondrial function in vivo.

Both exercise oxygen kinetics ($\dot{V}O_2$, tissue oxygenation) have an exponential response to an increase of energy demand as reported in Figure 1.1. The physiological variable response (e.g., $\dot{V}O_2$) to exercise is characterized by a set of parameters: the baseline value ($Y_{BL}$) represents resting conditions; at $t_0$, the muscle is stimulated and after a delay time (TD), the variable rises from the baseline value to reach a plateau $Y_{BL} + A$; $A$ is the amplitude from baseline to reach a plateau; $\tau$ is
a time constant that quantifies how fast the variable approaches the plateau value. The smaller \( \tau \) the faster kinetics. The characterization of the kinetics is currently used by the scientific community to infer oxygen delivery (heart rate, blood flow) and utilization (\( \dot{V}O_2 \), tissue oxygenation) responses to exercise in healthy and disease states. Nevertheless, this approach allows only indirect inferences on the O2 utilization.

Figure 1.1 Time profile of the physiological variable (i.e., venous oxygen concentration, NIRS measurements). Mono-exponential function used to characterize the physiological variable response to a stimulus and its parameters: baseline value (\( Y_{BL} \)), amplitude (\( A \)), time constant (\( \tau \)), onset of contraction (\( t_0 \)), time delay (TD).
1.2 Near-Infrared spectroscopy (NIRS) in disease states

In peripheral arterial disease (PAD), at the onset of exercise, the kinetics of oxygen desaturation, measured by NIRS, is slower than that of the healthy group (τ 21.9 ± 9.4 s vs 4.9 ± 2.2 s) (Bauer et al., 2004). It was suggested that the slow response could be related to an impairment of muscle oxidative metabolism which is consistent with alterations in ATP generation and activities of electron transport chain enzymes (Brass et al., 2001). In type 2 diabetes (T2D), the dynamics of deoxygenated NIRS signal at the onset of exercise, showed an overshoot which was not observed in the control group. This overshoot was attributed to a slow microvascular blood flow response to exercise (Bauer et al., 2007).

Another human study of patients with McArdle disease and Muscle Myopathy reported that that the maximal deoxygenated NIRS signal changes observed during incremental exercise were significantly lower than the healthy subjects (Grassi et al., 2019). The NIRS signal was used to quantify muscle O2 extraction. The peak of the NIRS signal at the end of exercise was correlated with $\dot{V}O_2$ peak, which is an index of maximal aerobic capacity (Grassi et al., 2019). Thus, low $\dot{V}O_2$ detected by indirect calorimetry and low extraction detected by NIRS can be used as diagnostic tools to identify a case of metabolic myopathy and support the clinician in evaluating whether a muscle biopsy is required for a conclusive diagnosis. In the same study, the dynamic response of the deoxygenated NIRS signal during exercise showed an overshoot as reported for patients with T2D (Porcelli et al., 2016). This mismatch between microvascular O2 delivery and utilization profile might be related to an impairment in the muscle oxygen delivery in both populations of patients (Grassi et al., 2019).
1.3 NIRS technology

The non-invasive nature and affordable price of this technology make NIRS stand out in translational medicine. NIRS uses the longer wavelength of the near-infrared region of the electromagnetic spectrum (700 nm to 900 nm), which experiences less scattering and easily penetrates living tissue. Light that is not absorbed is transmitted and detected by a detector placed close to the NIR source. This concept is illustrated in Figure 1.2 (Willingham & McCully, 2017).

Figure 1.2 Illustration of NIRS concept (Willingham & McCully, 2017). The light emitted by the source, travels through the tissue to reach the light detector of the NIRS probe. Part of the NIR is absorbed or scattered in the muscle before it reaches the detector. Copyright information in Appendix F.1.
NIR light is primarily absorbed by Hb and Mb which have a similar property to attenuate light at a given wavelength (Figure 1.3a). The NIRS technique exploits the Beer-Lambert law, reported by August Beer in 1851, which states:

$$OD_\lambda = \varepsilon_\lambda \ C \ L.$$ \hspace{1cm} 1.1

In this equation, $\lambda$ is the wavelength (nm) of the light; $\varepsilon_\lambda$ is the molar extinction coefficient of the chromophore that absorbs light (M$^{-1}$ cm$^{-1}$); $C$ is the concentration of the chromophore; and $L$ is the distance (cm) between light entry (source) and exit (detector) points. The Beer-Lambert law can be applied to a non-scattering medium. Since the skeletal muscle tissue scatters the light with the photons travelling along a banana-shaped path as in Figure 1.2, the shortest distance between the source and detector requires an additional correction factor called ‘differential pathlength factor’ (DPF) which supplements equation 1.1 as:

$$\Delta OD_\lambda = \varepsilon_\lambda \ \Delta C \ L \ DPF.$$ \hspace{1cm} 1.2

In this modified version of the Beer-Lambert law, $\Delta$ represents the relative change from a generic base line.

Two chromophores bind to oxygen (Figure 1.3c), four oxygen molecules are bound to Hb whereas only one binds to Mb. Hb and Mb in oxygenated ($HbO_2$, $MbO_2$) and deoxygenated ($HHb$, $HMb$) forms contribute to the oxygenated ($HbMbO_2$) and deoxygenated ($HHbMb$) NIRS signals, respectively.
Besides the scattering, as stated above, the oxygenation status of the chromophores affects absorption of light. Thus, the oxygenated ($\Delta HbO_2$, $\Delta MbO_2$) and deoxygenated ($\Delta Hb$, $\Delta Mb$) chromophores can be distinguished because of their different light absorption properties. $\Delta HbO_2$ and $\Delta MbO_2$ have a higher absorbance at a wavelength around 800 nm, whereas $\Delta Hb$ and $\Delta Mb$ have a higher absorbance at ~750 nm. The Beer-Lambert law, when applied to equation 1.2 for two different wavelengths, can be written as:

$$\Delta OD_{\lambda_1} = \varepsilon_{HHbMb\lambda_1} \Delta C_{HHbMb} \ L \ DPF + \varepsilon_{HbMbO_2\lambda_1} \Delta C_{HbMbO_2} \ L \ DPF$$

$$\Delta OD_{\lambda_2} = \varepsilon_{HHbMb\lambda_2} \Delta C_{HHbMb} \ L \ DPF + \varepsilon_{HbMbO_2\lambda_2} \Delta C_{HbMbO_2} \ L \ DPF.$$

Figure 1.3 a) Extinction coefficient of different chromophores in muscle (Li et al., 2015): oxygenated forms in blue and green (HbO$_2$, MbO$_2$) and deoxygenated from in red and grey (HHb, HMb), copyright information in Appendix F.2. c) Binding nature of Hb and Mb (Willingham & McCully, 2017). Copyright information in Appendix F.1.
where, $\lambda_1$ and $\lambda_2$ are two wavelengths chosen to detect the oxygenated and deoxygenated NIRS signals. Thus, these two equations allow us to quantify the relative change of tissue concentration $\Delta C_{HbMbo_2}$ and $\Delta C_{HbMb}$ from the optical density measured by NIRS. Usually, $L$ is known and DPF is unknown and generally assumed to estimate chromophore concentrations using Eq. 1.3 and 1.4 for continuous wave (CW) NIRS oximeters. Nevertheless, scattering and absorption of light in the tissue investigated affect DPF. The relationship between DPF and absorption ($\mu_a$) and scattering ($\mu'_s$) is given by (Fantini, 2002).

$$DPF = \frac{\sqrt{3\mu'_s}}{2\sqrt{\mu_a}}.$$  

Furthermore, it is not possible to quantify scattering changes during exercise or cuff occlusion. Cuff occlusion, which is performed to induce skeletal muscle ischemia, is typically obtained by a tourniquet that applies pressure to a limb limiting blood flow to the skeletal muscle. DPF has also been reported to be variable in different tissue and muscles such as: gastrocnemius (5.8 - 5.3), forearm (4.4-3.9), adult head (6.5-5.9) and infant head (5.4-4.7). Women with a larger layer of adipose tissue than men showed a higher DPF for forearm and calf muscle (Barstow, 2019; Duncan et al., 1995; Ferrari et al., 1992). Thus, DPF has a large variability, and it is a key parameter in quantifying the NIRS signals.

In addition to CW NIRS oximeters, there are time domain (TD) and frequency domain (FD) intensity-modulated NIRS oximeters. In CW NIRS, the light source has constant intensity whereas TD and FD NIRS oximeters are based on the modulation of light. TD uses light pulses with a duration of a few tens of picoseconds (Barstow, 2019) to identify the scattering and absorption coefficients as well as mean path length. FD NIRS is based on the modulation of the light amplitude at different frequencies to determine the average, amplitude, and phase of the
modulated light intensity at several source-detector distances. This allows continuous measurement of the absorption coefficient and scattering coefficient for each wavelength used. Thus, both TD and FD NIRS approaches provide measurement of absolute oxygenated and deoxygenated heme group concentrations without any assumption about the DPF required for CW NIRS which provide only relative changes from a base line.

1.4 NIRS limitation

A major limitation of the NIRS technology is the semi-quantitative information provided by the measurement. There are several factors contributing to NIRS signals: a) adipose tissue thickness, b) effect of scattering changes during muscle contraction, c) unknown Hb and Mb contribution to the signal, and d) change of blood volume. Because the NIRS signal does not distinguish the Hb chromophores from those of the Mb, the precise contributions of Hb and Mb to the NIRS are not directly quantifiable. Furthermore, the NIRS signal results from a weighted average of the oxygenation in microvascular (arteriole, capillaries, venule) and extravascular compartments. Their contributions to the NIRS signal are not known during exercise. Thus, the nature of the NIRS signals limit their clinical applications because NIRS signals provide only semi-quantitative information about the muscle oxygenation kinetics during contraction. Often to have more reliable quantification of the signal, a NIRS measurement obtained during muscle contraction is normalized by a NIRS measure obtained under ischemia by cuff occlusion upstream of the region of investigation.
1.4.1 Hb vs Mb relation between muscle and venous oxygenation

There is conflicting evidence to support a major contribution of Hb to the NIRS signal. Some experimental studies reported a linear relationship between $O_2$ saturation measured with NIRS and that obtained with the analysis of blood samples from femoral vein (Sun et al., 2016). In other studies, muscle and blood oxygenation values were not linearly related for some but not all experimental conditions (MacDonald et al., 1999).

![Figure 1.4](image)

Figure 1.4 a) Oxygenated NIRS signal vs venous oxygenation, b) deoxygenated NIRS signal vs venous oxygenation (Sun et al., 2016). Copyright information in Appendix F.3.

An NIRS study on a canine animal model of oxidative metabolism combined measures of NIRS and venous oxygenation (Figure 1.4). In this study, a linear relationship between venous oxygenation and NIRS signals was reported varying oxygen delivery in resting or contracting
muscle. In a human study of a leg kicking under different arterial oxygen contents (Figure 1.5) (MacDonald et al., 1999), the correlation between the NIRS signal and venous oxygenation was not always present. Under resting conditions, the NIRS and femoral oxygen saturation patterns were similar. At the onset of contraction, the correlation between the two variables was high (0.55) only for the first 40 seconds. Afterwards, the correlation of the two variables was low (0.02) for the rest of the contraction period, excluding the case of hypoxia (Figure 1.5C).

Figure 1.5 Temporal profiles of IR and femoral vein O₂ saturation in A) normoxia, B) hyperoxia and C) hypoxia. Open symbols are the venous O₂ saturation from femoral vein catheterization, whereas closed symbols are muscle oxygen saturation from NIRS (MacDonald et al., 1999). Copyright information in Appendix F.4.
The lack of correlation suggests that the NIRS signal does not always reflect the level of blood oxygenation. The NIRS difference obtained under hypoxic or normoxic conditions could be related to differences in the oxygenation of the microvascular (Hb) and extravascular compartments (Mb) contributing to the NIRS signal. Regarding the Mb and Hb contribution to the NIRS signal, a computational study (Lai et al., 2009) has shown significant contribution of Mb. Specifically, model simulations of oxygenated Hb and Mb dynamics to a step increase in work rate showed that the contribution of Mb to the oxygenated NIRS signal ($\Delta Mbo_2/\Delta HbMbO_2$) varied from 20% to 80% among different subjects according to the blood volume fraction change and oxygenated heme groups. The Mb contribution to the total heme groups was around 35% at rest, which decreased to 30% during contraction, irrespective of the O$_2$ saturation. Also, an experimental study indicated a significant Mb contribution to the NIRS signal (Davis & Barstow, 2013).

1.4.2 Blood volume effect on the NIRS signals

A typical NIRS kinetics profile from quadriceps measured during cycling exercise in humans is shown in Figure 1.6. During the unloaded pedaling exercise before contraction, NIRS signals were unchanged. At the onset of contraction (phase a), $\Delta[oxy(Hb + Mb)]$ decreased whereas $\Delta[deoxy(Hb + Mb)]$ increased during phase b. It took 60 s for $\Delta[deoxy(Hb + Mb)]$ to reach a plateau, whereas $\Delta[oxy(Hb + Mb)]$ continued to increase until the end of the exercise. Thus, $\Delta[deoxy(Hb + Mb)]$ kinetics appears consistent with venous oxygenation kinetics observed on a leg kicking exercise whereas $\Delta[oxy(Hb + Mb)]$ kinetics does not follow the same
pattern (Grassi et al., 2003). Typically, $\Delta[\text{deoxy}(Hb + Mb)]$ kinetics is considered to reflect muscle oxygen extraction and it is preferred to the $\Delta[\text{oxy}(Hb + Mb)]$ kinetics.

In the bottom panels of Figure 1.6, the sum of the oxygenated and deoxygenated signals ($\Delta[\text{oxy}(Hb + Mb)] + \Delta[\text{deoxy}(Hb + Mb)]$) increased during contraction (phase c) indicating that the total heme ($Hb + Mb$) in the region investigated has increased. This phenomenon is commonly attributed to a change of the Hb heme group concentration due to a local increase of blood volume because Mb heme group concentration in muscle cells do not change during exercise.

![Figure 1.6](image_url)

**Figure 1.6. A)** Time course of $\Delta[\text{deoxy}(Hb + Mb)]$ and $\Delta[\text{oxy}(Hb + Mb)]$ in a transition from unloaded pedaling to constant loading exercise (top), sum and difference of oxygenated and deoxygenated $\Delta[\text{oxy}(Hb + Mb)] + \Delta[\text{deoxy}(Hb + Mb)]$ NIRS signals. **B)** magnified abscissa of column A (Grassi et al., 2003). Copyright information in Appendix F.5.
The NIRS signals are affected by the microvascular and extravascular compartments. Thus, the oxygenated NIRS signal results from a weighted average of the oxygenation in arterioles, capillaries, venules of the microvascular domain and in myocytes of the extravascular domain. The contribution of these compartments can shift during exercise due to blood flow and volume changes. The composition of the oxygenated NIRS signal is like that of the deoxygenated NIRS signal. In some human studies, the blood volume changes observed in Figure 1.6 were attributed to blood flow changes of the skin under the NIRS probe. These blood volume changes were attributed to a cutaneous vasodilation related to thermoregulation. Often, it is assumed that the oxygenated NIRS signal is mainly affected by skin blood flow; thus, the deoxygenated signal is preferred as an index of muscle oxygenation, which is not “contaminated”. Nevertheless, this assumption is still debated.

A head-up tilt (HUT) study provided new insights on the effects of blood volume on the NIRS signals (Figure 1.7). Specifically, in the vastus lateralis (VL), the deoxygenated NIRS signal \([Hb] \) increases progressively (Fig. 1.7 A) while that of the oxygenated NIRS signal \([HbO_2]\) decreases as the tilt angle increases. The response in the gastrocnemius (GS) was similar to that of the VL but with greater magnitude (Fig 1.7 B). The increase in \([Hb] \) in both muscles was greater than the decrease in \([HbO_2]\) (57 µM vs -22µM). This evidence indicates a predominant contributor of \([Hb] \) to the blood volume changes \([THb]\) (Adami et al., 2015).
Figure 1.7. Temporal profile of the NIRS variable and femoral artery blood flow response to head-up tilt. Changes in oxygenated $HbO_2$, deoxygenated $HHb$ and total $THb$ (µM) in A): vastus lateralis, B): gastrocnemius as function of tilt angle. C): blood flow as a function of tilt angle (Adami et al., 2015). Copyright information in Appendix F.6.
1.4.3 Relationship between muscle volume composition and optical properties

O$_2$ saturation of the heme groups of Hb in the microvascular and Mb in the extravascular compartments contribute to the NIRS signal. Most of the contribution of the Hb heme group to NIRS signals come from small vessels less than 1 mm in diameter, which are considered the microvascular compartment in this work. The larger arteries and veins are believed to absorb all the NIR light (Grassi & Quaresima, 2016).

It has been widely believed that NIRS signal reflects the oxygenation of the venous-venular compartment in skeletal muscles (Boushel et al., 2001; McCully & Hamaoka, 2000). However, around 90% of the microvasculature consists of capillaries according to data by Poole et al., (Poole & Mathieu-Costello, 1989). Thus, uncertainties still exist regarding the microvascular compartment’s contributions to the NIRS signal (Grassi & Quaresima, 2016) because of the difficulties in quantifying the capillary and venule contributions.

Experimental studies reported an optical path length change during exercise (Endo et al., 2021) and cuff occlusion (Ferrari et al., 1992, Hammer et al., 2019). NIRS studies on skeletal muscle suggest that the specific muscle composition such as adipose tissue thickness (ATT) and muscle condition (contraction, arterial oxygen content) affect the differential path length factor (DPF). An investigation of the relationship between ATT and DPF shows that when the ATT increases, DPF increases for vastus lateralis muscle in humans (Pirovano et al., 2021). The relative change in oxygenated ($\Delta C_{HbMbO_2}$) and deoxygenated ($\Delta C_{HHbMb}$) signals are inversely related to DPF. Thus, with an increase of DPF due to a larger layer of ATT, $\Delta C_{HbMbO_2}$ and $\Delta C_{HHbMb}$ signals measured by NIRS would decrease and the total $[Hb + Mb]$ (Barstow, 2019). It is suggested that
the assumptions of a constant optical path length can underestimate both oxygenated and deoxygenated NIRS signals (Endo et al., 2021)

1.5 Computational model simulations

The possibility to infer mechanisms associated with biological and physiological processes is limited by the information available because some of the physiological variables of interest cannot be measured due to the nature of the experimental model and set-up. This limitation can be in part overcome with a complementary tool such as computational modeling. A mathematical model with appropriate validation provides a computational analysis of the system that fills the knowledge gap about the system investigated. By integrating a mechanistic mathematical model of oxygen transport and metabolism in skeletal muscle with experimental measurements, oxygen kinetics measurements at tissue-organ level were related with oxygen diffusion and utilization at a cellular level (Lai et al., 2007; Lai et al., 2009; Spires et al., 2012; Zhou et al., 2008).

These models describe the spatial and temporal changes of O₂, adenosine triphosphate (ATP) and phosphocreatine (PCr) concentrations in skeletal muscles during contraction (Lai et al., 2007). This model was extended by incorporating glycogenolysis that produces additional ATP at the onset of contraction for higher metabolic rate (Spires et al., 2012). These models were validated using arterio-venous differences of oxygen concentration measured from experiments. Model simulations have shown that the O₂ utilization response was faster than that measured with muscle oxygen uptake (Lai et al., 2007). In another computational study of the same animal model of oxidative metabolism, it is suggested that the permeability surface area is the main factor regulating O₂ diffusion determining the O₂ gradient during muscle contraction (Spires et al., 2012).
In some of these studies the approach focused on the integration and analysis of invasive measurement of the oxygen content in the blood domain whereas in others, the mathematical model was applied to quantify the oxygen content in the extravascular and microvascular compartments that contribute to the noninvasive measurements of muscle oxygenation by NIRS (Lai et al., 2009). The analysis indicated that Hb and Mb contributions to the NIRS signals were significant under different experimental conditions. Nevertheless, the effects of adipose tissue, blood volume and microvascular volume distribution on the NIRS signals were not systematically investigated.

A computational model of O₂ transport and metabolism with new features is proposed to quantitatively analyze the NIRS signals under different experimental conditions (Chapter 2). The computational analysis is based on NIRS data obtained from a canine model of oxidative metabolism briefly described in the next chapter.

The first part of the computational analysis focuses on the differential effects of blood flow on the Hb and Mb contributions to the oxygenated and deoxygenated NIRS signals in contracting muscles (Chapter 3). Then, the effects of blood flow and volume on the oxygenated and deoxygenated NIRS signals are investigated (Chapter 4). The computational model is then used to analyze the experimental evidence supporting a linear relationship between venous oxygen concentration (by blood sample) and muscle oxygenation measured by NIRS (Chapter 5). The analysis is used to identify those experimental conditions for which the two variables are not linearly related. Simulations quantify the Hb and Mb contribution to the relative (Chapter 5) and absolute (Chapter 6) oxygenated and deoxygenated NIRS signals. Finally, the effects of the microvascular distribution on the differential pathlength factor during contraction are quantified (Chapter 7). Thus, a strategy is proposed to overcome some of the limitations related to the
uncertainties of differential pathlength factor value. In the last chapter (Chapter 8), model applications and future works are presented.

1.6 Implication of outcomes from this research study

- In contrast to the common assumption that a deoxygenated NIRS signal is less affected by blood volume change than the oxygenated NIRS signal, model simulations in this study indicate that both oxygenated and deoxygenated NIRS signals are affected by the blood volume change.
- Both NIRS signals are sensitive to blood volume change with low capillary O₂ saturation. Blood volume change should be considered to evaluate O₂ extraction by NIRS under normal or reduced oxygen delivery in physiological or pathophysiological conditions.
- Even in the presence of a linear relationship between venous O₂ concentration and the NIRS signals, Mb contribution to the NIRS signals can be significant. Thus, Mb contribution to the signal should be quantified to analyze the NIRS kinetics during contraction.
- A quantitative relationship was proposed and validated between the changes in microvascular and extravascular tissue oxygenation and optical density measured in canine muscle. This approach provides quantitative information necessary to overcome the semi-quantitative nature of the signal measured with continuous wave NIRS spectrometer.
2.1 Animal model

The experimental data used in this computational study are obtained from an isolated canine gastrocnemius (GS) muscle that is electrically stimulated. It is an animal model of skeletal muscle oxidative metabolism. This model allows us to study oxygen transport and metabolism in skeletal muscles at the onset of contraction (Hernández et al., 2009). The isolated GS poses different advantages which include vascular isolation enabling the control of O₂ delivery by pump-perfused blood flow. The animal can inspire hyperoxic, normoxic or hypoxic air, which allows control of the O₂ concentration entering the muscle. The metabolic rate can be controlled by varying contraction frequency while inhibitors and activators can be perfused during contraction. Also, the isolated muscle allows us to simultaneously measure venous oxygen content and tissue oxygenation by NIRS. The NIRS probe is placed directly on the skeletal muscle to avoid any effects related to the skin and adipose tissue layers.

2.1.1. Experimental settings

Computational analysis was based on the data obtained from isolated GS muscles (Goodwin et al., 2011; Hernández et al., 2010; Sun et al., 2016). This skeletal muscle was canulated to the animal in the arterial and venous side, thereby allowing us to control the blood flow via
pump perfusion. Dogs were anesthetized by administering sodium pentobarbital via IV bolus of 30 mg kg$^{-1}$ body weight from the prominent cephalic vein of forelimb. This established a surgical plane, which was maintained throughout the experiment with additional doses of sodium pentobarbital to maintain the absence of pedal, palpebral and corneal reflexes. Dogs were ventilated using a respirator with an endotracheal tube. Temperature was maintained with a heat lamp and heating pad. Dogs were treated with heparin in divided doses totaling 3000 U kg$^{-1}$.

GS muscle in these dogs were isolated, and arterial circulation to the GS was maintained by routing blood from the popliteal/femoral artery to a peristaltic pump, which allowed experimental control of muscle perfusion whenever required. Likewise, the venous circulation was returned to the animal by returning the blood from the popliteal vein to a reservoir which drains into the jugular vein. Further, a probe was inserted by canulating the artery and vein to measure the blood flow as well as arterial and venous oxygen concentrations. A NIRS probe was also placed over the muscle to measure muscle oxygenation. The sciatic nerve was isolated to induce contractions in GS whenever required. A schematic representation of the experimentation is presented in Figure 2.1.
2.1.2. **Experimental studies**

Three different experimental studies were used to validate a computational model (section 2.2) and analyze the NIRS measurements under different experimental conditions (blood flow, contraction). The experimental studies, major outcome and the measured physiological variables are reported in Table 2.1.
Table 2.1. Summary of the experimental studies and their outcomes used in this study

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Physiological Variable</th>
<th>Experimental Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior Bout of contraction</td>
<td>Muscle venous oxygen</td>
<td>(Hernández et al., 2010)</td>
</tr>
<tr>
<td>speeds up $\dot{V}O_2$ and blood flow</td>
<td>concentration, blood flow and</td>
<td></td>
</tr>
<tr>
<td>kinetics causing faster oxygenation kinetics</td>
<td>tissue oxygenation (NIRS)</td>
<td></td>
</tr>
<tr>
<td>Slower O$_2$ delivery causes</td>
<td>Muscle venous oxygen</td>
<td>(Goodwin et al., 2011)</td>
</tr>
<tr>
<td>slower $\dot{V}O_2$ kinetics and faster oxygenation kinetics</td>
<td>concentration, blood flow and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tissue oxygenation (NIRS)</td>
<td></td>
</tr>
<tr>
<td>Muscle NIRS signals change</td>
<td>Muscle venous oxygen</td>
<td>(Sun et al., 2016)</td>
</tr>
<tr>
<td>linearly with increase in venous oxygeniation</td>
<td>concentration, blood flow</td>
<td></td>
</tr>
<tr>
<td></td>
<td>arterial oxygen concentration,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and tissue oxygenation (NIRS)</td>
<td></td>
</tr>
</tbody>
</table>

In the study by Hernández et al., 2010, two bouts of contraction were performed with a rest of 2 minutes in between. During these bouts, GS was self-perfused and contracted twice every 3 seconds. The experimental data are reported in Appendix A, Figures A.1 and A.2. The kinetics of $\dot{V}O_2$ and muscle blood flow in the second bout were faster in comparison to the first bout whereas the kinetics of muscle oxygenation measured by NIRS were slower in the second bout compared to the first bout.
In the study by Sun et al., 2016, the experiments were separated into five trials (T1-T5, T for trial). A schematic representation of the trials is shown in Figure 2.2, and the experimental data are reported in Appendix A, Figure A.3. T1 and T2 were performed in resting muscles, whereas T3 and T4 were performed in contracting muscles. T5 was performed in contracting muscle with cuff occlusion to induce an ischemic condition. This trial was designed to obtain the maximal change in NIRS signals from a resting condition. For T1 – T4, O₂ delivery was changed by either manipulating the blood flow (T1, T3 and T4) or the arterial O₂ partial pressure (T2).

In T1, the blood flow within the resting muscle was changed in four phases under normoxic conditions. In the first phase, a control blood flow was maintained. The blood flow was then increased by 50% (phase 2), decreased back to control condition (phase 3) and decreased by 50% (phase 4). For each phase, the blood flow was maintained and NIRS signals were monitored. The blood flow was changed only when the NIRS signals reached a steady state.

In T2, the arterial partial pressure of O₂ (pO₂) was changed while maintaining a constant blood flow in resting muscle. Like T1, T2 consisted of four phases. During phase 1, the arterial pO₂ was 92 mm Hg reflecting a normoxic condition. During phase 2, the arterial pO₂ was 537 mm Hg reflecting a hyperoxic condition. During phase 3, the arterial pO₂ was 87 mm Hg reflecting a second normoxic condition, and during phase 4, the arterial pO₂ was 43 mmHg reflecting a hypoxic condition. During each of these conditions, the NIRS signals were monitored, and only when the NIRS signals reached a steady state was the animal inspired a different level of oxygen (normoxia, hyperoxia or hypoxia).

In T3 and T4, the blood flow in contracting muscle was changed in four phases under normoxic conditions. During Phase 1 a control blood flow was maintained. The blood flow was then increased by 20% from the control blood flow (phase 2), decreased back to control blood flow
(phase 3) and decreased by 20% (phase 4). For T3, the contraction intensity was once every 2 seconds for 2 minutes whereas for T4, the contraction intensity was twice every 3 seconds.

T5 was designed to determine the maximal changes in NIRS signals. The animal was ventilated with hypoxic air, and the blood flow was increased by 50% above the control value. The
GS was then stimulated once every 2 seconds with the popliteal artery clamped to induce ischemia. Under these conditions, the measurements of $\Delta HHbMb$ and $\Delta HbMbO_2$ reached their maximal values. A linear correlation was observed between venous oxygen concentration and NIRS muscle oxygenation across a wide range of physiological conditions.

In the study by Goodwin et al., 2011, three different kinetics of blood flow were used for the same contraction intensity (1/2 seconds) and were characterized by time constant ($\tau$): control condition CT20 ($\tau = 20$ s), EX45 ($\tau = 45$ s) and EX70 ($\tau = 70$ s). The three trials were separated by 35 minutes of rest in between. The experimental data are reported in Appendix A, Figure A.4 and A.5. With slower O$_2$ delivery, slower $\dot{V}O_2$ kinetics were observed whereas the muscle oxygenation kinetics, measured by NIRS were faster.

### 2.2 Mathematical model

A mathematical model of oxygen transport and metabolism that predicts the dynamics of skeletal muscle in vivo to a change in energy demand (Lai et al., 2007; Lai et al., 2009; Spires et al., 2012) was used with some modifications to quantify and analyze the NIRS signals under different experimental conditions. The muscle volume ($V_{mus} = V_{b,m} + V_t + V_{b,v}$) consists of extravascular muscle tissue ($V_t$) and blood ($V_b$) which is assumed to have the vascular ($V_{b,v}$) and microvascular ($V_{b,m}$) compartments. The microvascular compartment includes arterioles, capillaries, and venules ($V_{b,m} = V_{art} + V_{cap} + V_{ven}$). In the absence of blood volume changes, the microvascular volume fraction ($f_{b,m}$) is equal to that at rest ($f_{b,m}^R$) and was calculated by the product of the blood volume fraction in the muscle ($f_b$) and microvascular volume fraction in blood ($f_m$). In the presence of blood volume changes, it is assumed that only the capillary volume
(\(V_{cap}\)) of the microvascular compartment varies with muscle perfusion changes and/or contraction. Thus, heme group concentration changes detected by the NIRS measurements are attributed to a capillary volume (\(V_{cap}\)) variation.

2.2.1. Temporal and spatial distribution of \(O_2\) in the microvascular and extravascular volumes

During muscle contraction, the \(O_2\) changes in the microvascular and extravascular volumes for differing \(O_2\) delivery are responsible for NIRS changes. The free oxygen concentration in capillary \([C_{b,0}^C(v, t)]\) and in muscle cells \([C_{t,0}^C(v, t)]\) depend upon time \((t)\) and location \((v)\) from artery \((v = 0)\) to vein \((v = V_m)\) as follows:

\[
f_{cap} \frac{\partial C_{b,0}^C}{\partial t} = -Q \frac{\partial C_{b,0}^C}{\partial v} + D_b \frac{\partial^2 C_{b,0}^C}{\partial v^2} - \frac{j_{b,t}^{\text{O}_2}}{\gamma_b}  \tag{2.1}
\]

where \(f_{cap} = V_{cap}/V_{mus}\), \(D_b\) is the axial dispersion coefficient in blood, \(j_{b,t}^{\text{O}_2}\) is transport of \(O_2\) between blood and tissue cells, and \(\gamma_b\) is the change of total oxygen concentration to the free oxygen concentration in blood. The first term on the right represents convective transport, due to delivery in the direction of blood flow \((Q)\). The second term is the axial dispersion in the capillary bed, and the third term represents oxygen transport between capillary and tissue. The dynamics of free \(O_2\) in muscle cells is described as:

\[
f_t \frac{\partial C_{t,0}^C}{\partial t} = D_t \frac{\partial^2 C_{t,0}^C}{\partial v^2} + \frac{j_{b,t}^{\text{O}_2}}{\gamma_t} - \frac{f_t \phi_{\text{O}_2}}{\gamma_t}  \tag{2.2}
\]

where \(f_t = V_{tiss}/V_{mus} = 1 - f_b\), \(D_t\) is the axial dispersion coefficient in the muscle cell, \(\gamma_t\) is the change of total oxygen concentration with respect to free oxygen concentration in muscle cells.
\( \phi_{Oxp} \) is the oxidative phosphorylation flux in tissue. The capillary-tissue transport depends upon the permeability surface-area (PS) and free \( O_2 \) concentrations.

\[
J_{O_2}^{b,t} = PS(C_{O_2}^{F} - C_{O_2,t}^{F}).
\]  

2.3

PS is linearly related to \( Q \) during muscle contraction according to:

\[
PS = PS_R + \eta(Q(t) - Q_R).
\]  

2.4

The index \( R \) indicates rest condition, and \( \eta \) is a parameter previously determined (Spires et al., 2013).

It is assumed that the input \( O_2 \), from arterial blood is known and the output oxygen concentration leaving the capillaries has a negligible gradient:

\[
v = 0 \quad C_{O_2,b}^F = C_{O_2,art}^F \quad \frac{\partial C_{O_2,t}^F}{\partial v} = 0
\]  

2.5

\[
v = V_m \quad \frac{\partial C_{O_2,t}^F}{\partial v} = 0 \quad \frac{\partial C_{O_2,t}^F}{\partial v} = 0.
\]  

2.6

The boundary conditions for lactate and pyruvate concentration are like those used for oxygen (Spires et al., 2012). Initially, the concentrations are distributed in a resting, steady state.

\[
t = t_0 \quad C_{O_2,b}^F = c_{O_2,b}^{F,Rest}(v), C_{O_2,t}^F = c_{O_2,t}^{F,Rest}(v), C_j^{Rest} = c_j^{Rest}(v).
\]  

2.7

The partial differential equations are solved using method of lines (Schiesser, 2012) of fourth order accuracy, and the ordinary differential equations are solved using ODE15s in MATLAB, which is a solver based on numerical differentiation formulas of order 1 to 5. The schematic, ODEs and fluxes are reported in Appendix A, Figure A.6, Appendix B and C, respectively (Spires et al., 2012). A schematic of the model used is reported in Figure 2.3.
The total (T) oxygen concentration in blood (b) and tissue (t) is the sum of the free (F) and bound (B) oxygen concentrations \( C_{O_2,x}^T = C_{O_2,x}^B + C_{O_2,x}^F, \) which are related by local equilibrium (Lai et al., 2007). In blood, the relationship is

\[
C_{O_2,b}^B = 4 \times Hct \times C_{rbc, Hb} \times SO_{2,b} \tag{2.8}
\]

where saturation in blood (\( SO_{2,b} \)) is given by:

\[
SO_{2,b} = \frac{K_{Hb} \times (C_{O_2,b}^F)^n}{1 + K_{Hb} \times (C_{O_2,b}^F)^n}. \tag{2.9}
\]

Subscript \( b \) refers to arterioles, capillaries, or venules. \( Hct \) is the hematocrit, \( C_{rbc, Hb} \) is the concentration of Hb in red blood cells, and \( K_{Hb} \) is the hill constant at which Hb is 50% saturated by \( O_2 \).
The equilibrium in the extravascular muscle is

\[ C_{O_2,t}^B = W_{mc} C_{mt,Mb} SO_{2,t} \]  

where saturation in tissue \((SO_{2,t})\) is given by:

\[ SO_{2,t} = \frac{K_{Mb} C_{O_2,t}}{1 + K_{Mb} C_{O_2,t}}. \]

Subscript \(t\) refers to the tissue domain. \(W_{mc}\) is the fraction of muscle cell in tissue, \(C_{mt,Mb}\) is the concentration of Mb in a muscle cell, and \(K_{Mb}\) is the hill constant at which Mb is 50% saturated by \(O_2\).

The parameters of the Hb and Mb saturation relationship are reported in Table 2.2. It should be noted that \(C_{c,Mb} = W_{mc} C_{mt,Mb}\), and \(C_{b,Hb} = Hct C_{rbc,Hb}\).

Table 2.2 Parameters of the Hb and Mb saturation relationship for blood and tissue, respectively.

<table>
<thead>
<tr>
<th>Notation</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_{Hb})</td>
<td>7210.9</td>
<td>[mM](^n)</td>
<td>(Lai, Saidel, et al., 2007)</td>
</tr>
<tr>
<td>(K_{Mb})</td>
<td>308.6</td>
<td>[mM](^{-1})</td>
<td>(Lai, Saidel, et al., 2007)</td>
</tr>
<tr>
<td>(n)</td>
<td>2.8</td>
<td>[-]</td>
<td>(Richardson et al., 1998)</td>
</tr>
<tr>
<td>(C_{c,Mb})</td>
<td>0.32</td>
<td>[mM]</td>
<td>(Lai, Saidel, et al., 2007)</td>
</tr>
<tr>
<td>(C_{b,Hb})</td>
<td>2.6-2.9</td>
<td>[mM]</td>
<td>This work</td>
</tr>
</tbody>
</table>
2.2.2. NIRS signals: Components of the oxygenated and deoxygenated heme group concentrations

The simulated oxygenated Hb and Mb in the microvascular and tissue volumes are used to compute the oxygenated Hb (\(HbO_2\)) and Mb (\(MbO_2\)) heme group concentrations in muscle. The oxygenated Hb (\(HbO_2\)) accounts for the individual arteriole, capillary, and venule contributions:

\[
HbO_2 = HbO_{2,art} + HbO_{2,cap} + HbO_{2,ven}
\]  \hspace{1cm} 2.12

where each contribution is computed as

\[
HbO_{2,art} = f_{b,m} C_{O2,art}^B \omega_{art}/4
\]  \hspace{1cm} 2.13

\[
HbO_{2,cap} = f_{b,m} < C_{O2,cap}^B > \omega_{cap}/4
\]  \hspace{1cm} 2.14

\[
HbO_{2,ven} = f_{b,m} C_{O2,ven}^B \omega_{ven}/4
\]  \hspace{1cm} 2.15

where \(< C_{O2,cap}^B >\) is the spatial average of the bound O\(_2\) concentration in the capillary blood. Substituting Eq. 2.13-2.15, equation 2.12 becomes:

\[
HbO_2 = f_{b,m} \left[ C_{O2,art}^B \omega_{art} + < C_{O2,cap}^B > \omega_{cap} + C_{O2,ven}^B \omega_{ven} \right]/4 = f_{b,m} C_{b,m}^B/4
\]  \hspace{1cm} 2.16

where \(C_{b,m}^B\) is the bound O\(_2\) concentration in the microvascular volume, \(f_{b,m}\) is the microvascular volume fraction and \(\omega_x (x \in \text{art, cap, ven})\) are the volume fraction of different compartments.

The oxygenated Mb (\(MbO_2\)) is expressed as:

\[
MbO_2 = f_t < C_{O2,t}^B >/4
\]  \hspace{1cm} 2.17

where \(f_t\) are the microvascular and tissue volume fraction in muscle, and \(< C_{O2,t}^B >\) is the spatial average of the bound O\(_2\) concentration in tissue. The spatial average of the bound O\(_2\) concentration depends on a specific volume as:

\[
<y> = \int_0^{V_x} y(v)dv /V_x \hspace{1cm} V_x = V_{cap},V_t.
\]  \hspace{1cm} 2.18
Likewise, the deoxygenated Hb (\(HHb\)) is expressed in terms of the individual microvascular components as:

\[
HHb = HHb_{art} + HHb_{cap} + HHb_{ven}
\]  

\[2.19\]

where each contribution can be computed as

\[
HHb_{art} = f_{b,m} \omega_{art} \left[ C_{rbc,Hb} Hct - \frac{c_{O_2,art}}{4} \right]
\]  

\[2.20\]

\[
HHb_{cap} = f_{b,m} \omega_{cap} \left[ C_{rbc,Hb} Hct - \frac{c_{O_2,cap}}{4} \right]
\]  

\[2.21\]

\[
HHb_{ven} = f_{b,m} \omega_{art} \left[ C_{rbc,Hb} Hct - \frac{c_{O_2,ven}}{4} \right]
\]  

\[2.22\]

Substituting Eq. 2.20-2.32, equation 2.19 becomes:

\[
HHb = f_{b,m} C_{b,Hb} - HbO_2.
\]  

\[2.23\]

The deoxygenated Mb (\(HMb\)) is expressed as:

\[
HMb = f_{t} C_{t,Mb} W_{mt}/4 - MbO_2.
\]  

\[2.24\]

In the presence of blood volume changes, \(f_{b,m}\) is a function of the relative heme concentration changes (\(\Delta C_{Heme,N}^{exp}\)) detected by the NIR\(S\) signals as:

\[
f_{b,m} = f_{b,m} \left( \Delta C_{Heme,N}^{exp} \right) = f_{b,m} R + \frac{\Delta C_{Heme,N}^{exp} HbMbO_2^R}{(C_{b,Hb} - C_{t,Mb}/4)}.
\]  

\[2.25\]

\(\Delta C_{Heme,N}^{exp}\) is calculated from the oxygenated and deoxygenated NIR\(S\) measurements:

\[
\Delta C_{Heme,N}^{exp} = \frac{\Delta HbMbO_2^{exp} + \Delta HHbMb^{exp}}{\Delta HbMbO_2^{T5}_{exp}}
\]  

\[2.26\]
2.2.3. Simulation strategy

The key model inputs are muscle blood flow ($Q$), arterial O$_2$ partial pressure ($P_{O_2,art}$), fraction of blood in muscle volume ($f_{b,m}$) and O$_2$ uptake ($V\dot{O}_2$) for each trial condition. $V\dot{O}_2$ was used to estimate $k_{ATPase}$ with the expression (Lai et al., 2007):

$$k_{ATPase} = \frac{5.4V\dot{O}_2}{fC_{ATP}}. \quad 2.27$$

The experimental data are compared with model simulations. These data represent the venous oxygen concentration ($C_{O_2,ven}^T$), the oxygenated ($\Delta HbMbO_2,N$) and deoxygenated ($\Delta HhMb$) NIRS measurements, and the rate of muscle oxygen uptake determined as:

$$V\dot{O}_2 = Q(t)[C_{O_2,art}^T - C_{O_2,ven}(t)]/V_{mus}, \quad 2.28$$

that are obtained under different conditions. The oxygenated and deoxygenated NIRS signals are normalized to the maximal variation of: oxygenated NIRS signals (Hernández et al., 2010) (eq. 2.29) or oxygenated and deoxygenated NIRS signals observed during ischemia (Sun et al., 2016) (eq. 2.30), respectively.

To compare the NIRS data of Hernández et al., 2010 with model simulation, the NIRS signals were normalized as follows:

$$\Delta HbMbO_2,N = \frac{HbMbO_2}{\Delta HbMbO_2^{max}}; \quad \Delta HhMb_N = \frac{\Delta HhMb}{\Delta HhMb^{max}}; \quad 2.29$$

where $\Delta HbMbO_2^{max}$ was the maximal amplitude of $\Delta HbMbO_2$ observed during contraction. To compare the NIRS data of Sun et al., 2016, the NIRS signals were normalized as follows:

$$\Delta HbMbO_2,N = \frac{HbMbO_2}{\Delta HbMbO_2^{T5}}; \quad \Delta HhMb_N = \frac{\Delta HhMb}{\Delta HhMb^{T5}}; \quad 2.30$$

where $\Delta HbMbO_2^{T5}$ and $\Delta HhMb^{T5}$ were the oxygenated and deoxygenated concentration changes from rest to ischemia. T5 is the trial corresponding to the ischemic conditions.
For equation 2.29, the Hb and Mb contribution to the oxygenated and deoxygenated NIRS signals are computed as

\[
\Delta HbMbO_{2,N} = \frac{\Delta HbMbO_{2}}{\Delta HbMbO_{2}^{max}} = \frac{(HbO_{2}^{max} - HbO_{2})+(MbO_{2}^{max} - MbO_{2})}{(HbO_{2}^{max} - HbO_{2})+(MbO_{2}^{max} - MbO_{2})}
\]  
\[2.31\]

\[
\Delta HHbMb_N = \frac{\Delta HHbMb}{\Delta HHbMb^{max}} = \frac{(HHb^{Bout} - HHb)^R+(HMB^{Bout} - HMB)^R}{(HHb^{max} - HbO_{2})+(MbO_{2}^{max} - MbO_{2})}
\]
\[2.32\]

where a superscript Bout designates the bout of muscle contraction performed. Similarly, for equation (2.30), the Hb and Mb contributions are computed at steady state as:

\[
\Delta HbMbO_{2,N} = \frac{\Delta HbMbO_{2}}{\Delta HbMbO_{2}^{TS}} = \frac{(HbO_{2}^{TS} - HbO_{2})+(MbO_{2}^{TS} - MbO_{2})}{(HbO_{2}^{TS} - HbO_{2})+(MbO_{2}^{TS} - MbO_{2})}
\]
\[2.33\]

\[
\Delta HHbMb_N = \frac{\Delta HHbMb}{\Delta HHbMb^{TS}} = \frac{(HHb^{TS} - HHb)^R+(HMB^{TS} - HMB)^R}{(HHb^{TS} - HHb)^R+(HMB^{TS} - HMB)^R}
\]
\[2.34\]

where the superscripts indicate resting (R), ischemic (T5) and each trial (T) conditions. The oxygenation changes in the microvascular and extravascular compartments are responsible for the NIRS measurement changes.
CHAPTER 3

EFFECT OF BLOOD FLOW ON HEMOGLOBIN AND MYOGLOBIN OXYGENATION IN CONTRACTING MUSCLE USING NEAR-INFRARED SPECTROSCOPY

3.1. Introduction

Imbalance between oxygen delivery and utilization in skeletal muscle produces significant mobility limitations for patients with chronic diseases. NIRS has been used to evaluate non-invasively the balance between these two physiological variables in contracting muscle (Davis & Barstow, 2013; Grassi & Quaresima, 2016). The interpretation of the NIRS signals (oxygenation and deoxygenation) depend on microvascular and extravascular O₂ concentration changes in response to blood flow and O₂ utilization rate kinetics. Mb and Hb in oxygenated ($HbO₂$, $MbO₂$) and deoxygenated ($HHb$, $HMb$) forms contribute to the oxygenated ($HbMbO₂$) and deoxygenated ($HHbMb$) NIRS signals.

It is unclear how $HbMbO₂$ or $HHbMb$ from NIRS signals could be used to evaluate muscle O₂ extraction (Grassi & Quaresima, 2016). Some consider only $HHbMb$ kinetics because the blood flow to the skin affects the HbMbO₂ signal resulting in a reoxygenation of the signal during contraction (Grassi et al., 2003). However, the $HHbMb$ signal is minimally affected by these changes. To avoid effects of skin blood flow in studying NIRS signals, an animal model of muscle oxidative metabolism (Hernández et al., 2010) can be used, allowing simultaneous venous O₂ content and NIRS signal measurements during contraction.
Since these responses depend on muscle O$_2$ utilization and delivery rates, the relative contributions to HbMbO$_2$ and $HHbMb$ can be quantitatively distinguished by analyzing experimental NIRS data. Using computer simulations with a mechanistic, mathematical model, we can analyse the underlying transport and metabolic processes that affect O$_2$ utilization in contracting skeletal muscle (Lai et al., 2007; Lai et al., 2009).

We validate the model by comparison of simulations with experimental venous O$_2$ and NIRS signals responses from contracting skeletal muscle. The simulated outputs are compared to experimental HbMbO$_2$ and $HHbMb$ kinetics obtained from contracting dog skeletal muscle under fast and slow blood flow kinetics (Hernández et al., 2010).

### 3.2. Methods

Our mechanistic model of O$_2$ transport and metabolism (Lai et al., 2007) simulates vascular and extravascular oxygenation in contracting muscle. By comparison to NIRS data we can quantify changes of muscle blood volume and contributions of Hb and Mb to the NIRS signals (Lai et al., 2009). We used this approach to analyze the effects of blood flow kinetics on the NIRS signals measured in contracting dog gastrocnemius (Hernández et al., 2010).

The O$_2$ transport and utilization dynamics in capillary and extravascular tissue of contracting muscle are modelled with dynamic mass balance equations of O$_2$ in the blood domain and O$_2$, adenosine diphosphate (ADP) and phosphocreatine (PCr) in the tissue domain. This model incorporates the metabolic fluxes of ATPase, oxidative phosphorylation and creatine kinase (Lai et al., 2007). At the onset of contraction, the dynamic response of blood flow is represented by an exponential function, and permeability-surface area is considered a function of blood flow (Spires
et al., 2013). The model simulates concentrations of free and bound O2 to Hb and Mb in capillary and tissue, respectively. Blood domain contributions to the NIRS signal have three compartments: arterial, capillary and venous (Lai et al., 2009). The change in muscle oxygenation during contraction incorporates the oxygenated Hb and Mb concentrations within the whole muscle as:

\[ HbO_2(t) = f_{b,m}(t) [ < C_{O_2, cap}^B > \omega_{cap}(t) + C_{O_2, art}^B \omega_{art}(t) + C_{O_2, ven}^B \omega_{ven}(t) ] / 4 \]  

\[ MbO_2(t) = f_{t}(t) < C_{O_2, t}^B > / 4 \]  

where \( C_{O_2, art}, < C_{O_2, cap}^B >, C_{O_2, ven}^B \) and \( C_{O_2, t}^B \) are the bound O2 concentrations in arteries, spatially averaged capillaries, veins, and muscle cells. \( \omega_{art}, \omega_{cap}, \omega_{ven} \) are the arterial, capillary, and venous volume fractions in blood; \( f_{b,m} \) and \( (f_t=1-f_b) \) are the vascular and extravascular volume fractions in muscle. Eqs. (3.1) and (3.2) can be rewritten in terms of oxygenated heme groups of Hb and Mb (\( C_{oxy,x} \)) as:

\[ HbO_2(t) = f_{b,m}(t) [ C_{oxy, cap} \omega_{cap}(t) + C_{oxy, art} \omega_{art}(t) + C_{oxy, ven} \omega_{ven}(t) ] \]  

\[ MbO_2(t) = f_{t}(t) C_{oxy,tis}. \]  

These equations can be used to calculate deoxygenated Hb and Mb heme groups (\( HHb, HMb \)) in muscle as:

\[ HHb(t) = f_{b,m}(t) [ C_{deoxy, cap} \omega_{cap}(t) + C_{deoxy, art} \omega_{art}(t) + C_{deoxy, ven} \omega_{ven}(t) ] \]  

\[ HMb(t) = f_{t}( C_{c,Mb} W_{mc} / 4 - C_{O_2,c}^B ) / 4 = f_{t} C_{deoxy,tis} \]  

where in blood compartments (x=cap, art, ven) \( C_{deoxy,x} = C_{b,Hb} - C_{oxy,x} \). \( W_{mc} \) is the fraction of muscle cells in the volume of tissue. The variable \( f_{b,m}(t) \) is related to the change of total heme
concentration $\Delta HbMb_{tot}(t)$ normalized by the maximal change measured during contraction ($\Delta HbMbO_{2,max}$) by NIRS (Lai et al., 2009):

$$f_{b,m}(t) = f^R_{b,m} + \frac{[\Delta HbMbO_{2,tot}(t)]_{\exp}}{C_{Hb} + C_{Mb} W_{mc}/4}$$

3.7

here, $f^R_{b,m}$ is the resting blood volume fraction, $C_{b,Hb}$ and $C_{c,Mb}$ are the Hb and Mb concentrations in blood and tissue, respectively. The vascular volume changes during contraction affect arterioles, capillary and venules as well as tissue volume fractions (Lai et al., 2009). The simulated oxy/deoxygenated Hb and Mb concentrations contributing to the NIRS signals are:

$$HbMbO_2(t) = HbO_2(t) + MbO_2(t); HHbMb = HHb + HMb(t).$$

3.8

Hb and Mb contributions to the $HbMbO_2$ and $HHbMb$ signals are quantified as:

$$y_{HbO_2}(t) = \frac{HbO_2(t)}{HbMbO_2(t)}; y_{MbO_2}(t) = \frac{MbO_2(t)}{HbMbO_2(t)}$$

3.9

$$y_{HHb}(t) = \frac{HHb(t)}{HHbMb(t)}; y_{HMb}(t) = \frac{HMb(t)}{HHbMb(t)}$$

3.10

where, $y_{HbO_2}(t)$ and $y_{MbO_2}(t)$ are the fraction of oxygenated Hb and Mb in the oxygenated heme group; $y_{HHb}(t)$ and $y_{HMb}(t)$ are the fractions of deoxygenated Hb and Mb in the deoxygenated heme group. To compare NIRS oxygenation and deoxygenation responses to contraction with those simulated, the changes in oxygenated ($\Delta HbMbO_{2}$) and deoxygenated ($\Delta HHbMb$) signals are normalized by the maximal oxygenated signal change during contraction ($\Delta HbMbO_{2,max}$)

$$\Delta HbMbO_{2,N}(t) = \Delta HbMbO_2(t)/\Delta HbMbO_{2,max}$$

3.11

$$\Delta HHbMb_N(t) = \Delta HHbMb(t)/\Delta HbMbO_{2,max}.$$
3.3. Results

The kinetics of muscle blood flow (Figure 3.1a) and volume (Figure 3.1b) are model inputs. The sum of the oxygenated and deoxygenated NIRS signals ($\Delta HbM_{btot}/\Delta HbMbO_{2,\text{max}}$) kinetics (Figure 3.1b) are used to quantify the microvascular volume fraction changes ($f_{b,m}$, Figure 3.1c) during contraction. Muscle $O_2$ uptake at steady state (Figure 3.2a) was also a model input to simulate the $\Delta HbMbO_2$ and $\Delta HbMb$ kinetics (normalized) (Figure 3.2b) (Hernández et al., 2010). The mathematical model was tested by comparing simulated muscle $O_2$ uptake (Figure 3.2a) and NIRS kinetics and experimental data obtained for two different contracting bouts (B1, B2) (Hernández et al., 2010). B1 is a prior bout of muscle contraction followed by two minutes of rest and a second bout of contraction (B2).
Figure 3.1. Model inputs and outputs for bouts 1, 2 and 3 of muscle contraction: a) blood flow kinetics (inputs) b) total NIRS signal kinetics normalized (inputs); c) microvascular blood volume fraction ($f_{b,m}$) (output).

The blood flow time constant in the first bout is larger ($\tau_{B_1} = 16.8$ s) than the second one ($\tau_{B_2} = 9$ s), whereas the amplitude of blood flow and $O_2$ uptake are the same in both bouts. Simulated oxygenated and deoxygenated kinetics based on Hb and Mb contributions follow closely those measured by NIRS.
Figure 3.2. Model outputs for bouts 1, 2 and 3 of muscle contraction: a) simulated and experimental muscle oxygen uptake; b) simulated and experimental NIRS signals normalized to maximal change of oxygenated NIRS signals.
The faster the blood flow kinetics, the slower the NIRS kinetics (Figure 3.2b) (Hernández et al., 2010). A third bout (B3) was simulated with the same conditions of B1 except blood flow time constant that was greater ($\tau_{B3} = 30s$). Under this condition, a concomitant undershoot of the oxygenated and an overshoot of the deoxygenated signals were observed.

The effect of blood flow kinetics on Hb and Mb contributions to the $HbMbo_2$ and $HHbMb$ (Figure 3.3a, b) are simulated for all bouts. At the onset of contraction, an overshoot of the deoxy/oxygenated Hb contributions and an undershoot of the deoxy/oxygenated Mb contribution are observed. Hb and Mb contribute similarly to the $HbMbo_2$ ($y_{Hbo_2}(t)$ and $y_{Mbo_2}(t)$): approximately 40% for Mb and 60% for Hb at rest. In contrast, the contributions of Hb and Mb to the deoxygenated NIRS signal ($y_{HHb}(t)$ and $y_{HMb}(t)$) are: 70-80% and 20-30%, respectively. Table 3.1 shows the concentration of oxygenated and deoxygenated heme groups in the vascular
and extravascular domains and whole muscle at rest. Under normal or fast O₂ delivery (B1 and B2) during muscle contraction, when NIRS signal approaches a plateau (ΔHbMbO₂,max), \(y_{MbO₂}(t)\) reaches 50% in less than 10s. In the presence of O₂ delivery impairment (B3), \(y_{MbO₂}(t)\) kinetics is slower than that observed for B1 and B2 while \(y_{HMb}(t)\) reaches its peak. Specifically, at 40s, in the presence of a ΔHbMbO₂,N undershoot of 60% of the plateau (ΔHbMbO₂,max), \(y_{MbO₂}(t)\) remains close to the rest value (40%) while \(y_{HMb}(t)\) increases from 20 to 37%.

Table 3.1 Concentration of oxygenated and deoxygenated heme groups in whole muscle (HbO₂, MbO₂, HHb, HMb) and skeletal muscle compartments (C_{oxy,x}, C_{deoxy,x}) (x=art, cap, ven, tis) and their volume fractions at rest.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Volume fraction [%]</th>
<th>(C_{oxy,x}) [(\mu M)]</th>
<th>(C_{deoxy,x}) [(\mu M)]</th>
<th>(HbO₂) or (MbO₂) [(\mu M)]</th>
<th>(HHb) or (HMb) [(\mu M)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>0.5</td>
<td>2360</td>
<td>100</td>
<td>11.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Capillary</td>
<td>0.75</td>
<td>2150</td>
<td>500</td>
<td>14.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Venous</td>
<td>3.75</td>
<td>2250</td>
<td>300</td>
<td>80.9</td>
<td>9.9</td>
</tr>
<tr>
<td>Myocytes</td>
<td>95</td>
<td>80</td>
<td>3</td>
<td>74.1</td>
<td>2.2</td>
</tr>
</tbody>
</table>

3.4. Discussion

Our mathematical model predicts the effects of blood flow kinetics on the oxygenated and deoxygenated kinetics by NIRS. For the same O₂ demand, fast muscle O₂ delivery slows both
oxygenated and deoxygenated kinetics. An imbalance between muscle O$_2$ delivery and utilization rate kinetics is responsible for the observed trend. Hb and Mb contributions ($y_{HbO_2}(t), y_{MbO_2}(t)$) to the $HbMbO_2$ at rest and during contraction differ from those ($y_{HHb}(t), y_{HMB}(t)$) to the $HHbMb$. The effect of blood flow kinetics on $y_{HbO_2}(t)$ and $y_{MbO_2}(t)$ kinetics was minimal while that on $y_{HHb}(t)$ and $y_{HMB}(t)$ determined overshoots and undershoots.

Simulations predict that in the presence of fast blood flow kinetics, an abundant muscle O$_2$ delivery in relation to its utilization (Figure 3.2b) is the main cause for slow muscle oxygenation and deoxygenation responses to contraction. To further establish the relationship between blood flow and NIRS kinetics, model simulation shows that slow muscle O$_2$ delivery causes undershoots and overshoots of oxygenated and deoxygenated kinetics, respectively (Figure 3.3a, b).

At rest, the contribution of Hb and Mb ($y_{HbO_2}(t)$ and $y_{MbO_2}(t)$) to the oxygenated heme groups are similar (Figure 3.3a, b). These contributions depend on heme concentration in the vascular and extravascular domains and on their volume fractions (Table 3.1). Thus, high heme concentration in blood domains with low microvascular volume fraction (<5%) or low heme concentration in the tissue domain with high tissue volume fraction (95%) resulted in a similar concentration of oxygenated heme group in muscle. The Hb contribution to the deoxygenated signal is higher than that of Mb. This is mainly related to the O$_2$ saturation of Hb (predominately in venous blood, 89%) compared to Mb (96.2%) that leads to a higher $HHb$ than $HMB$ (Table 3.1).

At the onset of contraction, the abrupt increase of $y_{HbO_2}(t)$ and $y_{HHb}(t)$ observed for both bouts 1 and 2 (Figure 3.3a, b) is due to the increase in blood volume detected by NIRS measurement (Figure 3.1b). This is quantified by the model with an increase of the vascular fraction $f_{b,m}$ (Figure 3.1c). The size of the microvascular and extravascular volume fractions
determines the amplitude of the changes in Hb and Mb contributions to the NIRS signal. During contraction the Hb and Mb kinetics are affected by the interplay between O\textsubscript{2} delivery (convection and diffusion) and utilization. Impaired O\textsubscript{2} delivery represented by slow blood flow kinetics was associated with a slow $y_{MbO_2}(t)$ and fast $y_{HMb}(t)$ kinetics during the first 20s of muscle contraction. Under this condition, the deoxygenation rate in tissue was faster than that in the blood domain. Tissue oxygenation increased only when the O\textsubscript{2} delivery was enough to balance the O\textsubscript{2} utilization.

In conclusion, the contribution of Mb to the NIRS signals is significant. This is consistent with other experimental (Davis & Barstow, 2013) and computational studies (Lai et al., 2009; Spires et al., 2011). Quantitative analysis indicates that oxygenated and deoxygenated kinetics are similarly affected by blood flow kinetics in the absence of a confounding factor such as blood flow to the skin. Hb and Mb contributions to the oxygenated and deoxygenated heme groups significantly change with O\textsubscript{2} delivery. These contributions are relevant in the interpretation of a NIRS signal used to evaluate chronic disease in which O\textsubscript{2} delivery is impaired. Approaches that combine NIRS with model simulation and devices quantifying hemodynamic and microvascular distribution can help to overcome NIRS’ inability to distinguish between Hb/Mb contributions.
CHAPTER 4

BLOOD VOLUME VS. DEOXYGENATED NIRS SIGNAL:
COMPUTATIONAL ANALYSIS OF THE EFFECTS OF MUSCLE O2 DELIVERY AND BLOOD VOLUME ON THE NIRS SIGNALS

4.1. Introduction

Skeletal muscle mitochondrial function can be assessed in vivo using near-infrared spectroscopy (NIRS) (Barstow, 2019; Grassi et al., 2019; Willingham & McCully, 2017). The non-invasive nature of this technology and its relatively affordable price make it a promising tool in translational medicine to complement approaches in vitro to assess bioenergetic function (Lai et al., 2019; Palmer et al., 1977; Veksler et al., 1987). Physiological parameters derived from NIRS signals are associated with mitochondrial respiratory rate measured in permeabilized fibers (Ryan et al., 2014).

Methods based on NIRS techniques are used to estimate mitochondrial function in contracting muscle with spontaneous blood flow (Barstow, 2019; Grassi et al., 2003) or arterial occlusion (Hamaoka et al., 1996; McCully et al., 2011; Ryan et al., 2012) under physiological and pathophysiological conditions, but uncertainties about quantitative information provided by the NIRS signals limit the physiological inferences. Besides the challenges related to the use of NIRS instrumentation based on different techniques (Ferrari et al., 2011; Grassi & Quaresima, 2016), the NIRS signal remains a semi-quantitative method for assessing relative oxygenation in skeletal muscle. Specifically, NIRS signals reflect changes of both hemoglobin (Hb) and myoglobin (Mb)
oxygenation and their contributions to the NIRS signals vary during muscle contraction (Lai et al., 2009; Spires et al., 2013). Other factors such as hematocrit, blood volume (Goodwin et al., 2011; Grassi et al., 2003) and arteriolar, capillary and venular distribution (Adami et al., 2015) affect the interpretation of the oxygenated and deoxygenated NIRS kinetics.

Different strategies were proposed to assess NIRS measurement in the presence of blood volume changes especially for a constant-wave (CW) NIRS system (Barstow, 2019). Some investigators prefer to use the deoxygenated NIRS signal to evaluate muscle O2 extraction (Grassi et al., 2019) arguing that it appears to be less affected by blood volume changes. Specifically, human studies have reported that the oxygenated signal was affected by an increase of skin blood flow whereas the deoxygenated signal was insensitive to the blood volume changes (Davis et al., 2006; Grassi et al., 2003; Grassi & Quaresima, 2016). The study of the factors responsible for the difference between oxygenated and deoxygenated NIRS signals is limited by the experimental setting of the NIRS measurement especially in human studies. An alternative approach to study this problem is to use a canine model of oxidative metabolism (Hernández et al., 2010; Sun et al., 2016). This animal model allows the study of the NIRS signal with an appropriate experimental setting without the effects related to skin blood flow and adipose tissue because the NIRS probe is placed directly on the skeletal muscle surface (Sun et al., 2016). Furthermore, with this animal model, it has been reported that the amplitude of the oxygenated and deoxygenated NIRS signals increased with a decrease in O2 delivery (i.e., varying arterial O2 concentration or blood flow) in the presence or absence of contraction. Also, the O2 delivery effects on the oxygenated NIRS signal differed from those on the deoxygenated counterpart. This observation suggests the presence of a blood volume effect on both NIRS signals (oxygenated and deoxygenated forms) (Sun et al., 2016) even in the absence of skin blood flow changes present in the experimental human study. The
effect of blood flow on the amplitude of the NIRS signals (oxygenated and deoxygenated forms) observed in the animal study is related to its effects on the oxygen saturation in the microvascular and extravascular compartments. Thus, it is possible that the oxygen saturation in these compartments may also have a role in determining how blood volume changes contribute to the NIRS signal amplitude. Moreover, it has also been reported that the deoxygenated signal is sensitive to microvascular volume changes (Adami et al., 2015). Thus, this evidence from animal and human studies challenges the view that the effects of blood volume change on the deoxygenated NIRS signal are negligible.

A challenge in studying blood volume effects on the NIRS signal is that it is difficult to control blood volume changes during the NIRS measurement. To overcome this limitation, a computational model of O₂ transport and utilization in skeletal muscle can be used to investigate the effect of blood volume. This mathematical model has been used to predict the skeletal muscle metabolic response to contraction under different experimental conditions (Grassi, Gladden, Samaja, et al., 1998; Grassi, Gladden, Stary, et al., 1998; Grassi et al., 2000). Specifically, the mathematical model was validated with kinetics of O₂ uptake and venous O₂ concentration data obtained from canine muscle under different arterial O₂ concentration, blood flow and metabolic rate conditions (Lai, Camesasca, et al., 2007; Spires et al., 2013; Spires et al., 2012). Thus, this model is ideal for studying the effects of blood flow and volume on the NIRS signals in animal models and is particularly appealing to relate blood and tissue oxygenations by NIRS. Other computational models were used to relate gas exchange with NIRS data in human studies (Lai et al., 2008; Lai et al., 2009; Zhou et al., 2008).

In this study we used a computational model validated with canine data with some modifications to analyze the effects of O₂ delivery on the NIRS oxygenation data to quantify the
effect of blood volume on the oxygenated and deoxygenated NIRS signals. The analysis focuses on NIRS measurements for conditions reflecting resting and contracting muscles under different blood flow without arterial occlusion. The hypothesis of this work is that the deoxygenated NIRS signal is significantly affected by blood volume changes during contraction or in the presence of reduced O_2 delivery. It is expected that both NIRS signals are significantly affected by blood volume changes when the microvascular oxygen saturation is reduced (60%) for contracting muscle, or impaired O_2 delivery. On the contrary, with sufficient O_2 delivery to have high microvascular oxygen saturation the effects of blood volume change on the deoxygenated NIRS signal are negligible.

4.2. Methods

4.2.1. Animal Model and Trials

The computational analysis was performed on the NIRS measurement under different experimental conditions (i.e., blood flow, contraction) reported in Figure 4.1 (Sun et al., 2016). It should be noted that the trial notation in this work is the same as that used in the experimental work (Sun et al., 2016) from which the NIRS data were used for the analysis proposed here. T1 and T3 trials were selected to analyze the effect of muscle oxygen (O_2) delivery and blood volume in resting (T1) and contracting muscle (T3). Also, T5 trial was considered because the NIRS signals obtained for this condition were used to normalize the NIRS signals of T1 and T3 trials. All experimental data were averaged from 6 measurements for each trial condition. A continuous-wave NIRS system (Oxymon Mk III, Artinis Medical Systems BV) was used to measure the muscle oxygenated (\(\Delta HbMbo_2\)) and deoxygenated (\(\Delta HbMb\)) heme group concentration changes relative to rest. The light at two different wavelengths (760 and 860 nm) were emitted and received
by two fiber-optic bundles. The optodes were placed over the medial head of the left gastrocnemius and held in place with an elastic band. The experimental control of muscle blood flow was obtained with a peristaltic pump connected to the gastrocnemius. The right carotid artery or the right femoral artery was cannulated to route blood to the peristaltic pump (Sun et al., 2016).

Figure 4.1. Experimental conditions simulated in this work and previously investigated by (Sun et al., 2016): Effect of blood flow at rest (T1) or during contraction (T3, 0.5Hz).
4.2.2. Volume distribution of the heme group in skeletal muscle

A mathematical model of skeletal muscle O$_2$ transport and metabolism (Lai et al., 2009; Spires et al., 2013; Spires et al., 2012) was used with some modifications to study the effect of O$_2$ delivery and blood volume on the NIRS signals. The muscle volume ($V_{mus}$) consists of extravascular tissue (cells and interstitial space, $V_t$) and blood ($V_b$) which is assumed to have the vascular ($V_{b,v}$) and microvascular ($V_{b,m}$) compartments (Figure 4.2).

![Skeletal muscle volume distribution and composition of the NIRS signal: vascular, microvascular (arteriole, capillary, venule) and extravascular volume compartments.](image)

$$f_{b,m} = \frac{V_{b,m}}{V_t+V_{b,m}+V_{b,v}}; f_t = 1 - f_{b,m} - f_{b,v}$$

$$\omega_{art} = \frac{V_{art}}{V_{b,m}}; \omega_{cap} = \frac{V_{cap}}{V_{b,m}}; \omega_{ven} = \frac{V_{ven}}{V_{b,m}}$$

The microvascular volume consists of arterioles, capillaries, and venules ($V_{b,m} = V_{art} + V_{cap} + V_{ven}$). In the absence of blood volume changes, the microvascular volume fraction ($f_{b,m}$) is equal to that at rest ($f_{b,m}^R$) and was calculated by the product of the blood volume fraction in the
muscle \( (f_p) \) and microvascular volume fraction in the blood \( (f_m) \) (Table 4.1). In the presence of blood volume changes, it is assumed that only the capillary volume \( (V_{cap}) \) of the microvascular compartment can vary with muscle perfusion changes in resting or contracting muscle. Thus, heme groups’ concentration changes detected by the NIRS measurements are attributed to a \( V_{cap} \) variation. The steps to calculate this variation are reported in section 4.2.5: Composition of the NIRS signal.

### 4.2.3. \( \text{O}_2 \) concentration in microvascular and muscle cells

The \( \text{O}_2 \) changes in the microvascular and extravascular volumes for different \( \text{O}_2 \) delivery are responsible for NIRS changes. The \( \text{O}_2 \) changes should account for the \( \text{O}_2 \) dissolved in plasma and tissue as well as that bound to the Hb and Mb. To quantify these changes a system of mass balance equations are used to simulate the concentration of \( \text{O}_2 \) dissolved in the microvascular \( (C_{\text{O}_2,\text{cap}}, C_{\text{O}_2,\text{ven}}) \) and extravascular \( (C_{\text{O}_2,t}) \) compartments. The Hill–Langmuir equation (Eq. 4.7, 4.8) is used to quantify the \( \text{O}_2 \) bound \( (C_{\text{O}_2}^B) \) either to Hb (arterioles, capillaries, venules) or Mb from the \( \text{O}_2 \) concentration dissolved \( (C_{\text{O}_2}^F) \) or also defined as free \( \text{O}_2 \) in the solution (Lai, Camesasca, et al., 2007; Lai, Saidel, et al., 2007; Lai et al., 2009). In Figure 4.3, a schematic representation of the model inputs (orange) required by the mathematical model (blue) to simulate the outputs such as the oxygenation within microvascular and extravascular compartments (green) with or without blood volume changes \( (f_{b,m} = f_{b,m}^R) \) are reported.
Figure 4.3. Algorithm steps of the method integrating experimental data and mathematical model to simulate NIRS measurement.

Under steady-state conditions, the free O$_2$ concentration in capillary [$C_{O_2,c}^F(v)$] and in muscle cells [$C_{O_2,c}^F(v)$] depends on location ($v$) from artery ($v = 0$) to vein ($v = V_{mus}$) as follows:

\[ Q \frac{\partial C_{O_2,c}^F}{\partial v} - D_b \frac{\partial^2 C_{O_2,c}^F}{\partial v^2} + f_{bc} \frac{C_{O_2,c}^F}{\gamma_b} = 0 \]  \hspace{1cm} 4.1

\[ D_c \frac{\partial^2 C_{O_2,c}^F}{\partial v^2} + f_c \frac{C_{O_2,c}^F}{\gamma_c} = 0 \]  \hspace{1cm} 4.2

where $D_b$ and $D_c$ are the axial effective dispersion coefficients in blood and tissue, while $\gamma_b$ and $\gamma_c$ are the changes of total O$_2$ concentration to the free O$_2$ concentration in blood and muscle cells, respectively. The derivation of these equations was previously reported in other studies (Lai et al.,...
In Eq. 4.1, the first term represents convective transport, due to delivery ($Q$) in the direction of blood flow. The second term is the axial dispersion in the capillary bed, and the third term represents O$_2$ transport rate between capillary and tissue as

$$J_{O_2}^b.c = PS(C_{O_2,b}^F - C_{O_2,c}^F).$$  \hspace{1cm} 4.3

The permeability surface-area ($PS$) is linearly related to $Q$ during muscle contraction according to:

$$PS = PS_R + \eta(Q(t) - Q_R).$$  \hspace{1cm} 4.4

The index $R$ indicates rest condition, and $\eta$ is a parameter previously determined (Spires et al., 2013).

For the boundary condition of the ordinary differential equations, it is assumed that the input O$_2$, from arterial blood is known and the output O$_2$ concentration leaving the capillaries is like that entering the venules (i.e., negligible gradient):

$$v = 0 \quad C_{O_2,b}^F = C_{O_2,art}^F \quad \frac{\partial c_t^F}{\partial v} = 0 \hspace{1cm} 4.5$$

$$v = V_m \quad \frac{\partial c_{O_2,b}^F}{\partial v} = 0 \quad \frac{\partial c_t^F}{\partial v} = 0. \hspace{1cm} 4.6$$

The boundary conditions for the other metabolites are like those used for O$_2$ (Spires et al., 2012). The details of the rest of the mass balance equations were previously reported in other studies (Lai, Camesasca, et al., 2007; Lai, Saidel, et al., 2007; Lai et al., 2009; Spires et al., 2013) and in Appendix B and C. The ordinary differential equation system was solved with 50 nodes using the method of lines with the MATLAB library ode15s.
4.2.4. Relationship between O₂ free and O₂ bound to either Hb or MB

Once the spatial profile O₂ in the microvascular and extravascular compartments are obtained from the solution of the ordinary differential equation system, the total (T) O₂ concentration for blood (b) and myocyte compartments (c), is calculated by the sum of the free (F) and bound (B) O₂ concentrations \( C_{O_2,x}^T = C_{O_2,x}^B + C_{O_2,x}^F, \ x = b, c \), which are related by local equilibrium (Lai, Saidel, et al., 2007). In the microvascular compartments (arteriole, capillary, venule) the relationship between the free and bound forms is

\[
C_b^B = 4 \text{ Hct } C_{rbc,Hb} SO_{O_2,b} = 4 C_{b,Hb} \frac{K_{Hb} (C_{O_2,b}^F)^n}{1 + K_{Hb} (C_{O_2,b}^F)_n^{0.7}} \tag{4.7}
\]

and in the extravascular muscle (i.e., myocyte) is

\[
C_t^B = W_{mc} C_{c,Mb} SO_{O_2,c} = C_{t,Mb} \frac{K_{Mb} C_{O_2,c}^F}{1 + K_{Mb} C_{O_2,c}^F} \tag{4.8}
\]

where \( SO_{O_2,b} \) and \( SO_{O_2,c} \) are the oxygen saturation (SO₂) in blood and muscle cell compartments.

The concentration of Hb in blood (\( C_{b,Hb} \)) and red blood cells (\( C_{rbc,Hb} \)) are related by Hct whereas the concentration of Mb in muscle tissue (cells and interstitial space) (\( C_{t,Mb} \)) and myocytes (\( C_{mc,Mb} \)) are related by \( W_{mc} \) (\( C_{b,Hb} = C_{rbc,Hb} \text{ Hct} ; C_{t,Mb} = C_{mc,Mb} W_{mc} \)). The parameters of the Hb and Mb saturation relationship are reported in Table 4.2 (Hogan et al., 1992; Lai, Saidel, et al., 2007; Richardson et al., 1998).

4.2.5. Composition of the NIRS signal

The oxygenated Hb and Mb in the microvascular and tissue volumes are used to quantify the oxygenated Hb (\( HbO_2 \)) and Mb (\( MbO_2 \)) heme group concentrations in muscle as:
\[ HbO_2 = f_{b,m} \left[ C_{O_2,art}^B \omega_{art} + C_{O_2,cap}^B \omega_{cap} + C_{O_2,ven}^B \omega_{ven} \right] / 4 = f_{b,m} (\Delta C_{Heme,N}^{exp}) C_{O_2,m}^B / 4 \]

\[ MbO_2 = f_t C_{O_2,t}^B / 4 \]

where \( f_{b,m} \) and \( f_t \) are the microvascular and tissue volume fractions in muscle, \( C_{O_2,m}^B \) is the bound O2 concentration in the microvascular blood and \( C_{O_2,cap}^B \) and \( C_{O_2,t}^B \) can be computed as the spatial average of the bound O2 concentration in capillaries and tissue, respectively.

The deoxygenated Hb (\( HHb \)) and Mb (\( HMb \)) heme group concentrations in muscle are computed as:

\[ HHb = f_{b,m} (\Delta C_{Heme,N}^{exp}) C_{b,Hb} - HbO_2 \]

\[ HMb = f_t C_{t,Mb}/4 - MbO_2. \]

The oxygenated (\( \Delta HbMbO_2 \)) and deoxygenated (\( \Delta HHbMb \)) NIRS signal changes relative to resting condition are computed as follows:

\[ \Delta HbMbO_2 = HbMbO_2 - HbMbO_2^R = HbO_2 + MbO_2 - HbO_2^R - MbO_2^R \]

\[ \Delta HHbMb = \Delta HHb + \Delta HMb - \Delta HHb^R - \Delta HMb^R. \]

The steps to calculate the \( f_{b,m} \) from NIRS measurement (\( \Delta C_{Heme,N}^{exp} \)) and \( V_{cap} \) from \( f_{b,m} \) are reported in Figure 4.3. When blood volume changes are considered, \( f_{b,m} \) is a function of the relative heme concentration changes (\( \Delta C_{Heme,N}^{exp} \)) detected by the NIRS signals as

\[ f_{b,m} = f_{b,m} (\Delta C_{Heme,N}^{exp}) = f_{b,m}^R + \frac{\Delta C_{Heme,N}^{exp} HbMbO_2^R}{(C_{b,Hb} - C_{t,Mb}/4)}. \]
\( \Delta C_{\text{Heme}, N}^{\text{exp}} \) is calculated from the oxygenated and deoxygenated NIRS measurements:

\[
\Delta C_{\text{Heme}, N}^{\text{exp}} = \frac{\Delta HbMbO_2^{\text{exp}} + \Delta HbMbO_2^{\text{eq}}}{\Delta HbMbO_2^{\text{eq}}}.
\]

4.16

Derivation of these equations is reported in section 4.2.7. The blood volume changes are attributed to the capillary compartment \( (V_{\text{cap}}) \) whereas arteriole and venule volumes are the same as those at rest (Table 4.1). \( f_{b,m} \) is used to quantify \( V_{\text{cap}} \) and the arteriole, capillary, and venule volume fractions \( (\omega_{\text{art}}, \omega_{\text{cap}}, \text{and } \omega_{\text{ven}}) \). Details to calculate the arteriole, capillary, and venule contributions to the NIRS signals are reported in Section 4.2.7.

4.2.6. Simulation strategy

The mathematical model of O2 transport and utilization requires specific inputs to simulate the outputs expected of the computational analysis (Figure 4.3). The model inputs that reflect the experimental conditions of each trial reported in Figure 4.1 are: 1) extravascular and microvascular volume distribution at rest and for blood volume changes; 2) metabolic rate \( (V\dot{O}_2) \) to estimate \( k_{\text{ATPase}} \) with the expression reported in Figure 4.3 (Lai, Saidel, et al., 2007); 3) muscle blood flow \( (Q) \) and arterial O2 partial pressure \( (P_{O_2}) \) for each trial (T1, T3) condition. Once the inputs are defined, the system of mass balance equations described at the beginning of the methods section (Eq. 4.1, 4.2) can be solved to generate the model outputs (Figure 4.3).

For each trial, to compute the NIRS signal changes relative to resting condition, the model simulations were obtained at rest and then for different blood flow with or without contraction. At rest and in the absence of blood volume changes, the extravascular and microvascular volume distributions are independent of the input conditions (i.e., arterial O2 concentration, blood flow).
and reported in Table 4.1; in the presence of blood volume changes the distribution is estimated from the NIRS measurement with the equation reported in Figure 4.3 (orange inputs). Similarly, $k_{ATPase}$ that represents the rate coefficient associated with the ATPase flux is estimated at rest and for each experimental condition investigated.

The value of the parameters for Hb and Mb saturation relationship reported in Table 4.2 are independent from the experimental conditions. The rest of the parameter values of this model are available from previous studies (Lai, Saidel, et al., 2007; Spires et al., 2013; Spires et al., 2012).

The experimental data compared with model simulations, are the rate of muscle O$_2$ uptake determined as:

$$\dot{V}O_2 = Q \left[ C_{art}^T - C_{ven}^T \right]/V_{mus}$$  \hspace{0.5cm} 4.17

and the normalized oxygenated ($\Delta HbMbO_2, N$) and deoxygenated ($\Delta HHbMb, N$) NIRS measurements. The $\Delta HbMbO_2$ and $\Delta HHbMb$ measurements are normalized to the maximal variation of the oxygenated and deoxygenated NIRS signals observed during blood flow occlusion of trial 5 (T5) in (Sun et al., 2016), respectively:

$$\Delta HbMbO_{2,N} = \frac{\Delta HbMbO_2}{\Delta HbMbO_{2,T5}}, \hspace{0.5cm} \Delta HHbMb_{N} = \frac{\Delta HHbMb}{\Delta HHbMb_{T5}}.$$  \hspace{0.5cm} 4.18

The strategy to calculate $\Delta HbMbO_{2,T5}$ and $\Delta HHbMb_{T5}$ is reported in section 4.2.9. Also, the simulated oxygenated and deoxygenated NIRS signals are computed with the Hb and Mb contribution as

$$\Delta HbMbO_{2,N} = \frac{\Delta HbMbO_2}{\Delta HbMbO_{2,T5}} = \frac{(HbO_2^T - HbO_2^R) + (MbO_2^T - MbO_2^R)}{(HbO_{2,T5} - HbO_2^R) + (MbO_{2,T5} - MbO_2^R)}$$  \hspace{0.5cm} 4.19

$$\Delta HHbMb_{N} = \frac{\Delta HHbMb}{\Delta HHbMb_{T5}} = \frac{(HHb^T - HHb^R) + (HMb^T - HMb^R)}{(HHb_{T5}^T - HHb^R) + (HMb_{T5}^T - HMb^R)}$$  \hspace{0.5cm} 4.20
where Hb and Mb contributions are computed at steady state for resting ($R$), blood flow occlusion ($T5$) and each trial (T1 resting, T3 contraction) condition. It should be noted that T5 indicates the trial corresponding to the blood flow occlusion condition (Sun et al., 2016); i.e., zero blood flow.

The mathematical model is used to simulate $\Delta HbMbO_2$ and $\Delta HHbMb$ obtained in the absence (no blood volume, $nbv$) or in the presence of blood volume changes (with blood volume, $wbv$) represented by a variation of the heme group concentration ($\Delta HbMb$). The relative contribution of blood volume changes to the amplitude of $\Delta HbMbO_2$ and $\Delta HHbMb$ is calculated as

\[
y-\text{HbMbO}_{2,\text{bv}} = \frac{\Delta HbMbO_{2}^{nbv} - \Delta HbMbO_{2}^{wbv}}{\Delta HbMbO_{2}^{nbv}} \quad 4.21
\]
\[
y-\text{HHbMb}_{\text{bv}} = \frac{\Delta HHbMb^{nbv} - \Delta HHbMb^{wbv}}{\Delta HHbMb^{nbv}} \quad 4.22
\]

### 4.2.7. Microvascular blood volume

The oxygenated and deoxygenated NIRS measurements are integrated in the mathematical model to quantify microvascular blood volume changes associated with the heme concentration changes detected by NIRS. For each physiological condition investigated, it is assumed that the measured ratio between blood volume changes relative to rest (sum of the oxygenated and deoxygenated NIRS measurements) and those observed under occlusion for trial $T5$ (maximal oxygenated signal change: $\Delta HbMbO_{2,\exp}^{T5}$) are the same as those used in the mathematical model to simulate the ratio between heme group concentration changes in the microvascular ($\Delta C_{Hb}$) and extravascular ($\Delta C_{Mb}$) compartments and oxygenated Hb and Mb changes during occlusion:

\[
\Delta C_{\text{Heme,}N}^{\text{exp}} = \frac{\Delta HbMbO_{2,\exp}^{T5} + \Delta HHbMb_{\exp}^{T5}}{\Delta HbMbO_{2,\text{exp}}^{T5}} = \Delta C_{\text{Heme,}N}^{\text{mod}} = \frac{\Delta C_{Hb} + \Delta C_{Mb}}{HbMbO_{2}^{R} - HbMbO_{2}^{T5}}. \quad 4.23
\]
The relative Hb and Mb concentration changes can be related to the microvascular blood volume fraction:

\[ \Delta C_{Hb} = \Delta f_{b,m} C_{b,Hb}; \quad \Delta C_{Mb} = \frac{\Delta f_{t,Mb}}{4} \]  \hspace{1cm} (4.24)

\[ \Delta C_{Hb} + \Delta C_{Mb} = (f_{b,m} - f_{b,m}^R) C_{b,Hb} + \frac{(f_t - f_t^R) C_{t,Mb}}{4} \]  \hspace{1cm} (4.25)

\[ \Delta C_{Hb} + \Delta C_{Mb} = (f_{b,m} - f_{b,m}^R)(C_{b,Hb} - C_{t,Mb}/4). \]  \hspace{1cm} (4.26)

Combining Eq. 4.23 with Eq. 4.26 yields

\[ f_{b,m}(\Delta C_{\text{Heme},N}) = f_{b,m}^R + \frac{\Delta C_{\text{Heme},N}(HbMbO_2^R - HbMbO_2^T)}{(C_{b,Hb} - C_{t,Mb}/4)}. \]  \hspace{1cm} (4.27)

The microvascular volume fraction in muscle \((f_{b,m})\) at rest is calculated by the product of the blood volume fraction in muscle \((f_b)\) and microvascular volume fraction in blood \((f_m)\) at rest. Since \(f_{b,m}\) changes are assumed to take place in capillaries, the volume change takes place in the microvascular compartment \((V_{b,m})\):

\[ f_{b,m} = \frac{V_{b,m}}{V_t + V_{b,m} + V_{b,v}}. \]  \hspace{1cm} (4.28)

The \(f_{b,m}\) change from rest \((R)\) to each trial condition investigated \((T1, T3, \text{and} T5)\) is computed as

\[ V_{b,m} = V_{b,m}^R + \Delta V_{\text{cap}} = V_{b,m}^R + V_{\text{cap}} - V_{\text{cap}}^R. \]  \hspace{1cm} (4.29)

When blood volume changes occur, the extravascular \((V_t)\), vascular \((V_{b,v})\), as well as arteriole \((V_{\text{art}})\) and venules \((V_{\text{ven}})\) volume remains constant at the resting value (See 4.1). Equation 4.28 and 4.29 can be rearranged as:

\[ V_{\text{cap}} = \frac{f_{b,m}(V_t + V_{b,v})}{(1 - f_{b,m})} - [V_{b,m}^R - V_{\text{cap}}^R] = \frac{f_{b,m}(V_t + V_{b,v})}{(1 - f_{b,m})} - [V_{\text{art}} + V_{\text{ven}}]. \]  \hspace{1cm} (4.30)
Once the capillary volume is determined, the total microvascular volume can be calculated \( V_{b,m} = V_{art} + V_{cap} + V_{ven} \) and thus, the arteriole, capillary, and venule volume fractions as:

\[
\omega_{art} = \frac{v_{art}}{v_{b,m}}; \quad \omega_{cap} = \frac{v_{cap}}{v_{b,m}}; \quad \omega_{ven} = \frac{v_{ven}}{v_{b,m}}.
\]

### 4.2.8. Arteriole, capillary, and venule contributions to the oxygenated and deoxygenated NIRS signals

The oxygenated Hb (Eq. 4.9) can be expressed in terms of the individual arteriole, capillary, and venule contributions:

\[
HbO_2 = HbO_{2,art} + HbO_{2,cap} + HbO_{2,ven}
\]

where each contribution can be computed as

\[
HbO_{2,art} = f_{b,m}(\Delta C^\text{exp}_{\text{Heme,N}}) C^B_{O2,art} \omega_{art}/4
\]

\[
HbO_{2,cap} = f_{b,m}(\Delta C^\text{exp}_{\text{Heme,N}}) < C^B_{O2,cap} > \omega_{cap}/4
\]

\[
HbO_{2,ven} = f_{b,m}(\Delta C^\text{exp}_{\text{Heme,N}}) C^B_{O2,ven} \omega_{ven}/4.
\]

The deoxygenated Hb (Eq. 4.11) can be expressed in terms of the individual arteriole, capillary, and venule contributions:

\[
HHb = HHb_{art} + HHb_{cap} + HHb_{ven}
\]

where each contribution can be computed as

\[
HHb_{art} = f_{b,m}(\Delta C^\text{exp}_{\text{Heme,N}}) \omega_{art} \left[ C_{b,Hb} - \frac{C^B_{O2,art}}{4} \right]
\]
\[ HHb_{cap} = f_{b,m}(\Delta C_{Heme,N}^{exp}) \omega_{cap} \left[ C_{b,Hb} - \frac{C_{O_{2,2,\text{cap}}}}{4} \right] \]  
\[ HHb_{ven} = f_{b,m}(\Delta C_{Heme,N}^{exp}) \omega_{ven} \left[ C_{b,Hb} - \frac{C_{O_{2,2,\text{ven}}}}{4} \right] \]

4.2.9. Trial 5: Blood flow occlusion

To compare published NIRS experimental data (Sun et al., 2016) with those simulated, the oxygenated (\(\Delta HbMbO_2\)) and deoxygenated (\(\Delta HHbMb\)) NIRS signals were normalized to the NIRS signal changes obtained with blood flow occlusion (\(\Delta HbMbO_2^{T5}\) and \(\Delta HHbMb^{T5}\), T5), respectively. The NIRS signals, \(\Delta HbMbO_2^{T5}\) and \(\Delta HHbMb^{T5}\), previously measured in canine muscle (Sun et al., 2016) during occlusion (-148.8±12.03, 78.3±6.58µM), was used in Eq. 4.27 to estimate the microvascular volume fraction \(f_{b,m}\) at occlusion. In Eq. 4.27, \(HbMbO_2^{T5}\) is assumed to be negligible at the end of the occlusion. Once \(f_{b,m}\) was estimated for ischemia, the vascular, extravascular and arteriole, capillary and venule volume fractions were calculated with Eq. 4.30 and 4.31 and reported in Table 4.1.

4.3. Results

The effects of muscle blood flow \(Q\) and volume \(\Delta HbMb\) on the NIRS signals were analyzed by comparing simulated and experimental data of blood and tissue oxygenation at rest (T1) and during contraction (T3) under steady-state conditions (Sun et al., 2016) (Figure 4.1). To accomplish this goal a computational model of \(O_2\) transport and utilization was used to simulate the oxygenation in the microvascular and extravascular compartments of skeletal muscle.
4.3.1. Estimate of microvascular blood volume change

The mathematical model relied on the estimate of the microvascular blood volume distribution to simulate the effects of blood volume changes on NIRS signals. Thus, in the absence of blood volume changes, the $f_{b,m}$ and microvascular volume distribution were the same for all trials at different blood flows whereas during occlusion $f_{b,m}$ was estimated from NIRS measurements (see Table 4.1). The details to estimate $f_{b,m}$ and microvascular volume distribution during ischemia are reported in section 4.2.9. The concentration of oxygenated ($HbO_2, MbO_2$) and deoxygenated ($HHb, HMb$) heme groups in the microvascular and extravascular compartments were calculated at rest and occlusion (Table 4.3) by Eq. (4.9-4.10) and (4.11-4.12). These concentrations and microvascular volume distribution were used to determine the simulated $\Delta HbMbO_2^{T5}$ and $\Delta HHbMb^{T5}$ which were -239.4 and 132.9 µM, respectively. The simulated $\Delta HHbMb^{T5}/\Delta HbMbO_2^{T5}$ ratio (55%) was similar to that determined with the experimental data (52%) of the NIRS signals (Sun et al., 2016) ($\Delta HbMbO_2^{T5}$=-148.8±12.03 µM, $\Delta HHbMb^{T5}$=78.3±6.58 µM). $\Delta HbMbO_2^{T5}$ and $\Delta HHbMb^{T5}$ were used to normalize the NIRS signals. The normalized oxygenated and deoxygenated heme group concentrations are indicated as $\Delta HbMbO_{2,N}$ and $\Delta HHbMb_{N}$. 
Table 4.1: Vascular, extravascular, and microvascular volume distributions at rest and ischemia.

<table>
<thead>
<tr>
<th>Notation</th>
<th>Description</th>
<th>Unit</th>
<th>Rest (R)</th>
<th>Ischemia (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{mus}$</td>
<td>Volume of muscle</td>
<td>[g]</td>
<td>62.5</td>
<td>62.5</td>
</tr>
<tr>
<td>$f_b$</td>
<td>Volume fraction of the blood in muscle</td>
<td>[%]</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>$f_t$</td>
<td>Volume fraction of tissue in muscle</td>
<td>[%]</td>
<td>93</td>
<td>97</td>
</tr>
<tr>
<td>$f_m$</td>
<td>Volume fraction of microvascular blood in blood</td>
<td>[%]</td>
<td>85</td>
<td>63.4</td>
</tr>
<tr>
<td>$f_{b,m}$</td>
<td>Volume fraction of microvascular blood in muscle</td>
<td>[%]</td>
<td>5.95</td>
<td>1.9</td>
</tr>
<tr>
<td>$\omega_{art}$</td>
<td>Volume fraction of arterioles in microvascular blood</td>
<td>[%]</td>
<td>10</td>
<td>32.7</td>
</tr>
</tbody>
</table>

Table 4.2 Parameters of the Hb and Mb saturation relationship for blood and tissue, respectively.

<table>
<thead>
<tr>
<th>Notation</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_{O_2}$</td>
<td>$1.34 \times 10^{-3}$</td>
<td>[mM mmHg$^{-1}$]</td>
<td>(Hogan et al., 1992)</td>
</tr>
<tr>
<td>$K_{Hb}$</td>
<td>7210.9</td>
<td>[mM]$^a$</td>
<td>(Lai, Saidel, et al., 2007)</td>
</tr>
<tr>
<td>$K_{Mb}$</td>
<td>308.6</td>
<td>[mM]$^{-1}$</td>
<td>(Lai, Saidel, et al., 2007)</td>
</tr>
<tr>
<td>$n$</td>
<td>2.8</td>
<td>[-]</td>
<td>(Richardson et al., 1998)</td>
</tr>
<tr>
<td>$C_{c,Mb}$</td>
<td>0.32</td>
<td>[mM]</td>
<td>(Lai, Saidel, et al., 2007)</td>
</tr>
<tr>
<td>$C_{b,Hb}$</td>
<td>2.6-2.9</td>
<td>[mM]</td>
<td>This work</td>
</tr>
</tbody>
</table>
Table 4.3 Oxygenated and deoxygenated heme group concentrations of Hb and Mb at rest and ischemia.

<table>
<thead>
<tr>
<th>Notation</th>
<th>Unit</th>
<th>Rest (R)</th>
<th>Ischemia (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$HbO_2$</td>
<td>[μM]</td>
<td>167.1</td>
<td>0</td>
</tr>
<tr>
<td>$MbO_2$</td>
<td>[μM]</td>
<td>72.3</td>
<td>0</td>
</tr>
<tr>
<td>$HHb$</td>
<td>[μM]</td>
<td>4.2</td>
<td>54.7</td>
</tr>
<tr>
<td>$HMb$</td>
<td>[μM]</td>
<td>2.7</td>
<td>78.2</td>
</tr>
<tr>
<td>$HbMb$</td>
<td>[μM]</td>
<td>246.3</td>
<td>132.9</td>
</tr>
</tbody>
</table>

For both T1 (Figure 4.4a) and T3 (Figure 4.4d), the muscle microvascular volume changes detected by NIRS measurements ($\Delta c_{\text{Heme},N}^{\text{exp}}$) were used to compute the changes in total heme groups concentration ($\Delta HbMb$) and $f_{b,m}$, by Eq. 4.23 and 4.15, respectively.
Figure 4.4. Microvascular volume changes: simulated (solid lines) and experimental data (open circle, by NIRS) of the relative heme group changes of Hb/Mb concentration ($\Delta C_{\text{Heme},N}$, black line), estimated heme group concentration changes ($\Delta HbMb$, grey line) with blood flow for (a) T1 and (d) T3; estimate of microvascular volume fraction ($f_{b,m}$) and capillary ($\omega_{\text{cap}}$) and venule ($\omega_{\text{ven}}$) volume fraction changes with blood flow for (b and c) T1 and (e and f) T3.

In general, for a $\Delta C^\text{exp}_{\text{Heme},N}$ increase of 10%, simulations predicted an increase of $\Delta HbMb$ and $f_{b,m}$ of 24µM (Figure 4.4a, 4.4d, grey line) and 1% (Figure 4.4b, 4.4e), respectively. These
changes were followed by 5.2% increase of capillary ($\omega_{cap}$) and 2.6% decrease of the arteriole ($\omega_{art}$) and venule ($\omega_{ven}$) volume fractions (Figure 4.4c, 4.4f). Although the volume changes are only related to $V_{cap}$, the extravascular, arteriole and venule volume fraction can change.

4.3.2. Model validation

The capability of the mathematical model to reproduce the experimental data was tested by comparing simulated and experimental data of muscle O$_2$ uptake ($\dot{V}O_2$) and O$_2$ concentration in muscle (i.e., NIRS) with different blood flow ($Q$) (Figure 4.5). The overall strategy was to simulate the NIRS signals of the muscle at rest and during contraction in the presence of blood volume changes (solid line) to mirror those detected by NIRS measurements and then repeat the simulations in the absence of blood volume changes (dashed line). For each trial, the model input ($k_{ATPase}$) associated with the energy demand (Figure 4.5a, 4.5d, grey line) was varied linearly to simulate the changes in $\dot{V}O_2$ (black line), oxygenated NIRS signals ($\Delta HbMbO_{2,N}$) (Figure 4.5b, 4.5e) and deoxygenated NIRS signals ($\Delta HHbMb_N$) (Figure 4.5c, 4.5f) with blood flow observed for T1 and T3. When the microvascular blood volume changes quantified in Figure 4.5 are considered the model predicted well the changes in $\Delta HbMbO_{2,N}$ and $\Delta HHbMb_N$ for T1 and T3 with changes in $Q$. For both T1 and T3, the higher the O$_2$ delivery, the lower the amplitude of the NIRS signal changes.
Figure 4.5. Model inputs: simulated and experimental data (open circle) of muscle oxygen uptake \(\dot{V}O_2\), black line) and rate coefficient \(k_{ATPase}\), grey line) associated to the metabolic rate to simulate \(\dot{V}O_2\) for (a) T1 and (c) T3 trials. Model validation for different blood flow \(Q\) in T1 and T3: comparison between model prediction with (solid line) and without (dashed line) blood volume \(\Delta HbMb < 0\) and experimental data (open circle) of oxygenated (panel b for T1; and panel e for T3; \(\Delta HbMbO_{2,N}\)) and deoxygenated (panel c for T1; and panel f for T3; \(\Delta HHbMb_N\)) NIRS signals.
4.3.3. Effect of blood flow on the components of the NIRS signal in absence of blood volume change

After the mathematical model validation, the effect of blood flow and volume on the components of the NIRS signals was further investigated (Figure 4.6-4.8). Specifically, the analysis focused on whether the oxygen saturation ($SO_2$) in the microvascular and extravascular compartments was a key factor in determining the amplitude of the NIRS signal changes for blood flow or volume changes. Thus, the concentration of heme forms in arteriole, capillary, venule ($\Delta HbO_2, \Delta HHb$) and in myocytes ($\Delta MbO_2, \Delta HMb$) contributing to the relative changes of the oxygenated ($\Delta HbMbO_2$) and deoxygenated ($\Delta HHbMb$) heme groups were simulated in the absence (dashed line) or presence (solid line) of volume changes for T1 (Figure 4.6a, b, c) and T3 (Figure 4.6d, e, f). For all trials, the simulation of $SO_2$ in the microvascular and tissue compartments for different $Q$ was not affected by blood volume changes for T1 (Figure 4.6a) and T3 (Figure 4.6d).
Figure 4.6. Blood and tissue oxygenation predictions with (solid line, BV variable) and without (dashed line, BV constant) blood volume changes for different $Q$: simulation of $O_2$ saturation ($SO_2$) in the microvascular (red, green, and blue refer to arterioles, capillaries, venules) and myocyte compartments (black) for (a) T1 and (d) T3; simulation of the relative changes of oxygenated ($\Delta HbO_2$, $\Delta MbO_2$) and deoxygenated ($\Delta Hb$, $\Delta Mb$) Hb and Mb components of the NIRS signals from the base line for (b and c) T1 and (e and f) T3. The green arrows indicate the effect of a decrease of blood volume on the NIRS components ($\Delta HbMb < 0$).
For T1, in the absence of blood volume, simulations obtained with an increase of $Q$, predicted a nonlinear increase of $SO_2$ (Figure 4.6a). The simulated relative oxygenated heme group concentration (dashed line) of Hb ($\Delta HbO_2$) and Mb ($\Delta MbO_2$) (Figure 4.6b) and that of the deoxygenated heme group of Hb ($\Delta Hb$) and Mb ($\Delta Mb$) (Figure 4.6c) decreased nonlinearly with an increase of $Q$. All variables began to reach a plateau for $Q$ greater than 0.1-0.2 L kg$^{-1}$ min$^{-1}$.

For T3, in the absence of blood volume, with an increase of $Q$, simulations predicted a quasi-linear increase of $SO_2$ (Figure 4.6d) and a decrease of $\Delta HbO_2$ (Figure 4.6e) and $\Delta Hb$ (Figure 4.6f) (dashed line) amplitudes in capillary and venule compartments. Only myocytes presented a non-linear relationship between $Q$ and $SO_2$ and between $Q$ and $\Delta MbO_2$. For both capillaries and venule compartments, the effects of $Q$ on $\Delta HbO_2$ change was like that of $\Delta Hb$ change but of the opposite sign.

For both trials, the arterioles’ contribution (red line) to the NIRS signals are negligible because two concomitant conditions: the arterioles’ oxygen saturation is unaltered (Figure 4.6a and d) and the changes of arteriole blood volume fraction in the muscle are negligible ($f_{b,m} \omega_{art} < 0.01\%$, for different blood flow with (Figure 4.4e and f) or without contraction (Figure 4.4b and c)).

### 4.3.4. Effect of blood volume on NIRS signals

The microvascular volume fraction changes quantified in Figures 4.4b and 4.4e were included in the mathematical model to quantify the effects of blood volume changes on the components of the NIRS signals (solid vs. dashed line) for T1 (Figure 4.6b and c) and T3 (Figures
4.6e and 4.6f). In general, the capillary domain was the main compartment (green line) affected by blood volume changes. Thus, capillary volume was responsible for both oxygenated and deoxygenated NIRS changes related to blood volume changes. Also, the capillary $\Delta HbO_2$ was more sensitive than the capillary $\Delta HHHb$ to the blood volume changes for different blood flow in T1 and T3.

In T1, the effect of blood volume changes (solid line) on capillary $\Delta HbO_2$ was significant whereas it was negligible on $\Delta HHHb$. Under this condition, the O$_2$ saturation in the capillary was above 90% (Figure 4.6a) for $Q$ greater than that at rest (0.2 L kg$^{-1}$ min$^{-1}$). In T3, the effect of blood volume on capillary $\Delta HbO_2$ (Figure 4.6e) was greater than that for $\Delta HHHb$ (Figure 4.6f), but the effect on $\Delta HHHb$ was not negligible. Specifically (for $Q=1$ L kg$^{-1}$ min$^{-1}$), $\Delta HbO_2$ amplitude increased from approximately -45 (no blood volume changes) to -70 $\mu$M whereas that of $\Delta HHHb$ decreased from 45 to 30 $\mu$M. The amplitude of $\Delta HbO_2$ increased and that of $\Delta HHHb$ remains almost constant with a decrease of capillary $SO_2$ (Figure 4.3d). For the range of $Q$ investigated, the capillary $SO_2$ was lower than 70%.

To further investigate the distinct effect of blood volume on oxygenated and deoxygenated NIRS components, capillary $\Delta HbO_2$ and $\Delta HHHb$ were simulated at rest (T1, Figure 4.7a) and during contraction (T3, Figure 4.7b) for different capillary $SO_2$ obtained varying the oxygen delivery ($Q= [0.01-4]$ L kg$^{-1}$ min$^{-1}$). The $\Delta HbO_2$ and $\Delta HHHb$ simulations were performed in the absence and in the presence of an increase of blood volume corresponding to a relative change of the concentration of the heme groups ($\Delta HbMb$) of 24 $\mu$M and 48 $\mu$M. For both T1 and T3, the amplitude of $\Delta HbO_2$ (grey line) and $\Delta HHHb$ (black line) increased with a decrease of $SO_2$. The effect of blood volume changes on $\Delta HHHb$ is comparable to that on $\Delta HbO_2$ at low $SO_2$ and was negligible for $SO_2$ above 85% (Figure 4.7a). In contracting muscle (T3), under normal delivery
(i.e., $Q=1.0$ L kg$^{-1}$ min$^{-1}$) corresponding to a $SO_2$ of 62%, an increase of blood volume equivalent to a $\Delta HbMb = 24$ μM caused a $\Delta HbO_2$ change of 16 μM from -41 (no blood volume change) to -25 μM (Figure 4.7b); i.e., almost twice as much as that observed for $\Delta Hb$ from 41 (no blood volume change) to 50 μM, Figure 4.7b.

Figure 4.7. Relative changes of capillary oxygenated ($\Delta HbO_2$) and deoxygenated ($\Delta Hb$) concentrations from a base line for different capillary oxygen saturation ($SO_2$) obtained varying muscle blood flow for (a) T1 (0.01-0.5 L kg$^{-1}$ min$^{-1}$) and (b) T3 (0.5-4.0 L kg$^{-1}$ min$^{-1}$). Simulations were obtained in absence of microvascular blood volume changes ($\Delta HbMb = 0$ μM) and in presence of an increase of blood volume equivalent to a heme group concentration variation ($\Delta HbMb$) of 24 μM or 48 μM. The black and grey arrows indicate the effect of an increase of blood volume on the NIRS components ($\Delta HbMb > 0$).
Additionally, for the experimental conditions investigated in Figure 4.7b, we also reported in Figure 4.8 the ΔHbMbO₂ changes (ΔHbMbO₂<sup>wbv</sup>, with blood volume changes) relative to ΔHbMbO₂ simulated in the absence of blood volume changes (ΔHbMbO₂<sup>nsv</sup>) using Eq. 4.21 (y-HbMbO₂<sub>2,bv</sub>). Similarly, the ΔHHbMb changes (ΔHHbMb<sup>wbv</sup>) relative to ΔHHbMb obtained in the absence of blood volume changes (ΔHbMbO₂<sup>nsv</sup>) were computed by Eq. 4.22 (y-HHbMb<sub>2,bv</sub>). We related these contributions to venous oxygen saturation to facilitate the comparison with physiological conditions defined by measurable variables. The y-HbMbO₂<sub>2,bv</sub> was more sensitive than y-HHbMb<sub>2,bv</sub> to a decrease of venous oxygen saturation (i.e., oxygen delivery). Under normal delivery (i.e., Q=1.0 L kg⁻¹ min⁻¹) corresponding to a venous oxygen saturation SO₂ of 34% (or capillary SO₂=62%), a blood volume increase (ΔHbMb=24 μM) determined a ΔHbMbO₂ decrease equivalent to almost 20% of ΔHbMbO₂ without blood volume change and an ΔHHbMb increase equivalent to 10% of ΔHHbMb without blood volume change. y-HbMbO₂<sub>2,bv</sub> and y-HHbMb<sub>2,bv</sub> decreased almost linearly with a decrease of venous oxygen saturation, and they had a similar variation (10% and -10%) for reduced oxygen delivery.
Figure 4.8. Contribution of blood volume changes to the $\Delta HbMbo_2$ ($y-HbMbo_2, bv$) and $\Delta HbMb$ ($y-HbMb_{bv}$) amplitudes with venous oxygen saturation ($SO_2$) obtained varying muscle blood flow for T3 for the same range reported in Figure 3.6.

4.4. Discussion

A mathematical model of muscle $O_2$ transport and metabolism was validated (Figure 4.5) with experimental data (Sun et al., 2016) obtained under different experimental conditions and was used to quantify the effects of blood flow and volume on oxygenated and deoxygenated NIRS signal changes. The primary finding of this study was that microvascular blood volume changes estimated from the total heme group concentration measured by NIRS can significantly affect both oxygenated and deoxygenated NIRS signals during contraction in the absence of skin blood flow. Our analysis confirms the hypothesis proposed. When skeletal muscle perfusion is adequate, the oxygenated signal is more sensitive than the deoxygenated signal to blood volume changes. Nevertheless, the deoxygenated signal is also significantly affected by the blood volume changes.
In the presence of impaired oxygen delivery with a consequent low microvascular oxygen saturation, both NIRS signals are similarly affected by blood volume changes. Accordingly, blood volume changes should be considered when the oxygenated or deoxygenated NIRS signals are used to quantify O₂ extraction and consumption.

4.4.1. Blood flow and volume effect on NIRS signals

Capillary recruitment (Clark et al., 2008), or expansion of already-recruited capillaries (Poole et al., 2008), skin blood flow and capillary hematocrit changes (Grassi & Quaresima, 2016) during muscle contraction can contribute to a microvascular volume change. This blood volume change is considered to have smaller effects on the deoxygenated than the oxygenated signal (Grassi & Quaresima, 2016); thus, the deoxygenated signal is generally preferred to the oxygenated signal. However, a human study has reported that the deoxygenated signal is also affected by blood volume and flow changes (Adami et al., 2015). In an animal study the blood flow effect on the deoxygenated NIRS signal differed from that on the oxygenated NIRS signal (Sun et al., 2016), but the NIRS signal’s sensitivity to blood volume changes could not be determined. To infer the cause for this different sensitivity under different conditions, model simulations were obtained in the absence or presence of blood volume changes (Figure 4.6 and 4.7). Thus, our approach overcomes experimental limitations, providing the opportunity to distinguish between blood volume and flow effects on the NIRS signal.

In T1, the blood volume change had an additional effect on ΔHbMbO₂,N (Figure 4.5b). This effect was mainly related to the capillary ΔHbO₂ (Figure 4.6b) due to an increase in \( f_{b,m} \) and \( \omega_{cap} \) (Figure 4.4b, 4.4c) which contributed to the amplitude of the NIRS signal. In contrast, \( f_{b,m} \)
effects on $\Delta HHbMb_N$, were not evident (Figure 4.5c) because $\Delta HHb$ and $\Delta HMb$ concentrations were small (Figure 4.6c) due to the high O$_2$ saturation of $Hb$ and $Mb$ (Figure 4.6a) related to the abundant O$_2$ delivery. The sensitivity of the oxygenated NIRS signal differed from the deoxygenated signal because capillary $HbO_2$ was four-fold greater than $HHb$ (data not reported). $SO_2$ in the microvascular (i.e., Hb) compartments appears to be a key factor in determining the NIRS signal amplitude in presence of blood volume changes. Consistent with this view, during contraction (T3) when capillary oxygen saturation dropped to less than 70%, the capillary $HbO_2$ concentration was comparable to that of $HHb$; thus, the effect of blood volume on $\Delta HbO_2$ (Figure 4.6e) and $\Delta HHb$ changes (Figure 4.6f) were both significant although the effect on $\Delta HbO_2$ was 40% greater than that on $\Delta HHb$. Although the venule oxygen saturation was also low ($SO_2 < 50\%$, Figure 4.6d), the blood volume effect was not observed in this compartment (blue line, Figure 4.6e and 4.6f) because its volume fraction was smaller than that of the capillaries (12% vs. 77%). Furthermore, our analysis indicates that a decrease in blood volume, as for T3, would determine a higher $\Delta HbMbO_2$ amplitude than that of $\Delta HHbMb$ (Figure 4.6e, 4.6f). This inference is consistent with a greater $\Delta HbMbO_2$ than $\Delta HHbMb$ amplitude obtained from NIRS measurements in contracting canine muscle (Sun et al., 2016). Specifically, $\Delta HbMbO_2$ was 50 $\mu$M whereas $\Delta HHbMb$ was 37 $\mu$M for $Q=1.0$ L kg$^{-1}$ min$^{-1}$.

Because capillary O$_2$ saturation determines the $HbO_2$ and $HHb$ concentrations, it is the key factor in determining the sensitivity of the oxygenated and deoxygenated NIRS signals to blood volume changes. This finding was further supported by an enhanced sensitivity of $\Delta HHb$ to blood volume increase (Figure 4.7) for a decrease of $Q$. Specifically, when $Q$ was decreased, capillary O$_2$ saturation (Figure 4.7) decreased with a consequent increase of $\Delta HHb$ and increase of blood volume effects on the deoxygenated Hb concentration changes from rest (Figure 4.7b). The
different sensitivity of the capillary oxygen saturation on the two NIRS signals observed in our simulations is consistent with the NIRS study on passive head-up tilt (Adami et al., 2015). In this NIRS study, consistently with the primary role of capillary oxygen saturation suggested by our analysis, the \( HHbMb \) contribution to the blood volume change increase with a decrease in the oxygen saturation induced by a reduced muscle oxygen delivery.

Our results indicate that in contracting muscle with normal or reduced \( O_2 \) delivery representing physiological or pathophysiological conditions (Grassi et al., 2019; David C. Poole et al., 2011), the effect of blood volume on the deoxygenated signal should be quantified when NIRS signals are used to derive information about muscle \( O_2 \) transport and utilization. These findings are consistent with the view that the deoxygenated NIRS signal is reflecting the \( O_2 \) extraction only when there is no blood volume change (Quaresima & Ferrari, 2009). The integrative approach proposed in this work provides for the first time a strategy based on a theoretical framework to correct both oxygenated and deoxygenated NIRS measurements for blood volume changes in protocol with spontaneous blood flow. These corrections are expected to affect the kinetics of the NIRS signals in normal and impaired skeletal muscle perfusion.

At the onset of muscle contraction, the kinetics of the deoxygenated NIRS signal increases exponentially while the oxygenated NIRS signal often shows an undershoot (Jones et al., 2009; Kowalchuk‡ et al., 2002; Lai et al., 2009) accompanied by an increase of blood volume associated with the increase of the sum of oxygenated and deoxygenated signals. An interpretation of this finding is to consider the oxygenated signal affected and the deoxygenated signal unaffected by the blood volume change (Jones et al., 2009; Kowalchuk‡ et al., 2002). Nevertheless, this interpretation lacks robust experimental evidence. Quantitative analysis using experimental approaches to evaluate the differential effects of blood volume on the oxygenated and
deoxygenated NIRS signal appears challenging. While waiting for technology and methods to overcome the semi quantitative nature of the signal, the deoxygenated NIRS signal allows fitting procedures with exponential functions to characterize the kinetics of the muscle response to contraction even in the presence of an increase in blood volume. In this case, an increase in blood volume (Figure 4.7) is added to that of the imbalance between O₂ delivery and utilization. In contrast, the effect of an increase in blood volume on the oxygenated NIRS signal contribute to the undershoot mentioned earlier, thus deviating from the exponential pattern. On the other hand, the effect of a decrease of blood volume on the oxygenated and deoxygenated signals is opposed to the previous case. In this condition, a decrease of the heme groups concentration would cause an underestimation of the deoxygenated and an overestimation of the oxygenated NIRS signals. The quantitative analysis proposed in our work suggests that either an increase or decrease of heme group concentration can lead to underestimating or overestimating the amplitude of the muscle oxygen extraction measured by NIRS signals during contraction. The mathematical model is a unique and complementary tool that integrating measurable and non-measurable information can be used to correct the NIRS signals in assessing muscle oxygen utilization in contracting muscle (Koirala et al., 2021; Lai et al., 2009; Zhou et al., 2009). The knowledge of microvascular blood volume distribution and flow changes is crucial information for computational and experimental approaches to quantifying the unknown contribution of blood volume to the NIRS signals.

Our results were obtained with different O₂ delivery in the absence of factors such as adipose tissue (Ryan et al., 2012) and skin blood flow (Davis et al., 2006) that contribute to the NIRS signal. Thus, the inferences made on the NIRS measurement were robust and highlighted the value of investigating NIRS measurement at differing O₂ deliveries, particularly to quantifying the blood volume effect. To our knowledge, there is a human (Adami et al., 2015) study and no
other animal models beside that used in our study that showed a distinct effect of blood volume on NIRS signals (Sun et al., 2016). To further investigate blood volume effects on the NIRS signals, animal studies allow use of experimental designs with different O₂ delivery and methods to quantify microvascular blood volume.

Another study has reported the importance of correcting NIRS measurements for blood volume changes and proposed a method to quantify these changes (McCully et al., 2011; Ryan et al., 2012). Nevertheless, this method was developed for NIRS measurements with arterial occlusion, and it is not directly applicable to NIRS measurement with spontaneous blood flow. Also, the method proposed represents an acceptable compromise between the current NIRS limitations and outcome provided; the assumption of equally redistributed blood volume change between the oxygenated and deoxygenated NIRS signals is not always satisfied. In this regard, our model simulations showed that blood volume change effects on the oxygenated NIRS signal are in general different from those on the deoxygenated NIRS signal (Figure 4.7 and 4.8). This difference was affected by O₂ delivery and metabolic rate via $SO_2$.

Another aspect to consider is the applicability of our findings to spontaneous human exercise. The mathematical model was validated with data obtained from an animal model of oxidative metabolism in electrically stimulated muscle which presents some differences (Hernández et al., 2010) in comparison to the voluntary muscle contraction in humans. Nevertheless, it is expected that our findings are also applicable to other mammalian species, including humans, because the mathematical model does not include any specific features related to the muscle electrical stimulation of the animal model. Any change in muscle perfusion and metabolic intensity are simulated with a variation of blood flow and oxygen consumption, respectively. The heme group concentration variation detected by NIRS measurements at the onset
of contraction decreased in the animal model whereas it typically increases in human exercise. The pump blood flow that was turned up before the contractions could have affected the initial decrease of the heme group concentration. Model simulations were obtained for both a decrease (Figure 4.6) and increase (Figure 4.7) of blood volume changes; thus, our findings can be applied to animal and human studies with an increase of blood volume detected by NIRS. To further confirm our findings in human contracting muscle, the mathematical model can be tested with additional invasive and non-invasive (i.e., NIRS) exercise data.

4.4.2. Microvascular and extravascular volumes

Model simulation provided an estimate of the total heme group concentration (246.3 μM) in resting skeletal muscle that was close to the values (190-240 μM) reported in studies using time resolved NIRS (Adami et al., 2015; Marcinek et al., 2003). Furthermore, the simulated muscle O₂ saturation accounting for microvascular and extravascular oxygenation at rest (97%) was consistent with that measured in both animal (Marcinek et al., 2003) and human (Costes et al., 1996) NIRS studies under similar conditions. The blood volume change estimated for T3 (35 μM) or assumed in the analysis of blood volume change reported in Figures 4.6 and 4.7 corresponded to $\Delta HbMb$ of 20-50 μM which was comparable to that observed in a human study on resting (Adami et al., 2015) and contracting quadriceps (Chance et al., 1997).

The composition of the NIRS signal is affected by the extravascular and microvascular volume ($f_{b,m}$, $\omega_{art}$, $\omega_{cap}$, $\omega_{ven}$) distributions as well as by the oxygen saturation in these compartments. Although the oxygen saturation value in the four compartments appears consistent with the values observed for conditions like those of T1 and T3, uncertainties exist regarding the
simulated microvascular and extravascular volume distributions. Here, the volume distribution assumed in the model are discussed. The capillary-fiber geometry data reported in the literature is consistent with the volume fraction of 6% used in our study. It was determined assuming a capillary volume fraction in canine gastrocnemius of 5% (Bebout et al., 1993) and that this volume fraction was 80% of the microvascular volume (Poole & Mathieu-Costello, 1989). Other canine skeletal muscle studies reported lower values than those reported for gastrocnemius: capillary volume density was 1.5-2.5% (Conley et al., 1987), and active vascular volume in gracilis was 4-5% (Baker et al., 1976). With regard to the microvascular volume distribution, the 10% arterial volume fraction used in our work is similar to that reported for human (Ruotsalainen et al., 1997) and animal skeletal muscle (Poole & Mathieu-Costello, 1989) whereas the capillary volume fraction of 80% was similar to that reported for rat skeletal muscle (Poole & Mathieu-Costello, 1989). A different microvascular volume distribution with 80% venous fraction was used in the past (Kocsis et al., 2006; Lai et al., 2009) and based on anatomical studies of the brain (van Lieshout et al., 2003).

The heme group concentration changes detected by NIRS may not only be related to blood volume but also to the hematocrit in blood vessels. At rest, the microvascular hematocrit is smaller than that observed in the large vessel due to the Fåhraeus’s effect (Fåhraeus, 1929) and during contraction reaches a value comparable to that of the systemic hematocrit. Hematocrit in the microvascular volume was suggested to be 22% (Davis & Barstow, 2013) or 60-90% (Fantini, 2002; Pries et al., 1996) of that in large vessels. In our study, because NIRS signals reflect the small blood vessel, Hb contribution to the NIRS signal was corrected using microvascular volume fraction rather than the systemic fraction whereas microvascular and systemic hematocrit was assumed to be the same. This assumption is consistent with a similar capillary and systemic
hematocrit observed in well perfused and contracting muscle (Poole et al., 2013). Nevertheless, this assumption should be further analyzed for NIRS kinetics in future computational studies including the mathematical model’s proposed additional features reflecting Fåhraeus’s effect. Beside the increase of microvascular hematocrit, it has been also suggested that capillary surface area can increase with a length increase of capillary already recruited “longitudinal recruitment” (D. C. Poole et al., 2011). In our model, we assumed a blood volume change which would be consistent with an increase of capillary length.

It was assumed that the extravascular volume was unchanged during contraction or different blood flow rate, but the extravascular volume can increase during exercise (Fotedar et al., 1990). An MRS study reported an increase of extravascular volume of approximately of 5-10% of that at resting condition (Fotedar et al., 1990). These changes related to fluid shifts between vasculature and tissue of contracting muscle are expected to have a minimal effect on the NIRS signals. Specifically, if we assume an increase of the extravascular volume of 10% of the resting value, the extravascular volume fraction increases from 93% to 93.6%. Also, because this increase is due to a fluid shift, the heme group content associated with Mb does not change whereas its concentration within the whole muscle should slightly decrease.

The comparison between simulated and experimental data indicates that the assumptions made about volume distribution of extravascular and microvascular compartments are consistent with experimental evidence. The scenario proposed in the model reflects specific constraints related to the oxygen transport and biochemical processes that limit the possibilities to compose the NIRS signal with different and arbitrary anatomical distribution and oxygen saturation. Furthermore, among the microvascular compartments, capillary and venule volume distribution appears to play a major role in contributing to the NIRS signal because the effects of arteriole on
the NIRS signal are negligible as previously discussed. We can infer that with a higher venules volume fraction in muscle \( f_{b,m} \omega_{ven} \) represented by a higher \( \omega_{ven} = 50\% \) (and \( \omega_{cap} = 40\% \)) in comparison to that proposed \( \omega_{ven} = 10\% \) (and \( \omega_{cap} = 80\% \), Table 4.1) the simulated \( \Delta HbMbO_{2,N} \) and \( \Delta HHbMb_N \) overestimate the measured \( \Delta HbMbO_{2,N} \) and \( \Delta HHbMb_N \) (Figure 4.5). Thus, with the assumptions proposed on \( f_{b,m} \omega_{ven} \) and \( f_{b,m} \omega_{cap} \), it appears that there is no other microvascular volume distribution that can be used in the model to provide \( \Delta HbMbO_{2,N} \) and \( \Delta HHbMb_N \) simulations close to those measured. It should be noted that different volume distributions are possible if other scenarios are considered as in the case of a systemic hematocrit different from that in capillaries and venules. They should be simulated to check their consistency with the experimental data. Even if a different volume distribution may affect the amplitude of both NIRS signals, the microvascular \( SO_2 \) remains a key factor in determining the sensitivity of \( \Delta HbMbO_2 \) and \( \Delta HHbMb \) to blood volume changes; thus, the main findings presented in this work still hold.

The quantitative analysis proposed in this study indicates that oxygen delivery is a key factor in determining the effects of blood volume on oxygenated and deoxygenated NIRS signals. Under high oxygen delivery followed by high microvascular oxygen saturation above 80%, blood volume contribution to the oxygenated NIRS signal is much greater than that of the deoxygenated NIRS signal. In contrast, under physiological or pathophysiological conditions characterized by microvascular oxygen saturation less than 80% due to normal or reduced oxygen delivery, the deoxygenated NIRS signal becomes more sensitive to blood volume changes. Thus, contrary to common assumptions that only oxygenated NIRS is affected by blood volume changes, both NIRS signals are affected by blood volume changes even in the absence of skin blood flow effects. These
findings should be considered in developing NIRS methods and technologies that are quantitative rather than semi-quantitative.
CHAPTER 5

QUANTITATIVE ANALYSIS OF THE HB AND MB CONTRIBUTIONS TO THE RELATIONSHIP BETWEEN MUSCLE VENOUS BLOOD OXYGENATION AND NEAR-INFRARED SPECTROSCOPY SIGNALS

5.1. Introduction

The assessment of skeletal muscle function in healthy and disease states by Near-infrared spectroscopy (NIRS) technology has continuously gained attention during the last decades (Grassi et al., 2019; Hamaoka et al., 1996; McCully et al., 2011; Wilson et al., 1989). Nevertheless, its clinical application has been limited because of the semi-quantitative nature of the NIRS signal in quantifying the oxygen (O$_2$) level in skeletal muscle. Specifically, NIRS signals reflect the O$_2$ saturation of hemoglobin (Hb) and myoglobin (Mb) within the microvascular and extravascular compartments (Davis & Barstow, 2013; Lai, Zhou, et al., 2009; Masuda et al., 2010), but their contributions to the NIRS signals vary during muscle contraction (Lai, Zhou, et al., 2009; Spires et al., 2011) and cannot be easily quantified. Although quantitative analysis of the temporal profile of muscle NIRS signals provides a valuable parameter characterizing the NIRS kinetics (i.e., time constant), inferences are limited by the unknown contribution of Hb and Mb to the kinetics.

NIRS measurement in animals have reported that the contribution of Mb could be in the range of 20-50% (Marcinek et al., 2007; Masuda et al., 2010; Nioka et al., 2006) whereas human studies reported a contribution close to 50-80% (Davis & Barstow, 2013; Marcinek et al., 2007).
Integrative approaches that combine experimental and computational methods were used to analyze the NIRS measurement in contracting muscle during human exercise (Binzoni et al., 1999; Lai, Saidel, et al., 2009; Lai, Zhou, et al., 2009; Spires et al., 2011; Zhou et al., 2008). It was reported that Mb contribution to the signal varies from 20 to 80% during contraction (Lai, Zhou, et al., 2009). The significance of the Mb contribution inferred by the analysis of the computational model was also confirmed by a quantitative analysis reported by another group (Davis & Barstow, 2013).

Another quantitative and experimental approach used to evaluate the Hb/Mb contribution to NIRS is to examine the correlation between venous and muscle oxygenation by NIRS. The correlation or linear relationship between these two variables, which is considered evidence of a major Hb contribution to the NIRS signal, is not always present for measurement in skeletal muscle. The presence of this relationship was reported in canine gracilis muscle (Wilson et al., 1989) and human forearm muscle (Mancini et al., 1994). In other studies, the relationship was not always present and was affected by oxygen delivery (Boushel et al., 1998; Wüst et al., 2014) to the contracting muscle. In particular, the correlation between venous and NIRS oxygenation improved at high oxygen delivery (i.e., blood flow) in forearm flexor (Boushel et al., 1998) and pump perfused canine muscle (Wüst et al., 2014). In contrast, a decrease of oxygen delivery (i.e., hypoxia) improved the (Costes et al., 1996; MacDonald et al., 1999) correlation between the NIRS signal and venous blood oxygenation in vastus lateralis. Some of the differences observed could be in part related to confounding factors such as adipose tissue, skin blood flow and blood volume changes that complicate the interpretation of this relationship in human studies.

To avoid the effects of these confounding factors, an animal model (Hernández et al., 2010) was used to quantify the effects of O₂ delivery (i.e., varying arterial O₂ content or blood flow) on
the relationship between venous O$_2$ content and NIRS signal (Sun et al., 2016) in the presence or absence of contraction. In this experimental setting with all sets of data obtained under different O$_2$ delivery, a decrease in O$_2$ delivery determined a linear decrease of venous oxygen content and an increase of the NIRS signal (oxygenated and deoxygenated forms) changes. Thus, these results support a predominant contribution of Hb to the NIRS signal. However, because O$_2$ transport and utilization are intimately coupled and followed by both O$_2$ content changes in blood and tissue compartments, it is possible that the oxygenation changes detected by the NIRS signals are related to concurrent Hb and Mb oxygenation changes. Physiologically based mathematical models of energy metabolism can be used to quantify Hb and Mb contributions to the NIRS signals (Lai et al., 2008; Lai, Saidel, et al., 2009; Lai et al., 2010). The metabolic response to contraction in human and animal models has been predicted by a computational model of O$_2$ transport and utilization in skeletal muscle under different experimental conditions (Grassi, Gladden, Samaja, et al., 1998; Grassi, Gladden, Stary, et al., 1998; Grassi et al., 2000). Specifically, the mathematical model was validated with kinetics of O$_2$ uptake, and venous O$_2$ content data obtained from canine muscle under different arterial O$_2$ content, blood flow and metabolic rate conditions (Lai, Camesasca, et al., 2007; Spires et al., 2013; Spires et al., 2012). The capability of this model was enhanced with the quantification of the oxygenation in the microvascular and extravascular compartments to study the effects of blood flow and volume on the NIRS (Chapter 4). Thus, this tool can be used to quantifying the Hb and Mb contribution to NIRS signals for different O$_2$ delivery.

In the present study, we propose to use this computational model validated with canine data to analyze the effects of O$_2$ delivery on the relationship between venous and NIRS oxygenation to determine whether Mb contribution to the NIRS signal can be significant even in the presence of a linear relationship between venous O$_2$ content and the NIRS signals.
5.2. Methods

5.2.1. Animal model

The experimental data for the computational analysis of our study were obtained from a previous investigation that used an animal model of muscle oxidative metabolism. The experimental setting of this animal model provided venous ($C_{ven}^T$) and muscle oxygenation measurements under different experimental conditions (i.e., arterial O$_2$ content, blood flow, contraction) reported in Figure 5.1 (Sun et al., 2016). NIRS measurements included the oxygenated ($\Delta HbMbO_2, N$) and deoxygenated ($\Delta HHbMb_N$) signals. $N$ indicates that $\Delta HbMbO_2$ and $\Delta HHbMb$ measurements were normalized to the maximal variation of the oxygenated and deoxygenated NIRS signals observed during blood flow occlusion.

![Figure 5.1](image.png)

Figure 5.1. Experimental conditions simulated in this work and previously investigated by (Sun et al., 2016): Effect of blood flow at rest (T1) or during contraction (T3, 0.5Hz) with constant arterial partial pressure of oxygen ($P_{O2}$=100 mmHg); Effect of $P_{O2}$ (T2) with constant blood flow 0.2 L kg$^{-1}$ min$^{-1}$).
5.2.2. Mathematical model

A mathematical model of skeletal muscle O$_2$ transport and metabolism (Lai, Saidel, et al., 2007; Spires et al., 2013; Spires et al., 2012) was used to quantify the Hb and Mb contribution to the NIRS measurement obtained with the animal model introduced in the previous section (Sun et al., 2016). The muscle volume ($V_{mus}$) consists of extravascular tissue (cells and interstitial space, $V_t$) and blood ($V_b$) which is assumed to have the vascular ($V_{b,v}$) and microvascular ($V_{b,m}$) compartments. The microvascular volume consists of arterioles, capillaries, and venules ($V_{b,m} = V_{art} + V_{cap} + V_{ven}$). The model simulates the O$_2$ concentration in the microvascular and extravascular compartments, and they are used to quantify the oxygenated Hb ($HbO_2$) and Mb ($MbO_2$) concentration in muscle as:

$$HbO_2 = f_{b,m}(\Delta C_{Heme,N}^{exp})C_{b,m}^B/4$$  \hspace{1cm} 5.1

$$MbO_2 = f_t C_t^B/4$$  \hspace{1cm} 5.2

where $f_{b,m}$ and $f_t$ are the microvascular and tissue volume fraction in muscle and $C_{b,m}^B$ and $C_t^B$ are the bound O$_2$ concentration in the microvascular and tissue compartments, respectively.

The deoxygenated Hb ($HHb$) and Mb ($HMb$) contributions to the deoxygenated ($\Delta HHbMb$) NIRS signal are computed as:

$$HHb = f_{b,m}(\Delta C_{Heme,N}^{exp})C_{b,Hb} - HbO_2$$  \hspace{1cm} 5.3

$$HMb = f_t C_{t,Mb}/4 - MbO_2.$$  \hspace{1cm} 5.4

$f_{b,m}$ is a function of the relative heme concentration changes ($\Delta C_{Heme,N}^{exp}$) detected by the NIRS signal and used to quantify the microvascular volume changes. Details of the models are reported in Chapter 2 and Chapter 4.
5.2.3. Model simulations

The details of the strategy to simulate the conditions of the three trials is like that reported in Chapter 4. The model inputs (i.e., arterial $O_2$ content, blood flow) reflect the experimental condition of each trial and are reported in Figure 5.1. The parameter values in Table 5.1 apply to all experiments and are independent of the input conditions. The rest of the parameter values of this model are available from previous studies (Lai, Saidel, et al., 2007; Spires et al., 2013; Spires et al., 2012).

Table 5.1. Vascular, extravascular, and microvascular volume distribution as well as oxygenated and deoxygenated heme group concentrations of Hb and Mb at rest and ischemia.

<table>
<thead>
<tr>
<th>Notation</th>
<th>Unit</th>
<th>Rest (R)</th>
<th>Ischemia (I)</th>
</tr>
</thead>
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<tr>
<td>$V_{mus}$</td>
<td>[g]</td>
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</tr>
<tr>
<td>$f_b$</td>
<td>[%]</td>
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<td>3</td>
</tr>
<tr>
<td>$f_t$</td>
<td>[%]</td>
<td>93</td>
<td>97</td>
</tr>
<tr>
<td>$f_m$</td>
<td>[%]</td>
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<td>$f_{b,m}$</td>
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<td>$\omega_{art}$</td>
<td>[%]</td>
<td>10</td>
<td>32.7</td>
</tr>
<tr>
<td>$\omega_{cap}$</td>
<td>[%]</td>
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<td>34.6</td>
</tr>
<tr>
<td>$\omega_{ven}$</td>
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<td>10</td>
<td>32.7</td>
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*Volume distribution*

<table>
<thead>
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<th>Ischemia (I)</th>
</tr>
</thead>
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<tr>
<td>$HbMb$</td>
<td>[mM]</td>
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<td>132.9</td>
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</table>

*Heme group concentrations (Hb/Mb)*
The experimental data compared with model simulations, are the venous O$_2$ concentration ($C_{ven}^T$) and the rate of muscle O$_2$ uptake ($\dot{V}O_2$) and the normalized oxygenated ($\Delta HbMbO_{2,N}$) and deoxygenated ($\Delta HHbMb_N$) NIRS measurements:

$$\Delta HbMbO_{2,N} = \frac{\Delta HbMbO_2}{\Delta HbMbO_2^{TS}}, \quad \Delta HHbMb_N = \frac{\Delta HHbMb}{\Delta HHbMb^{TS}}.$$  \hspace{1cm} (5.5)

T5 represents the condition during blood flow occlusion. The contribution of Hb and Mb to the oxygenated ($\Delta HbMbO_2$) and deoxygenated ($\Delta HHbMb$) NIRS signals under different experimental conditions are quantified as:

$$y_{\Delta HbO_2} = \Delta HbO_2/\Delta HbMbO_2; \quad y_{\Delta MbO_2} = 1 - y_{\Delta HbO_2}$$ \hspace{1cm} (5.6)

$$y_{\Delta HHb} = \Delta HHb/\Delta HHbMb; \quad y_{\Delta HMb} = 1 - y_{\Delta HMb}.$$ \hspace{1cm} (5.7)

5.3. Results

The overall strategy to analyze the Hb/Mb contribution to the relationship between venous and NIRS signal oxygenations were the following: a) validate the mathematical model with blood and NIRS experimental data (Sun et al., 2016) (Figure 5.2), b) relate muscle venous oxygenation to Hb and Mb contribution to NIRS signal (Figure 5.4), and c) analyze the nature of this relationship for different oxygen delivery (Figure 5.5-5.6).

5.3.1. Mathematical model validation

The capability of the mathematical model to reproduce the experimental data was tested with measurements of venous O$_2$ content ($C_{ven}^T$) and tissue oxygenated ($\Delta HbMbO_{2,N}$) and deoxygenated ($\Delta HHbMb_N$) heme group concentrations obtained for different blood flow ($Q$) at
rest (T1, Figure 5.2 a, b) and during contraction (T3, Figure 5.2 g, h) and for different arterial O$_2$ content ($P_{art}$) at rest (T2, Figure 5.2 d, e). For each trial simulation, the parameter ($k_{ATPase}$) associated with the energy demand was used to simulate the changes in $V\dot{O}_2$ (Figure 5.3, a, c, e) and O$_2$ content in the blood and tissue compartments. Simulations accounted for microvascular blood volume changes detected by the sum of the oxygenated and deoxygenated NIRS signals (Figure 5.3, b, d, f).
Figure 5.2. Model validation and predictions of blood and tissue oxygenation for different blood flow ($Q$) at rest (T1, a, b), arterial partial pressure (T2, d, e) and different $Q$ during contraction (T3, g, h): comparison between model prediction (solid line) and experimental data (open circle) of $C_{ven}^T$ (panel a–T1; panel d–T2; panel g–T3) and (open circle) $\Delta HHbMb_N$ and (filled circle) $\Delta HbMbO_{2,N}$ (panel b–T1; panel e–T2; panel h–T3;).

The model predicted well the changes of $C_{ven}^T$ (Figure 5.2a, d, g), $\Delta HbMbO_{2,N}$ and $\Delta HHbMb_N$ (Figure 5.2b, e, h) with $O_2$ delivery ($Q$, arterial $P_{O_2}$) changes. The higher the $O_2$ delivery, the higher $C_{ven}^T$ and the lower the amplitude of the NIRS signal changes. In T1, for reduced $O_2$ delivery ($Q<0.1 \text{ L kg}^{-1} \text{ min}^{-1}$), simulations predicted an abrupt nonlinear decline in $C_{ven}^T$ (Figure 5.2a) and $\Delta HbMbO_{2,N}$ and an increase in $\Delta HHbMb_N$ (Figure 5.2b). Similarly, in T2, for reduced $O_2$ delivery obtained under hypoxia, simulations predicted an abrupt nonlinear decline
in $C_{ven}^{T}$ (Figure 5.2d) and $\Delta HbMbO_{2,N}$ (Figure 5.2e) and a rise in $\Delta HhMbN$. In T3, $C_{ven}^{T}$ (Figure 5.2g), $\Delta HbMbO_{2,N}$ and $\Delta HhMbN$ (Figure 5.2h) showed a quasi-linear relationship with blood flow $Q$.

Figure 5.3. Model inputs: simulated (black line) and experimental data (open circle) of muscle oxygen uptake ($\dot{V}O_2$) and relative heme group changes of Hb/Mb concentration ($\Delta C_{Heme,N}$) for (a, b) T1, (c, d) T2, and (e, f) T3 trials; rate coefficient ($k_{ATPase}$, grey line) associated to the metabolic rate to simulate $\dot{V}O_2$ for (a) T1, (c) T2, and (e) T3 trials; microvascular volume fraction ($f_{b,m}$) changes with (b, T1) $Q$ or (d, T2) $P_{O2}$ at rest, or (f, T3) $Q$ during contraction.
Figure 5.4. (a) Relationship between venous O₂ content ($C_{ven}$) and $\Delta HHbMb_N$, (b) relationship between $C_{ven}$ and $\Delta HbMbO_{2,N}$: comparison between model prediction (solid lines: black - T1; light grey - T2; grey - T3) and experimental data (symbol: open circle - T1; filled circle - T2; filled square - T3) of (a) $\Delta HHbMb_N$, and (b) $\Delta HbMbO_{2,N}$ obtained for different $C_{ven}$. Simulation of the (c) relationship between $C_{ven}$ and Hb contribution to the $\Delta HHbMb_N$ NIRS signal ($y_{\Delta HbO_2}$); simulation of the (d) relationship between $C_{ven}$ and Hb contribution to the $\Delta HbMbO_{2,N}$ NIRS signal ($y_{\Delta HHb}$): (solid lines: black - T1; light grey - T2; grey - T3). It should be noted that the Mb contribution to the oxygenated ($y_{\Delta MbO_2}$) or deoxygenated ($y_{\Delta HMb}$) signals is related to that of Hb by the expressions $y_{\Delta MbO_2} = 1 - y_{\Delta HbO_2}$, and $y_{\Delta HMb} = 1 - y_{\Delta HHb}$. 
5.3.2. NIRS signal and venous $O_2$ content

The model predicted well $\Delta HbMbO_{2,N}$ and $\Delta HHbMb_N$ changes with $C_{ven}^T$ that correspond with the experimental observations of $\Delta HHbMb_N$ and $\Delta HbMbO_{2,N}$ (Figures 5.4a and 5.4b). Whereas the sets of experimental data for T1, T2 and T3 trials covered a different region of the $C_{ven}^T$-NIRS signal relationship, model simulations of each trial were performed for the entire experimental range (5-25 mLO2 100mL$^{-1}$). To cover this $C_{ven}^T$ range the blow flow range used for T1 and T3 simulations was 0.01-0.4 and 0.7-4.0 L kg$^{-1}$ min$^{-1}$, respectively. For T2 it was 40-500 mmHg. These ranges were larger than those used in Figure 5.2. An overall linear relationship is notable between $C_{ven}^T$ and $\Delta HHbMb_N$ or $\Delta HbMbO_{2,N}$ when all sets (T1, T2, T3) of data are considered. Nevertheless, when the sets of data are independently considered at low oxygen delivery ($C_{ven}^T < 7-9$ mLO2 100mL$^{-1}$), the model predictions showed that the relationship between the venous and muscle oxygenation ($\Delta HHbMb_N$) was still linear for T1 (black line) but was not linear for T3 (grey line) (Figure 5.4a, 5.4b). A similar difference between T1 and T3 was also observed for the relationship between $C_{ven}^T$ and $\Delta HbMbO_{2,N}$.

The $\Delta HbMbO_{2,N}$ and $\Delta HHbMb_N$ were further analyzed to relate the changes of the Hb contribution to the $\Delta HbMbO_{2,N}$ ($y_{\Delta HbO_2}$) or $\Delta HHbMb_N$ ($y_{\Delta HHb}$) signal with $C_{ven}^T$ (Figures 5.4c, 5.4d). At high oxygen delivery (i.e., high $C_{ven}^T$), the Hb contribution to both signals was above 80% for all trials, reaching a plateau close to 80% for $y_{\Delta HHb}$ and close to 100% for $y_{\Delta HbO_2}$. At low oxygen delivery ($C_{ven}^T < 7-9$ mLO2 100mL$^{-1}$), for a $C_{ven}^T$ decrease, the Hb contribution to both signals decreased linearly for T1 and non-linearly for T3 (Figure 5.4c, 5.4d).
5.3.3. Hb and Mb contribution to the signals

To further investigate the nonlinearity between $C_{ven}^T$ and Hb contribution to the NIRS signals, the components of the deoxygenated ($\Delta HHbMb$) or oxygenated ($\Delta HbMbO_2$) forms within the microvascular ($\Delta Hb$ or $\Delta HbO_2$: capillary, venule) and extravascular ($\Delta Mb$ or $\Delta MbO_2$) compartments were analyzed in relationship to $C_{ven}^T$ changes in T1 (Figures 5.5a, 5.5b) and T3 (Figures 5.5c, 5.5d).

Figure 5.5. Relationship between venous O2 content ($C_{ven}^T$) and the relative changes of deoxygenated (a, c), ($\Delta Hb$, $\Delta Mb$ – dashed line) and oxygenated (b, d), ($\Delta HbO_2$, $\Delta MbO_2$ – solid line) Hb and Mb from rest obtained for different blood flow of T1 (a, b) and T3 (c, d); (green, blue and black refer to capillaries, venules and myocytes compartments).
For both T1 and T3 trials, with a decrease of $C_{ven}^T$, the amplitude of both deoxygenated (Figure 5.5a, c) and oxygenated (Figure 5.5b, d) forms in the capillary (green line) and venules (blue line) increased linearly whereas those in myocytes increased nonlinearly (black line) with a biphasic pattern. Also, for both trials, the change of slope of $\Delta MbO_2$ and $\Delta HMb$ occurred for $PO_2$ in capillary, venule, and myocyte approximately 45, 25 and 20 mmHg which corresponded to a $C_{ven}^T$ of 7 and 9 mLO$_2$ 100mL$^{-1}$ in T1 (Figure 5.5a, b) and T3 (Figure 5.5c, d), respectively. This change of slope indicates a higher sensitivity of Mb than Hb to oxygen delivery changes. Thus, the Hb contribution to the NIRS signals (Figure 5.5c d) decreased although capillary $\Delta HbO_2$ and $\Delta HHb$ amplitudes increased with lower $C_{ven}^T$. In T3, the effect of blood flow (i.e., decrease of $C_{ven}^T$) on the amplitude of both deoxygenated and oxygenated forms in myocytes was even larger than that observed in T1 and led to a non-linear increase of the Mb contribution to the NIRS signal.

Figure 5.6. Relationship between venous O$_2$ concentration ($C_{ven}^T$) and the oxygen saturation of Hb and Mb in different compartments for a) rest (T1) and b) contraction (T3) (green, blue, and black refer to capillaries, venules, and myocytes compartments).
Microvascular and extravascular $PO_2$ were in the range that corresponds to a large variation of their oxygen saturation ($SO_2$) curves which are strongly related to the NIRS signals. Thus, the sensitivity of $SO_2$ in the microvascular and extravascular compartments to $C_{ven}^{T}$ changes was analyzed. The Hb and Mb saturation changes were plotted against $C_{ven}^{T}$ (Figure 5.6a and b).

For both T1 and T3 a decrease of $C_{ven}^{T}$ with a reduction of O$_2$ delivery determined a linear decrease of the $SO_2$ of Hb in venules and capillary compartments and a non-linear decrease of the $SO_2$ in the myocytes. $SO_2$ in capillary and venules is more sensitive than myocytes to $C_{ven}^{T}$ changes for $C_{ven}^{T} > 7-9$ mLO$_2$ 100 mL$^{-1}$. For further $C_{ven}^{T}$ decrease followed by $SO_2$ decrease of Mb below 80-85%, $SO_2$ of Mb changes become more sensitive than those observed for the capillary and venule. This sensitivity in T3 is even larger than T1: a $C_{ven}^{T}$ decrease of 1 mLO$_2$ 100mL$^{-1}$ from 7 (T1) or 9 (T3) mLO$_2$ 100mL$^{-1}$ was followed by a decrement of 4% (81 to 77%) and 13% (80 to 67%) of the Mb saturation in T1 and T3, respectively. The $SO_2$ changes in venules and capillaries in T1 were comparable to those observed for T3.

5.4. Discussion

A computational model of muscle O$_2$ transport and metabolism was validated with experimental data to analyze how Hb and Mb contributions to the NIRS signal are affected by O$_2$ delivery. The primary finding of this study was that for adequate O$_2$ delivery, Mb contribution to the NIRS signals was significant even in the presence of a linear $C_{ven}^{T}$-NIRS relationship; for reduced O$_2$ delivery, the non-linearity of the $C_{ven}^{T}$-NIRS relationship was related to the Mb contribution. O$_2$ saturation in a capillary and myocytes is a key factor affecting Hb and Mb contributions to both oxygenated and deoxygenated NIRS signals. With reduced O$_2$ delivery, Mb
saturation varied from 80 to 40% corresponding to a variation of the Mb contribution to the NIRS from 15 to 50%.

5.4.1. Venous vs NIRS signals relationship

The linearity between the venous O$_2$ content and tissue oxygenation measured by NIRS has been reported in animal (Sun et al., 2016; Wüst et al., 2014) and human (Mancini et al., 1994; Vogiatzis et al., 2015) studies. This evidence supports the idea that the NIRS signal is affected by oxygenation of Hb within the venous compartment but does not exclude the Mb contribution. Our model simulations indicate that the Mb contribution to the NIRS signal can be significant and depends on the balance between O$_2$ delivery and utilization (Figure 5.6). Other studies suggested a tight interplay between O$_2$ convection, diffusion, and utilization kinetics; thus, both capillary/venous and tissue O$_2$ content change simultaneously (Lai, Camesasca, et al., 2007; Takakura et al., 2015) with blood flow during muscle contraction. Thus, it appears that NIRS changes reflect not only Hb, but also Mb oxygenation adjustments. In contracting rat gastrocnemius muscle with a well-oxygenated hemoglobin-free buffer Mb desaturation kinetics were detected by NIRS measurements (Takakura et al., 2010).

The concurrent change in Hb and Mb oxygenation predicted by the simulations is supported by a human study with simultaneous measurement of the Mb signal by $^1$H NMR and the Hb/Mb signal by NIRS (Bendahan et al., 2017). Another human study (Boushel et al., 1998) reported that muscle oxygenation changes by NIRS did not follow those of the venous O$_2$ saturation, thus suggesting that Mb contribution to the NIRS could have been in part responsible for the differences.
The specific balance between O₂ delivery and utilization determined the linear or non-linear relationship between NIRS signals and \( C_{ven}^T \). Model simulations suggest that a linear relationship between NIRS signals and \( C_{ven}^T \) can coexist with a Mb contribution to the NIRS signal smaller than 35% observed in resting muscle (Figure 5.4). This is possible because both Hb and Mb saturation are linearly affected by O₂ delivery for the range considered. In the presence of contraction with a more pronounced imbalance between O₂ delivery and utilization than that observed at rest, the relationship between NIRS signals and \( C_{ven}^T \) became non-linear with high Mb contribution to the NIRS signal (Figure 5.4c). In contrast, the experimental data obtained for the T3 condition showed a linear relationship between venous and NIRS signal oxygenations. Two aspects can be considered to interpret these apparent conflicting results: a) model simulations deviated from the linearity, but they are still within the range of the experimental data standard deviation; b) the oxygen delivery range used by the computational model was larger than that investigated in the experimental study. The nonlinear relationship between NIRS signals and \( C_{ven}^T \) simulated by the computational model and characterized by Mb contribution that can reach 50% is consistent with a similar Mb contribution to NIRS signal observed in rat muscle perfused with a hemoglobin free buffer (Masuda et al., 2010) and in other animal and human studies (Marcinek et al., 2007; Nioka et al., 2006).

### 5.4.2. Blood flow effects on oxygen saturation: Hb and Mb contribution to the NIRS signals

The oxygen saturation (\( SO_2 \)) in the microvascular (i.e., Hb) and extravascular (i.e., Mb) compartments appears to be a key factor in determining the O₂ content redistribution within the microvascular and tissue compartments and, thus, the Hb/Mb contribution to the NIRS signals (\( \gamma_{\Delta HbO_2}, \gamma_{\Delta MbO_2} \)) at different O₂ delivery. It has been suggested that the difference in O₂...
desaturation between Hb and Mb during muscle contraction could in part explain a slower muscle
deoxygenated NIRS kinetics than that of the microvascular O$_2$ content by phosphorescence-quenching (Koga et al., 2011). This finding was related to a faster O$_2$ desaturation of Hb than that of Mb during contraction. In comparison to this study (Koga et al., 2011), our results offer a different perspective in studying the effects of $SO_2$ on Hb/Mb contribution because our simulations were obtained for steady-state conditions. Thus, any Mb contribution to the NIRS signal related to slower O$_2$ desaturation kinetics of Mb compared to when Hb were not present. Nevertheless, an important effect of blood flow on $SO_2$ of Hb and Mb affecting the NIRS signals was still present; this finding is discussed in the following section.

Our results suggest that an $SO_2$ characteristic difference between blood and tissue contributes to determining the O$_2$ redistribution in the NIRS compartments with O$_2$ delivery changes. Under resting or contracting conditions when the O$_2$ delivery was sufficient or in excess ($C_{ven}^T > 7$-9 mLO$_2$ 100 mL$^{-1}$), the $SO_2$ changes in the microvascular compartments were more sensitive than those in the extravascular compartment. Meanwhile, with reduced O$_2$ delivery, the $SO_2$ changes in the extravascular compartment became more sensitive than those in the microvascular compartments (Figure 5.6). The $SO_2$ difference between microvascular and extravascular compartments can be attributed to two factors: a) a higher affinity of Mb than Hb for O$_2$ and b) the different characteristics of the oxygen saturation curve ($SO_2-PO_2$). Although the partial pressure of O$_2$ required to achieve 50% Hb and Mb saturation ($P_{50}$) is approximately 28 and 3 mmHg, respectively (Feher, 2017; Takakura et al., 2010), the effects of blood flow on the microvascular and extravascular $SO_2$ are also significant at higher $PO_2$ than $P_{50}$. Specifically, for the oxygen saturation curve, the $SO_2-PO_2$ slope changes approximately at 50 and 20 mmHg for Hb and Mb respectively (Dash & Bassingthwaigte, 2004; Feher, 2017). Consistent with the Hb
and Mb $SO_2$ characteristics, the variation of the Mb contribution to the NIRS signal became more sensitive than that of Hb in the capillary and venules (Figure 5.4 and 5.5) when capillary $PO_2$ was 43 mmHg and myocyte was 20 mmHg. Our interpretation is consistent with the finding of the NIRS kinetics study (Koga et al., 2011) that suggested an earlier $O_2$ desaturation of Hb than Mb during contraction; our analysis indicates that the higher affinity of Mb than Hb for $O_2$ contributes to the initial and preferential $O_2$ desaturation of Hb during contraction.

5.4.3. Microvascular volume

The capillary-fiber geometry data reported in the literature is consistent with the volume fraction of 6% used in our study. It was determined assuming a capillary volume density in canine gastrocnemius muscle of 5% (Bebout et al., 1993) a capillary volume fraction that was 80% of the microvascular volume (Poole et al., 1995). Other canine skeletal muscle studies reported lower values than those reported for gastrocnemius: capillary volume density was 1.5-2.5 % (Conley et al., 1987) and active vascular volume in gracilis was 4-5% (Baker et al., 1976). If a microvascular volume fraction lower than 6% were considered in our simulation, the contribution of Mb could have been greater than that estimated for the conditions investigated (Figure 5.4-5.6).

With regard to the microvascular volume distribution, the 10% arterial volume fraction used in our work is similar to that reported for human (Ruotsalainen et al., 1997) and animal skeletal muscle whereas the capillary volume fraction of 80% was similar to that reported for rat skeletal muscle (Poole et al., 1995). A different microvascular volume distribution with 80% venous fraction was used in the past (Kocsis et al., 2006; Lai, Saidel, et al., 2009) and based on anatomical studies of the brain (van Lieshout et al., 2003).
Another factor affecting the Hb contribution to the NIRS signal is the hematocrit in blood vessels. In our work, we assumed that the systemic hematocrit was like that in the microvascular compartment. Nevertheless, the microvascular hematocrit is smaller than that observed in the large vessel due to the Fåhraeus’s effect (Fåhraeus, 1929). Hematocrit in the microvascular volume was suggested to be 22% (Davis & Barstow, 2013) or 60-90% (Fantini, 2002; Pries et al., 1996) of that in large vessels. In our study, because NIRS signals reflect the small blood vessel, Hb contribution to the NIRS signal was corrected using microvascular volume fraction rather than the systemic fraction although hematocrit was assumed to be the same (45-50%). Thus, the model prediction of Hb contribution to the NIRS signal would decrease by 50% if a microvascular hematocrit of 25% was considered.

In conclusion, investigators are challenged by the difficulties in interpreting the nature of the NIRS signals under physiological and pathophysiological conditions. The integrative approach proposed in our work allows quantification of the oxygenation from the blood and tissue domains that cannot be measured but are essential to evaluate O₂ delivery effects on Hb and Mb contributions to the NIRS signals. The robustness of our approach was validated with direct comparison between simulated and experimental data of venous and tissue oxygenation at steady states. The computational analysis indicates that physiological or pathophysiological conditions leading to a decline of O₂ saturation in myocytes below 80% affect significantly both oxygenated and deoxygenated NIRS signals. Our approach can quantify the effects of blood flow on the NIRS kinetics and overcome the limitation of the NIRS measurements accounting for blood volume and distinguishing Hb and Mb signals. The integrative approach allows a quantitative assessment of mitochondrial O₂ utilization *in vivo* from NIRS measurements.
CHAPTER 6

QUANTITATIVE ANALYSIS OF HB AND MB CONTRIBUTION TO THE NIRS SIGNALS IN EXERCISING MUSCLES: ABSOLUTE VS. RELATIVE HEME GROUP CONCENTRATION CHANGES FROM A BASELINE

6.1. Introduction

Muscle oxygenation can be measured non-invasively by near-infrared spectroscopy (NIRS) to evaluate physiological and pathophysiological conditions (Grassi et al., 2019; Willingham & McCully, 2017). There are different types of spectrometers: continuous wave (CW) provides heme group concentration changes relative to a baseline as well as time domain (TD) and frequency domain (FD) intensity modulated NIRS that with an appropriate calibration provide absolute concentration (Barstow, 2019; Ferrari et al., 2011; Grassi & Quaresima, 2016). In CW NIRS, the concentration changes are relative to a base line and are typically normalized to the concentration changes relative to an ischemic condition.

For all NIRS spectrometers, the technology is limited by the uncertainties related to the hemoglobin (Hb) and myoglobin (Mb) contribution to the NIRS signal. Experimental (Davis & Barstow, 2013; Marcinek et al., 2007) and computational studies (Koirala et al., 2021; Lai et al., 2009; Spires et al., 2011), indicate that Mb can significantly contribute to the NIRS signals. These works focused on the Hb and Mb contribution to the relative changes detected by a continuous wave spectrometer. It was reported that Hb and Mb contributions to the oxygenated heme group differed from those to the deoxygenated heme group. Nevertheless, it is not known whether these
contributions to the NIRS concentration changes are like those of the absolute NIRS concentration measured by TD and FD NIRS. It is expected that Hb and Mb contribution to the deoxygenated NIRS concentration is similar to that expressed in terms of relative changes from rest because the absolute and relative concentration values are similar since the deoxygenated NIRS concentration at rest is negligible. On the other, the oxygenated NIRS concentration at rest is not negligible, thus the relative concentration of the oxygenated heme groups differs from the absolute concentration.

A quantitative approach based on a mathematical model of oxygen transport and utilization can be used to quantify Hb and Mb contributions to both relative and absolute oxygenated and deoxygenated heme group concentration measured in skeletal muscle. Also, this approach provides a promising theoretical framework to identify the differences among CW, TD and FD NIRS which currently provide information that cannot be directly compared. The mathematical model can quantify the venous and myocytes oxygen concentration and thus integrate measurable and unmeasurable variables to estimate Hb and Mb contribution to the absolute oxygenated and deoxygenated concentration under different physiological and pathophysiological conditions. Hb and Mb contributions to the NIRS are not only affected by the physiological conditions such as exercise but also by the microvascular volume distribution (arteriole, capillaries, and venules) (Barstow, 2019; Davis & Barstow, 2013). Especially during muscle contraction, the microvascular volume composition can change due to an increase of capillary surface occurring in an already recruited capillary; i.e., “longitudinal recruitment” (Poole et al., 2011).

In this study, a computational model validated with canine data is used to: 1) analyze the effects of blood flow on absolute oxygenated and deoxygenated heme group concentrations and on their relative changes from a base line; 2) evaluate the differences of Hb and Mb contribution
to the NIRS signal between absolute and relative concentration changes by NIRS; and 3) examine the effects of the microvascular volume fraction on the NIRS signals.

6.2. Methods

6.2.1. Animal model

The experimental data used for this analysis was obtained from an animal model of muscle oxidative metabolism (Sun et al., 2016). The trial notation in this work is the same as that used in the experimental work (Sun et al., 2016) from which the NIRS data were taken for the analysis proposed here. The T4 trial was selected (Figure 6.1) to analyze the effects of muscle blood on the amplitude of the oxygenated and deoxygenated NIRS signals in contracting muscle (2 contractions every 3 seconds). Also, the T5 trial was considered because the NIRS signals obtained for this condition were used to normalize the NIRS signals of the T4 trial. All experimental data were averaged from 6 measurements for each trial condition. A continuous-wave NIRS system (Oxymon Mk III, Artinis Medical Systems BV) was used to measure the muscle oxygenated ($\Delta HbMbo_2$) and deoxygenated ($\Delta HHbMb$) heme group concentration changes relative to rest. The light at two different wavelengths (760 and 860 nm) was emitted and received by two fiber-optic bundles. The optodes were placed over the medial head of the left gastrocnemius and held in place with an elastic band. Experimental control of muscle blood flow was made possible by a peristaltic pump connected to the arterial inflow to the gastrocnemius. The right carotid artery or the right femoral artery was cannulated to route blood to the peristaltic pump (Sun et al., 2016).
6.2.2. Mathematical model

To analyze the heme group concentration differences between absolute and relative concentration changes from a base line (i.e., rest) measured by CW NIRS, a mathematical model of skeletal muscle oxygen transport and metabolism (Koirala et al., 2021) discussed in Chapters 3, 4 and 5 is used. The mathematical model is used to simulate the oxygenation in the extravascular tissue \( V_{ts} \) and microvascular volume \( V_{b,m} \) under different muscle blood flow reported in Table 6.1 (Sun et al., 2016). The microvascular volume of the mathematical model consists of arterioles, capillaries, and venules \( V_{b,m} = V_{art} + V_{cap} + V_{ven} \). The derivation of the equations to calculate the physiological variables of interest was previously reported in the method section of Chapter 4 and in another study (Koirala et al., 2021) using previous models (Lai et al., 2007; Spires et al., 2013; Spires et al., 2012). With the mathematical model of \( \text{O}_2 \) transport and utilization, the \( \text{O}_2 \)
concentrations in blood and tissue are simulated to compute the oxygenated heme group concentrations in the microvascular ($C_{art}^B$, $C_{cap}^B$, $C_{ven}^B$) and extravascular ($C_t^B$) domains. The oxygenation changes in the microvascular (arteriole, capillary, and venule) and extravascular compartments are responsible for NIRS measurement changes.

Table 6.1: Vascular, extravascular, and microvascular volume distribution at rest and ischemia

<table>
<thead>
<tr>
<th>Notation</th>
<th>Description</th>
<th>Unit</th>
<th>Rest (R)</th>
<th>Ischemia (I)</th>
</tr>
</thead>
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<tr>
<td>$V_{mus}$</td>
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<td>[g]</td>
<td>62.5</td>
<td>62.5</td>
</tr>
<tr>
<td>$f_b$</td>
<td>fraction of systemic blood in muscle</td>
<td>[%]</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>$f_t$</td>
<td>fraction of tissue in muscle</td>
<td>[%]</td>
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<td>97</td>
</tr>
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<td>fraction of microvascular blood in systemic blood</td>
<td>[%]</td>
<td>85</td>
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<tr>
<td>$f_{b,m}$</td>
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<td>[%]</td>
<td>5.95</td>
<td>1.9</td>
</tr>
<tr>
<td>$\omega_{art}$</td>
<td>fraction of arterioles in microvascular blood</td>
<td>[%]</td>
<td>10</td>
<td>32.7</td>
</tr>
</tbody>
</table>

The oxygenated Hb ($HbO_2$) and Mb ($MbO_2$) concentrations in muscle are computed as:

$$HbO_2 = f_{b,m}[C_{art}^B \omega_{art} + C_{cap}^B \omega_{cap} + C_{ven}^B \omega_{ven}]/4$$  \hspace{1cm} (6.1)

$$MbO_2 = f_t C_t^B/4$$  \hspace{1cm} (6.2)

where $f_{b,m}$ is the microvascular volume fraction in muscle, $C_{art}^B$ and $C_{ven}^B$ are the bound O$_2$ concentration of arteriole and venules, respectively, and $C_{cap}^B$ and $C_t^B$ are the spatial average of the bound O$_2$ concentration in capillary and tissue, respectively. The $f_{b,m}$ is the microvascular volume fraction in muscle and is a function of the relative heme concentration changes detected by the
NIRS signal ($\Delta C_{\text{heme,N}}^\text{exp}$) (Koirala et al., 2021); $\omega_{\text{art}}$, $\omega_{\text{cap}}$, and $\omega_{\text{ven}}$ represent arteriole, capillaries and venule volume fractions in the microvascular compartment. Both $HbO_2$ and $MbO_2$ contribute to the NIRS signal $HbMbO_2$.

The deoxygenated Hb ($Hb$) and Mb ($Mb$) contributions to the NIRS signal $HbMb$ are computed as:

\[
HHb = f_{b,m} C_{b,Hb} - HbO_2
\]

\[
HMb = f_{t} C_{t,Mb}/4 - MbO_2.\]

The simulated oxygenated and deoxygenated heme group concentration of Hb and Mb relative to the rest condition are calculated as:

\[
\Delta HbO_2 = HbO_2 - HbO_2^R
\]

\[
\Delta MbO_2 = MbO_2 - MbO_2^R
\]

\[
\Delta HHb = HHb - HHb^R
\]

\[
\Delta HMb = HMb - HMb^R
\]

where ($R$) refers to rest. The contribution of Hb and Mb to the oxygenated ($HbMbO_2$) and deoxygenated ($HHbMb$) heme groups under different experimental conditions are quantified as:

\[
y_{HbO_2} = HbO_2/(HbO_2 + MbO_2); \quad y_{MbO_2} = 1 - y_{HbO_2}
\]

\[
y_{HHb} = HHb/(HHb + HMb); \quad y_{HMb} = 1 - y_{HMb}.
\]

Also, the contribution of Hb and Mb to the oxygenated ($\Delta HbMbO_2$) and deoxygenated ($\Delta HHbMb$) NIRS signals relative to the rest condition are quantified as:

\[
y_{\Delta HbO_2} = \Delta HbO_2/\Delta HbMbO_2; \quad y_{\Delta MbO_2} = 1 - y_{\Delta HbO_2}
\]

\[
y_{\Delta HHb} = \Delta HHb/\Delta HHbMb; \quad y_{\Delta HMb} = 1 - y_{\Delta HMb}.
\]
6.2.3. Model simulation

The experimental data are compared with model simulations. These data represent the venous oxygen concentration \( C^T_{ven} \), the normalized oxygenated \( \Delta HbMbO_{2,N} \) and deoxygenated \( \Delta HHbMb_N \) NIRS measurements, and the rate of muscle oxygen uptake determined as:

\[
\dot{V}O_2 = Q(t)[C^T_{art} - C^T_{ven}] \cdot V_{mus}
\]

obtained under different conditions. The oxygenated and deoxygenated NIRS signals are normalized to the maximal variation of the oxygenated and deoxygenated NIRS signals observed during ischemia (Sun et al., 2016), respectively:

\[
\Delta HbMbO_{2,N} = \frac{\Delta HbMbO_{2}}{\Delta HbMbO_{TS}} = \frac{(HbO_2^{T4} - HbO_2^{R}) + (MbO_2^{T4} - MbO_2^{R})}{(HbO_2^{I} - HbO_2^{R}) + (MbO_2^{I} - MbO_2^{R})}
\]

\[
\Delta HHbMb_N = \frac{\Delta HHbMb}{\Delta HHbMb^{TS}} = \frac{(HHb^{T4} - HHb^{R}) + (HMb^{T4} - HMb^{R})}{(HHb^{I} - HHb^{R}) + (HMb^{I} - HMb^{R})}
\]

where Hb and Mb contributions are computed at steady state for resting \( (R) \), ischemic \( (I) \) and trial \( (T4) \). It should be noted that Trial 5 \( (T5) \) indicates the trial corresponding to the ischemic condition (Sun et al., 2016).

6.3. Results

6.3.1. Model validation

The mathematical model was validated comparing the simulation and experimental data for tissue oxygenated \( \Delta HbMbO_{2,N} \) and deoxygenated \( \Delta HHbMb_N \) NIRS signals (Figure 6.2a), and venous O2 concentration \( C^T_{ven} \) (Figure 6.2b) obtained for different blood flow \( (Q) \) during contraction for T4.
The comparison shows the capability of the mathematical model in reproducing the experimental data for both blood and tissue domains. An increase of muscle O2 delivery \( (Q) \) increased the venous oxygen concentration, \( C_{\text{ven}}^T \), and decreased the amplitude of the NIRS signal changes. \( C_{\text{ven}}^T, \Delta HbMb_N \) and \( \Delta HbMbO_{2,N} \) showed a quasi-linear relationship (Figure 6.2) with \( Q \).

Figure 6.2. Model validation: comparison between simulation and experimental data for (a) oxygenated \( \Delta HbMbO_{2,N} \) and deoxygenated \( \Delta HHbMb_N \) NIRS signals, and (b) venous oxygenation \( C_{\text{ven}}^T \) for different blood flow.

6.3.2. Absolute and relative heme groups concentration changes from rest

With an increase of \( Q \), simulations predicted a quasi-linear increase of \( HbO_2 \) (Figure 6.3a) in capillary and venule compartments and non-linear increase of \( MbO_2 \). Also, with an increase of \( Q HHb \) was almost unchanged whereas \( HMb \) decreased nonlinearly (Figure 6.3b). The effect of
$Q$ on the amplitude of oxygenated ($\Delta HbO_2, \Delta MbO_2$, Figure 6.3c) and deoxygenated ($\Delta HHb, \Delta HMb$, Figure 6.3d) heme group concentration changes from rest were like that observed for the absolute heme group concentrations (Figure 6.3a and b). The amplitude of $HbO_2$ and $MbO_2$ increased whereas that of $\Delta HbO_2$ and $\Delta MbO_2$ decreased with $Q$. In contrast, both $HHb$ and $\Delta HHb$ increased with $Q$ and had a similar value because $HHb$ was negligible at rest. $HMb$ and $\Delta HMb$ values were similar because $HMb$ was negligible at rest.

Figure 6.3. Model simulations of the absolute oxygenated (a, $HbO_2, MbO_2$) and deoxygenated (b, $HHb, HMb$) heme groups as well as the model simulations of the relative oxygenated (c, $\Delta HbO_2$, $\Delta MbO_2$) and deoxygenated (d, $\Delta HHb, \Delta HMb$) heme groups concentration changes from rest at different $Q$. 
The relative change of the heme group concentration in the arteriole compartment was almost negligible because the oxygen concentration changes in the arteriole were negligible in comparison to the other compartments. The major Hb contribution to the NIRS was due to the capillary in the microvascular compartment (Figure 6.3c green line). The non-linearity of extravascular oxygenation was primarily due to the change of slope in extravascular O₂ saturation when \( Q \) decreases. During low or impaired O₂ delivery, the contribution towards the NIRS signal by the extravascular domain was significant because the imbalance between the O₂ delivery and utilization was prominent. This imbalance diminishes as the delivery is increased; thus, the amplitude in relative change of oxygenation in myocytes decreased (Figure 6.3c).

6.3.3. Hb and Mb contribution to the heme groups and NIRS signals

At low O₂ delivery, the concentrations of \( HbO₂ \) are higher in the microvascular domain than the extravascular domain, which resulted in a larger Hb contribution to the NIRS signal (\( yHbO₂ \), Figure 6.4a). As \( Q \) increased, the concentration of the oxygenated heme groups in both capillary (\( HbO₂ \)) and extravascular (\( MbO₂ \)) domains increased. Because \( MbO₂ \) changes were more sensitive than those of \( HbO₂ \), Hb contribution to the oxygenated heme groups decreased with an increase of \( Q \). An opposite trend is observed when the oxygenated heme group concentration changes relative to rest are considered. In this case, the Hb contribution to the NIRS signal (\( y\Delta HbO₂ \)) increased with \( Q \) because \( \Delta MbO₂ \) decreases with \( Q \).
Figure 6.4. The contribution of Hb to the absolute and relative (a) oxygenated heme group concentration ($y\Delta HbO_2$ vs $yHbO_2$) and (b) deoxygenated heme group concentration ($y\Delta HHb$ vs $yHHb$) for different $Q$.

At low $O_2$ delivery, the concentrations of $HMb$ in the extravascular domain were close to that in the microvascular domain (Figure 6.3b), which resulted in a similar Hb (55%) and Mb (45%) contribution to the deoxygenated heme groups ($yHHb$, Figure 6.4b). With an increase of $Q$, $yHHb$ increased because the $HMb$ decreased whereas $HHb$ remained almost unchanged. The effect of $Q$ on $yHHb$ was like that on $y\Delta HHb$ (Figure 6.4b) because the variation of deoxygenated heme group concentrations $HHb$ and $HMb$ with $Q$ was similar to that observed for the variations of the deoxygenated heme groups ($\Delta HHb$ and $\Delta HMb$) relative to rest (Figure 6.3b and 6.2d).

6.3.4. Effect of microvascular volume fraction ($f_{b,m}$)

The effects of $f_{b,m}$ on absolute and relative contributions of Hb were simulated in contracting muscle with $Q$ of 1.3 [L Kg$^{-1}$ min$^{-1}$] (Figure 6.5) with and without blood volume
changes. With an increase of $f_{b,m}$ both the Hb contribution to the absolute and relative concentration of oxygenated ($y_{HbO_2}$ and $y_{HHb}$) and deoxygenated ($y_{\Delta HbO_2}$ and $y_{\Delta HHb}$) heme groups increased (Figure 6.5a, b). In addition to the effect, due to a low $f_{b,m}$, a decrease of blood volume caused a larger decrease of $y_{HbO_2}$ (red solid line Figure 6.5a) than in the case with unchanged blood volume (red dashed line Figure 6.5a). In contrast, a decrease of blood volume caused a smaller decrease of the $y_{\Delta HbO_2}$ in comparison to the case with constant blood volume (black dashed line Figure 6.5a). The upward shift of the curve quantifying the relationship between $f_{b,m}$ and $y_{\Delta HbO_2}$ was due to an increase of the $\Delta HbO_2$. This increase was associated with the decrease of the microvascular blood volume from rest. The blood volume decrease contributed to a decrease in the hemoglobin concentration in the muscle region investigated. The total heme group’s concentration decreased when the microvascular blood volume change was considered. Indeed, the microvascular volume fraction decreased from rest to contraction by 1%, leading to a decrease of $y_{HbO_2}$ (Figure 6.4a). In contrast, $y_{\Delta HbO_2}$ had an opposite trend to $y_{HbO_2}$. When microvascular blood volume decreased, $y_{\Delta HbO_2}$ increased because the amplitude of the relative oxygenated heme group’s concentration changes from rest increased in the capillary. With low (i.e., rest) $f_{b,m}$ at the onset of contraction and in the presence of a decrease in blood volume, the contribution of Hb to both absolute and relative deoxygenated heme groups’ concentration (Figure 6.5b) was less than in the case with unchanged blood volume.
Figure 6.5. Effect of microvascular volume fraction on Hb contribution to the absolute and relative (a, $yHbO_2$, $y\Delta HbO_2$) oxygenated heme groups concentration; and to the absolute and relative deoxygenated heme groups concentration (a, $yHHb$, $y\Delta HHb$) with and without blood volume change.

6.4. Discussion

A mathematical model of muscle $O_2$ transport and utilization was validated with experimental data obtained under different muscle blood flow and used to analyze the Hb and Mb contribution to the oxygenated and deoxygenated heme group concentrations and their relative changes from rest. Simulations indicated the effect of blood flow on the Hb contribution to the absolute differs from that of the relative oxygenated heme group concentration changes. With an increase of blood flow, the Hb contribution to the relative concentration increased whereas the Hb contribution to the absolute concentration decreased.
6.4.1. Hb and Mb contribution to the absolute vs relative heme groups concentration

The decrease of $yHbO_2$ (Figure 6.4a) is due to a more sensitive increase of Mb oxygenation than Hb oxygenation in response to an increase in $O_2$ delivery. When blood flow was increased from 0.9 to 1.7 L kg$^{-1}$ min$^{-1}$, the oxygenated Mb increased more than twice (30 µM to 60 µM; Figure 6.3a, black line) whereas the capillary oxygenated Hb increased only 0.6 times (45 µM to 75 µM; Figure 6.3a, green line). This caused an increase of the Mb contribution in response to an increase of blood flow. On the other hand, the amplitude of the relative change in the oxygenated Mb was much smaller compared to Hb when the $O_2$ delivery increased. As a result, $y\Delta HbO_2$ increased.

6.4.2. Theoretical framework to overcome NIRS oximeter limitation

With the widespread application of the three main categories of NIRS oximeters (continuous wave, time domain, and frequency domain) to evaluate skeletal muscle metabolic function, new challenges related to the inconsistencies among the different instruments are emerging. Because of the semi-quantitative nature of the NIRS, there are experimental and device-specific factors accountable for measurement differences between NIRS devices. These uncertainties raise the need of new standards and tools to understand and minimize the inter-instrument inconsistencies.

To provide absolute heme group concentrations even from continuous wave (CW) NIRS, some approaches have been proposed (Pollonini, 2018; Rovati et al., 2004). Also, the approach proposed in our work integrates measurement of optical density by a CW NIRS and venous oxygenation with a mathematical model of $O_2$ transport and utilization to quantify absolute heme group concentrations from NIRS measurements. Simulations allowed us to analyze the Hb and Mb
contribution difference between absolute and relative oxygenated or deoxygenated heme groups concentrations. These two scenarios resemble those of a CW and frequency domain (FD) or time domain (TD) NIRS. Thus, our model can be used to analyze the difference between NIRS oximeters used in the analysis of the NIRS kinetics during muscle contraction (Pollonini, 2018).

CW NIRS assumes a constant differential path length factor (DPF) and provides only heme groups concentration changes from an arbitrary baseline, whereas TD and FD NIRS are capable of measuring the absolute concentrations of heme groups in the muscle region interrogated (Barstow, 2019). There is a need for standardization of measurement using either CW or TD and FD NIRS. Ideally, these devices should provide similar and comparable information about muscle oxygen kinetics. However, there exists discrepancy and inconsistency between different devices. Tissue oxygen saturation obtained using CW was reported to be significantly different than that from FD NIRS (Komiyama et al., 2001; van Essen et al., 2020). In another study, absolute concentration of oxygenated Hb obtained by CW NIRS and FD NIRS was similar, whereas the deoxygenated Hb in CW NIRS significantly differed from that observed with FD NIRS (Pollonini, 2018).
CHAPTER 7

RELATING DIFFERENTIAL PATHLENGTH FACTOR TO MICROVASCULAR AND EXTRAVASCULAR OXYGENATION

7.1. Introduction

Near-Infrared spectroscopy (NIRS) can be used non-invasively to measure muscle oxygenation to evaluate physiological and pathophysiological conditions (Grassi et al., 2019; Willingham & McCully, 2017). The oxygenation of the heme group concentrations can be measured from different types of spectrometers: continuous wave (CW) NIRS provides concentration changes relative to a baseline; frequency domain (FD) or time domain (TD) NIRS provide absolute concentration (Barstow, 2019; Ferrari et al., 2011; Grassi & Quaresima, 2016). Often, CW NIRS measurements are normalized to the concentration changes from rest to ischemia.

The CW NIRS devices are limited by the uncertainties related to the differential path length factor (DPF) which is used in the Beer-Lambert law to quantify oxygenated \((\Delta HbMBO_2)\) and deoxygenated \((\Delta HHbMb)\) heme groups’ concentration changes from the measurement of the optical density at different wavelengths. The estimates of the concentration changes can be either overestimated or underestimated (Endo et al., 2021; Pirovano et al., 2021) because the concentrations are based on a constant DPF although it can vary with the experimental conditions and during contraction. The typical range of values for DPF in brain and skeletal muscle tissues are: gastrocnemius (5.8 -5.3), forearm (4.4-3.9), adult head (6.5-5.9) and infant head (5.4-4.7) (Duncan et al., 1995; Ferrari et al., 1992). It has been reported that with a larger adipose tissue
layer DPF is higher for forearm and calf muscle (Barstow, 2019; Duncan et al., 1995; Ferrari et al., 1992). Thus, accurate DPF should be determined for each individual muscle group, taking into consideration the thickness of adipose tissue present (Pirovano et al., 2021), water content (Ferreira et al., 2007), and optical properties such as attenuation of light due to absorption and scattering (Barstow, 2019; Endo et al., 2021; Ferreira et al., 2007; Pirovano et al., 2021) in the muscle.

Besides the challenge related to the estimate of DPF which might in part be overcome with TD and FD NIRS spectrophotometers, the application of this technology is limited by the semi-quantitative nature of the signal obtained either with CW or TD and FD NIRS spectrophotometers. Specifically, the absolute or relative oxygenated ($HbMBO_2$) and deoxygenated ($HHbMb$) concentrations measured by NIRS devices result from the contribution of the oxygen content within an unknown microvascular and extravascular volume distribution. In addition, these distribution changes during exercise might be altered under disease states. Thus, the quantitative relationship between the NIRS signal and the heterogeneous oxygen distribution in the muscle region investigated is not known whereas quantitative calibration of the signal is not available yet.

A quantitative approach based on a mathematical model of oxygen transport and metabolism has been proposed to quantify the contribution of Hb and Mb contribution to the NIRS signals (Koirala et al., 2021; Lai et al., 2009). The analysis was based on concentration changes normalized to the changes from rest to ischemia because DPF was unknown. Nevertheless, this approach has not been applied to analyze absolute and relative concentration change measured by NIRS.

We propose to use a physiologically based model of oxygen transport and utilization to relate optical density measured by NIRS to the oxygenated/deoxygenated heme group concentration changes in the microvascular (arteriole, capillary, venules) and extravascular
compartments. To demonstrate this quantitative relationship the analysis was based on NIRS data obtained from a canine model of oxidative metabolism to avoid adipose tissue effects on the NIRS signals.

In this study, the computational model was used to: 1) estimate DPF using optical density measurements obtained for different blood flow; 2) determine, using the estimated DPF, whether the mathematical model can predict NIRS measurements obtained with independent experiments from those used to estimate DPF; and 3) analyze the effect of microvascular volume fraction on DPF estimation.

7.2. Methods

7.2.1. Animal model

The experimental data obtained from an animal model of oxidative metabolism reported in previous studies (Goodwin et al., 2011; Hernández et al., 2010; Sun et al., 2016) were used to estimate and validate the DPF. It should be noted that the trial or experiment notation of this work is the same as that used in the experimental works (Goodwin et al., 2011; Hernández et al., 2010; Sun et al., 2016) from which the NIRS data are used for the analysis proposed here (Table 7.1).

All experimental data were averaged from 5 or 6 measurements for each trial condition. A continuous-wave NIRS system (Oxymon Mk III, Artinis Medical Systems BV) was used to measure the optical density ($OD_{\lambda_1}$ and $OD_{\lambda_2}$) at two different wavelengths ($\lambda_1 = 760$ and $\lambda_2 = 860$ nm). The light at two different wavelengths (760 and 860 nm) was emitted and received by two fiber-optic bundles. The optodes were placed over the medial head of the left gastrocnemius and held in place with an elastic band.
Table 7.1. Experimental condition of each trial.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Blood Flow</th>
<th>Metabolic rate</th>
<th>Work</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Contraction frequency</td>
<td></td>
</tr>
<tr>
<td>B1/B2</td>
<td>Self-perfused</td>
<td>0.66 Hz</td>
<td>(Hernández et al., 2010)</td>
</tr>
<tr>
<td>CT20</td>
<td>Pump-perfused</td>
<td>0.5 Hz</td>
<td>(Goodwin et al., 2011)</td>
</tr>
<tr>
<td>EX45</td>
<td>Pump-perfused</td>
<td>0.5 Hz</td>
<td>(Goodwin et al., 2011)</td>
</tr>
<tr>
<td>EX70</td>
<td>Pump-perfused</td>
<td>0.5 Hz</td>
<td>(Goodwin et al., 2011)</td>
</tr>
<tr>
<td>T3</td>
<td>Pump-perfused</td>
<td>0.5 Hz</td>
<td>(Sun et al., 2016)</td>
</tr>
<tr>
<td>T4</td>
<td>Pump-perfused</td>
<td>0.66 Hz</td>
<td>(Sun et al., 2016)</td>
</tr>
</tbody>
</table>

7.2.2. Estimation of DPF

A mathematical model of skeletal muscle oxygen transport and metabolism proposed in Chapters 4, 5 and 6 and in another study (Koirala et al., 2021) was used to estimate the DPF of the continuous wave NIRS used to measure the muscle oxygenation under different experimental conditions. The derivation of the equations to calculate the physiological variables of interest are based on previous models (Lai et al., 2007; Spires et al., 2013; Spires et al., 2012) with some modifications reported in Chapter 4.

The oxygenated Hb \( (HbO_2) \) and Mb \( (MbO_2) \) concentrations in the microvascular and extravascular compartments of the muscle are computed as:

\[
HbO_2 = f_{b,m} \left[ \frac{C_{art}^B \omega_{art} + C_{cap}^B \omega_{cap} + C_{ven}^B \omega_{ven}}{4} \right] 
\]

\[
MbO_2 = f_t \frac{C_t^B}{4}. 
\]
Similarly, the deoxygenated Hb ($HHb$) and Mb ($HMb$) contributions to the NIRS signal $HHbMb$ are computed as:

$$HHb = f_{b,m} C_{b,Hb} - HbO_2 \tag{7.3}$$
$$HMb = f_{t} C_{t,Mb}/4 - MbO_2. \tag{7.4}$$

The simulated oxygenated and deoxygenated heme group concentrations of Hb and Mb relative to the rest condition are calculated as:

$$\Delta HbO_2 = HbO_2 - HbO_2^R \tag{7.5}$$
$$\Delta MbO_2 = MbO_2 - MbO_2^R \tag{7.5}$$
$$\Delta HHb = HHb - HHb^R \tag{7.6}$$
$$\Delta HMb = HMb - HMb^R$$

where ($R$) refers to rest. The total oxygenated ($\Delta HbMbO_2$) and deoxygenated ($\Delta HHbMb$) heme groups relative to the resting conditions are then computed as:

$$\Delta HbMbO_2 = \Delta HbO_2 + \Delta MbO_2 \tag{7.7}$$
$$\Delta HHbMb = \Delta HHb + \Delta HMb. \tag{7.8}$$

The simulated $\Delta HbMbO_2$ and $\Delta HHbMb$ concentrations are used to calculate the optical density with the modified Beer-Lambert law for Oxymon Mk III, Artinis Medical Systems BV as follows:

$$\Delta OD_{\lambda 1} = \varepsilon_{HHbMb\lambda 1} \cdot \Delta HHbMb \cdot L \cdot DPF + \varepsilon_{HbMbO_2\lambda 1} \cdot \Delta HbMbO_2 \cdot L \cdot DPF \tag{7.9}$$
$$\Delta OD_{\lambda 2} = \varepsilon_{HHbMb\lambda 2} \cdot \Delta HHbMb \cdot L \cdot DPF + \varepsilon_{HbMbO_2\lambda 2} \cdot \Delta HbMbO_2 \cdot L \cdot DPF \tag{7.10}$$

where, $OD_{\lambda 1}$ and $OD_{\lambda 2}$ are the optical density at two different wavelengths $\lambda 1$ and $\lambda 2$, respectively; $\varepsilon_{HbMbO_2\lambda 1}$ (1.207 mM$^{-1}$ cm$^{-1}$) and $\varepsilon_{HHbMb\lambda 1}$ (0.7977 mM$^{-1}$ cm$^{-1}$) are the extinction
coefficient for $HbMbo_{2}$ and $HHbMb$ at $\lambda_1$ and $\varepsilon_{HbMbo_{2}\lambda_2}$ (0.7613 mM$^{-1}$ cm$^{-1}$) and $\varepsilon_{HHbMb\lambda_2}$ (1.0173 mM$^{-1}$ cm$^{-1}$) are the extinction coefficient for $HbMbo_{2}$ and $HHbMb$ at $\lambda_2$.

7.2.3. Microvascular blood volume change

The oxygenated and deoxygenated NIRS measurements are integrated in the mathematical model to quantify microvascular blood volume changes associated with the heme concentration changes detected by NIRS. For each physiological condition investigated, it is assumed that the measured blood volume changes obtained by converting optical density are the same as the blood volume change simulated by the mathematical model by estimating the DPF.

$$\Delta C_{Heme}^{\text{exp}} = \Delta HbMbo_{2exp} + \Delta HHbMb_{exp} = \Delta C_{Heme}^{mod} = \Delta C_{Hb} + \Delta C_{Mb}. \tag{7.11}$$

The relative Hb and Mb concentration changes can be related to the microvascular blood volume fraction:

$$\Delta C_{Hb} = \Delta f_{b,m} C_{b,Hb}; \Delta C_{Mb} = \frac{\Delta f_{C_{b,Mb}}}{4} \tag{7.12}$$

$$\Delta C_{Hb} + \Delta C_{Mb} = (f_{b,m} - f_{b,m}^R) C_{b,Hb} + \frac{(f_t - f^R_t) C_{t,Mb}}{4} \tag{7.13}$$

$$\Delta C_{Hb} + \Delta C_{Mb} = (f_{b,m} - f_{b,m}^R)(C_{b,Hb} - C_{t,Mb}/4). \tag{7.14}$$

Combining Eq. 7.11 with Eq. 7.14 yields

$$f_{b,m}(\Delta C_{Heme}^{\text{exp}}) = f_{b,m}^R + \frac{\Delta C_{Heme}^{\text{exp}}}{(C_{b,Hb} - C_{t,Mb}/4)}. \tag{7.15}$$

The microvascular volume fraction in muscle ($f_{b,m}$) at rest is calculated by the product of the blood volume fraction in muscle ($f_b$) and microvascular volume fraction in blood ($f_m$) at rest. Since $f_{b,m}$
changes are assumed to take place in capillaries, the volume change takes place in the microvascular compartment \((V_{b,m})\):

\[
f_{b,m} = \frac{V_{b,m}}{V_t + V_{b,m} + V_{b,v}}. \tag{7.16}
\]

The \(V_{b,m}\) change from rest (R) to each trial condition investigated is computed as:

\[
V_{b,m} = V_{b,m}^R + \Delta V_{cap} = V_{b,m}^R + V_{cap} - V_{cap}^R. \tag{7.17}
\]

When blood volume changes occur, the extravascular \((V_e)\), vascular \((V_{b,v})\), as well as arteriole \((V_{art})\) and venules \((V_{ven})\) volume remains constant at the resting value (See Table 7.2). Equations 7.16 and 7.17 can be rearranged as:

\[
V_{cap} = f_{b,m} \frac{(V_e + V_{b,v})}{(1 - f_{b,m})} - [V_{b,m}^R - V_{cap}^R] = \frac{f_{b,m} (V_e + V_{b,v})}{(1 - f_{b,m})} - [V_{art} + V_{ven}]. \tag{7.18}
\]

Once the capillary volume is determined, the total microvascular volume can be calculated \((V_{b,m} = V_{art} + V_{cap} + V_{ven})\); thus, the arteriole, capillary, and venule volume fractions are:

\[
\omega_{art} = \frac{V_{art}}{V_{b,m}}; \quad \omega_{cap} = \frac{V_{cap}}{V_{b,m}}; \quad \omega_{ven} = \frac{V_{ven}}{V_{b,m}}. \tag{7.19}
\]

The optimal value of \(DPF\) is that which minimizes the least-square objective function:

\[
f(dp) = \sum_{i=1}^{n} (OD_{exp,i} - OD_{mod,i})^2 \tag{7.20}
\]

where \(n\) indicates the number of available experimental data points.

The minimization is accomplished by numerical optimization using the ‘lsqcurvefit’ library in MATLAB. The algorithm allows us to impose the constraints using lower and upper bounds. The estimation of DPF is obtained with the T3 data. The optimal estimate of DPF from this data is applicable to simulations of all experimental conditions in Table 7.1. After the parameter
estimation, the mathematical model was used to predict the optical density for the NIRS measurements at steady states obtained for T4, B1/B2, CT20, EX45 and EX70 (Goodwin et al., 2011; Hernández et al., 2010; Sun et al., 2016).

Table 7.2: Vascular, extravascular, and microvascular volume distribution at rest

<table>
<thead>
<tr>
<th>Notation</th>
<th>Description</th>
<th>Unit</th>
<th>Rest (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{mus}$</td>
<td>Volume of muscle</td>
<td>[g]</td>
<td>62.5</td>
</tr>
<tr>
<td>$f_b$</td>
<td>fraction of systemic blood in muscle</td>
<td>[%]</td>
<td>7</td>
</tr>
<tr>
<td>$f_t$</td>
<td>fraction of tissue in muscle</td>
<td>[%]</td>
<td>93</td>
</tr>
<tr>
<td>$f_m$</td>
<td>fraction of microvascular blood in systemic blood</td>
<td>[%]</td>
<td>85</td>
</tr>
<tr>
<td>$f_{b,m}$</td>
<td>fraction of microvascular blood in muscle</td>
<td>[%]</td>
<td>5.95</td>
</tr>
<tr>
<td>$\omega_{art}$</td>
<td>fraction of arterioles in microvascular blood</td>
<td>[%]</td>
<td>10</td>
</tr>
</tbody>
</table>

7.3. Results

7.3.1. Parameter estimation

The DPF parameter value was estimated using the optical density measured in T3 for different blood flow. As model inputs in these simulations, the parameter ($k_{ATPase}$) associated with the energy demand was used to simulate the changes in $\dot{V}O_2$ (Figure 7.1a), and the total heme group concentration change was used to simulate the microvascular blood volume changes (Figure 7.1b).
Figure 7.1. Model inputs: simulated (black line) and experimental data (symbols) of (a) muscle oxygen uptake ($\dot{V}O_2$) and relative (b) heme group concentration changes ($\Delta C_{\text{Heme}}$) for T3. $k_{\text{ATPase}}$, (grey line, c) is associated to the metabolic rate to simulate $\dot{V}O_2$ with $Q$; and the microvascular volume fraction ($f_{b,m}$) changes with $Q$ are estimated from $\Delta C_{\text{Heme}}$ (Eq. 7.15)
Figure 7.2. Comparison (a) between model simulation (solid line) and experimental data (filled circles) for optical density ($OD_{A1}$ and $OD_{A2}$) at different blood flow ($Q$), and between model simulation and experimental data for oxygenated ($\Delta HbMbO_2$) and deoxygenated ($\Delta HHbMb$) heme group concentrations changes. By fitting simulated optical density $OD_{A1}$ and $OD_{A2}$ to experimental data (Figure 7.2a) obtained during different blood flow (T3), the optimal estimate was obtained for DPF (2.1). The
corresponding oxygenated ($\Delta HbMbo_2$) and deoxygenated ($\Delta HbMb$) heme group concentration changes were reported in Figure 7.2b.

Figure 7.3. Model inputs: simulated (black line) and experimental data (symbols) of (a) muscle oxygen uptake ($V_{O2}$) and relative (b) heme group concentration changes ($\Delta C_{Heme}$) for T4; simulated (black line) and experimental data (symbols) of (c) $V_{O2}$ and (d) $\Delta C_{Heme} \cdot k_{ATPase}$, (grey line, c) is associated to the metabolic rate to simulate $V_{O2}$ and $f_{b,m}$ is the microvascular volume fraction with $Q$. 
The capability of the mathematical model to reproduce the experimental data was further tested simulating the NIRS data of T4, CT20, EX45, EX70 and B1&B2 obtained for different blood flow (Q) during contraction. For these simulations, the parameter \( k_{ATPase} \) associated with the energy demand was used to simulate the changes in \( \dot{V}O_2 \) (Figure 7.3a, c), and total heme group concentration change was used to simulate the microvascular blood volume changes (Figure 7.3b, d).

In the simulations, the previously estimated value of DPF and other common parameters were kept constant. The model predicted well the \( \Delta HbMbO_2 \) and \( \Delta HbMb \) and the optical density \( OD_{A1} \) and \( OD_{A2} \) each blow flow of the trial T4, CT20, EX45, EX70, B1 and B2. The optical densities were obtained by converting the concentration changes using the modified Beer-Lambert law. The model predicted well the changes of \( \Delta HbMbO_2 \) and \( \Delta HbMb \) (Figure 7.4a; c) with \( O_2 \) delivery (Q) changes. The higher the \( O_2 \) delivery, the lower the amplitude of the oxygenated and deoxygenated heme groups relative to the resting conditions. In both cases, the amplitude of \( OD_{A1} \) decreased and \( \Delta OD_{A2} \) increased slightly in response to an increase of \( O_2 \) delivery.
Figure 7.4. Comparison between model prediction and experimental data for (a, c) oxygenated ($\Delta HbMbo_2$) and deoxygenated ($\Delta HHbMb$) heme groups concentration changes and (b, d) optical density ($OD_{\lambda_1}$ and $OD_{\lambda_2}$) for different blood flow ($Q$) during contraction (Goodwin et al., 2011; Hernández et al., 2010; Sun et al., 2016).

7.3.2. Effect of microvascular blood volume on DPF

Because of the microvascular volume fraction $f_{b,m}$ uncertainties, we analyzed the effects of $f_{b,m}$ on the estimates of DPF. With a decrease of $f_{b,m}$ from 7 to 3%, the estimated value of DPF decreased from 1.5 to 3 (Figure 7.5).
Experiential evidence showed that DPF is affected by blood volume changes; thus, an alternative strategy to that of considering all T3 data is evaluating the potential effects of blood volume on DPF. Specifically, the mathematical model was used to estimate DPF for two different blood flow and volume changes in T3 reported in Table 7.3. For an increase in blood volume of 8 μM (from -42 to -34 μM), the DPF decreased by 3%. The negative value of the blood volume changes indicates that in T3 the blood volume decreases from rest to contraction (Fig. 7.3b).
Table 7.3 Effect of blood volume changes on the estimate of DPF

<table>
<thead>
<tr>
<th>Blood flow L kg⁻¹m⁻¹</th>
<th>Blood volume changes μM</th>
<th>DPF -a</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.82</td>
<td>-42.5</td>
<td>2.1</td>
</tr>
<tr>
<td>1.25</td>
<td>-34.1</td>
<td>2.04</td>
</tr>
</tbody>
</table>

7.4. Discussion

A mathematical model of muscle O₂ transport and metabolism was used to estimate the DPF for measurements of oxygenated and deoxygenated heme groups’ concentration changes measured with a constant wave oximeter. With the estimated DPF, model simulations well predicted the optical density of NIRS data measured under different experimental conditions. This result indicates the capability of the model to quantify the NIRS data and relate it to O₂ transport and utilization conditions and suggests that the approach used can overcome the semi quantitative nature of the NIRS with sufficient information about the microvascular volume distribution.

7.4.1. Blood volume effect on DPF

Model simulations showed a decrease in DPF with an increase of microvascular blood volume (Table 7.3). This finding is consistent with other experimental studies that reported a reduced optical path length with an increase in blood volume during exercise (Endo et al., 2021) or cuff occlusion (Ferrari et al., 1992; Hammer et al., 2019). In particular, for a blood volume increase of 28 μM detected by a Time-resolved NIRS during contraction, DPF decreased by 5-10% (Endo et al., 2021). Our analysis showed that an increase of microvascular volume
corresponding to 8 μM determined a decrease in DPF of approximately 3%. Thus, the quantitative relationship between blood volume and DPF predicted by our model is close to that observed in the experimental study. Also, another human study attributed a DPF decrease of 7% to an increase in blood volume during cuff occlusion (Ferrari et al., 1992). The relative changes observed in these studies are consistent with those quantified by the mathematical model proposed.

7.4.2. Differential pathlength factor

Besides the motion artifact related to the muscle contractions, the DPF is affected by the optical properties of the skeletal muscle including extravascular tissue (myocytes), adipose tissue, and blood volume. The depth of light penetration into the tissue has been reported to be in the range between 4-6 and varies among different tissues (Chance et al., 1992). The DPF value (2.1) estimated in our work is lower than the values reported in the literature. In a human NIRS study, DPF for skeletal muscle was reported to be 4.3 (Ferrari et al., 1992). A possible difference between our DPF value and those reported in several studies could be related to the absence of adipose tissue in the skeletal muscle region investigated in our animal model. Indeed, the NIRS probe was placed directly on the skeletal muscle. In support of this view, several studies reported a relationship between the adipose tissue layer and DPF. In a study on humans’ vastus lateralis, DPF was linearly correlated with adipose tissue thickness (Pirovano et al., 2021). According to this relationship, in the absence of adipose tissue, the DPF value is expected to be 2.6 which is close to that estimated in our work. Furthermore, another NIRS study on heart muscle reported a DPF value of 1.8 (Stephen et al., 2005).

Another uncertainty about the estimation of DPF is related to the microvascular volume distribution which was assumed to simulate the NIRS signals. Because DPF is particularly
sensitive to $f_{b,m}$ (Figure 7.5), even small variation of $f_{b,m}$ determined important changes in the estimate of DPF. Thus, our finding should be confirmed with more accurate information on $f_{b,m}$ even though $f_{b,m}$ used in the simulations is consistent with the value range reported in the literature. Existing experimental techniques such as MRI (Hindel et al., 2017) can be used to acquire quantitative information on the microvascular volume in skeletal muscle.

Another important factor that could affect the estimate of DPF is the microvascular hematocrit which has been reported to be 50% (20-30%) of the systemic hematocrit (45-50%). In a NIRS study, this hematocrit has been considered to affect the NIRS signals (Davis & Barstow, 2013). Regardless of these current limitations in estimating DPF, it should be noted that the model is capable of predicting different experimental conditions with the same DPF, indicating that a unique DPF value is consistent with all NIRS measurements. The results of our simulations of the NIRS signal based on a volume average of the heme group concentration within the microvascular and extravascular compartments suggest that it is possible to overcome the semi-quantitative nature of the NIRS signal with accurate measurements of the microvascular blood volume fraction and distribution in skeletal muscles. The analysis of the NIRS data reported in Chapter 5 indicated a primary role of capillary oxygen saturation in determining the sensitivity of the oxygenated and deoxygenated NIRS signal to blood volume change.

Our NIRS experimental data were obtained assuming constant optical path length within the range of experimental conditions considered. It has been suggested that the optical path length varies during contraction; thus, the current assumption of considering a constant DPF would underestimate both NIRS signals (Endo et al., 2021). Further computational analysis could be performed with our mathematical model in combination with measurements of DPF.
CHAPTER 8

CONCLUSION AND FUTURE DIRECTIONS

8.1. Summary of Conclusion

A mathematical model of oxygen transport and metabolism in skeletal muscle was integrated with invasive and non-invasive measurements of oxygen content in blood and tissue domains to analyze the NIRS signals for different blood volume and oxygen delivery. The model was validated by testing its ability to predict the data obtained under different experimental conditions. Based on model simulations, the outcomes of the computational analysis are:

- Oxygen delivery has a key role in determining the effect of blood volume on NIRS signals. The deoxygenated NIRS signal is not sensitive to blood volume changes when the microvascular oxygen saturation is above 80%. This saturation value can be obtained with high oxygen delivery. On the other hand, when oxygen delivery is impaired, the microvascular oxygen saturation decreases and both oxygenated and deoxygenated NIRS signals are sensitive to the blood volume changes. This finding is particularly significant when evaluating oxygen extraction in skeletal muscle of patients under different pathophysiological conditions. Generally, under these conditions, oxygen delivery either by convection and/or diffusion is impaired. Thus, it is suggested that the blood volume changes should be quantified to properly evaluate muscle oxygen extraction in disease states.

- Mb contribution to both NIRS signals is significant even in the presence of a linear relationship between venous oxygen concentration and NIRS signals. Under physiological
or pathophysiological conditions characterized by reduced oxygen delivery, the oxygen saturation in microvascular and extravascular compartments is also reduced and leads to a high Mb contribution to the NIRS signal.

- The effects of oxygen delivery on Hb and Mb contributions to the absolute oxygenated and deoxygenated heme group concentrations differ from those to the relative oxygenated and deoxygenated heme group concentrations. This finding is particularly important in comparing the muscle oxygen extraction kinetics obtained with constant wave and time domain or frequency domain intensity-modulated NIRS oximeters. The popularity of these devices presents new challenges related to the need to quantitatively compare the measurements obtained with oximeters that use different technologies.

- The integrative approach proposed allows us to estimate the differential pathlength factor (DPF) by relating the tissue oxygenation measurement by NIRS to the microvascular and extravascular volume distribution. This unique DPF predicts the oxygenated and deoxygenated heme group concentration changes from rest for different blood flow during contraction. This approach was further tested in predicting the oxygenated and deoxygenated heme group concentrations obtained with additional and independent experiments. This finding indicates that it is possible to relate the heme group concentration measured by the NIRS device to the microvascular and extravascular volume distribution within the muscle region investigated.

8.2. Future directions

The permeability surface area (PS) changes during muscle contraction are quantified in the model with a phenomenological expression relating blood flow to PS. The PS parameter is a key
regulator of the dynamic response of oxygen diffusion to contraction. The mathematical model proposed predicts well the oxygenated and deoxygenated signals in contracting muscle at steady state, but it is less accurate for resting muscle at different blood flow. A quantitative relationship that describes more closely the mechanisms leading to an increase of PS with or without contraction should be considered to improve the model predictions of the NIRS kinetics. It should be further tested with NIRS kinetics under different experimental conditions.

Another aspect that was not considered in the mathematical model proposed was the difference between the microvascular and systemic hematocrit and how they change with an increase in blood flow with or without contraction. These features can be included and tested with additional modification to the mathematical model.

**8.3. Model applications**

Currently, NIRS measurements are used for semi-quantitative information about the muscle oxygen extraction. In this work, the integrative approach proposed can be used to estimate the kinetics of muscle oxygen consumption from NIRS measurements. This approach overcomes the NIRS uncertainties related to the unknown contribution of the microvascular and extravascular compartment to the NIRS signals. Furthermore, the mathematical model presented here can be used to assess how oxygen delivery impairment can affect the NIRS signals and their components. The mathematical model can be used as a complementary tool for diagnosis to quantify the transport and metabolic limitations in pathophysiological conditions and to explore therapeutical protocols or to assess the impact of a treatment.
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APPENDIX A

Figure A.1. A: Oxygen uptake ($\dot{V}O_2$) and B: blood flow ($Q$) during two bouts of contraction (Hernández et al., 2010). Copyright information in Appendix F.9.
Figure A.2. NIRS muscle oxygenation data, A: change in oxygenated heme groups, B: change in deoxygenated heme groups (Hernández et al., 2010). Copyright information in Appendix F.9.
Figure A.3. Experimental data from (Sun et al., 2016). a, d and e: Venous oxygenation, b, e and h oxygenated NIRS signal normalized to the maximal oxygenated signal obtained during ischemic conditions, c, f and i: deoxygenated NIRS signal normalized to the maximal deoxygenated signal obtained during ischemic condition. a, b and c represent Trial 1, d, e and f represent Trial 2, g, h and i represent Trial 3.
Figure A.4. a: Blood flow ($Q$) and b: oxygen uptake ($V\dot{O}_2$) for different experimental settings-CT20, EX45 and EX70 (Goodwin et al., 2011). Copyright information in Appendix F.10.

Figure A5. Oxygenated NIRS signal $\Delta[HbMbO_2]$, deoxygenated NIRS signals $\Delta[HHbMb]$ and total NIRS signal $[HbMb_{tot}]$ for a: CT20, b: EX45 and c: EX70 (Goodwin et al., 2011). Copyright information in Appendix F.10.
Appendix A.6. Schematic representation of extended mechanistic model used in this study (Spires et al., 2012). Copyright information in Appendix F.11. All the ODEs and fluxes are reported in appendix B and C, respectively.
APPENDIX B

Dynamic Mass Balance

The model is spatially distributed in both blood and tissue compartments. All metabolites are present in the tissue compartment. The blood compartment only includes $O_2$, lactate, and pyruvate.

**Blood compartment**

\[
 f_b \frac{\partial c_{O_2,b}^c}{\partial t} = -Q \frac{\partial c_{O_2}^c}{\partial y} + D_{O_2,b} \frac{\partial^2 c_{O_2,b}^c}{\partial y^2} - \frac{j_{O_2}^{b,c}}{\gamma_{O_2,b}} \quad B.1
\]

\[
 f_b \frac{\partial c_{lac,b}^c}{\partial t} = -Q \frac{\partial c_{lac}^c}{\partial y} - j_{lac}^{b,c} \quad B.2
\]

\[
 f_b \frac{\partial c_{pyr,b}^c}{\partial t} = -Q \frac{\partial c_{pyr}^c}{\partial y} - j_{pyr}^{b,c} \quad B.3
\]

**Tissue compartment**

\[
 f_c \frac{\partial c_{O_2,c}^c}{\partial t} = \frac{j_{O_2}^{b,c}}{\gamma_{O_2,c}} + D_{O_2,c} \frac{\partial^2 c_{O_2,c}^c}{\partial y^2} - \frac{f_c \phi_{OXPhos}}{\gamma_{O_2,c}} \quad B.4
\]

\[
 \frac{\partial c_{lac,c}}{\partial t} = \frac{j_{lac}^{b,c}}{f_c} + \phi_{LDH} \quad B.5
\]

\[
 \frac{\partial c_{pyr,c}}{\partial t} = \frac{j_{pyr}^{b,c}}{f_c} + \phi_{PK} - \phi_{LDH} \quad B.6
\]

\[
 \frac{\partial c_{gap}}{\partial t} = \Psi_{GP} - \phi_{PGLM} \quad B.7
\]

\[
 \frac{\partial c_{g6p}}{\partial t} = \phi_{PGLM} - \phi_{PGI} \quad B.8
\]

\[
 \frac{\partial c_{f6p}}{\partial t} = \phi_{PGI} - \phi_{PFK} \quad B.9
\]

\[
 \frac{\partial c_{f6p}}{\partial t} = \phi_{PFK} - \phi_{ALD} \quad B.10
\]

\[
 \frac{\partial c_{dihap}}{\partial t} = \phi_{ALD} + \phi_{TPI} \quad B.11
\]

\[
 \frac{\partial c_{gap}}{\partial t} = \phi_{ALD} - \phi_{TPI} - \phi_{GAPDH} \quad B.12
\]

\[
 \frac{\partial c_{13bpg}}{\partial t} = \phi_{GAPDH} - \phi_{PGK} \quad B.13
\]
\[ \frac{\partial C_{3pg}}{\partial t} = \phi_{PGK} - \phi_{PGM} \]  
B.14

\[ \frac{\partial C_{2pg}}{\partial t} = \phi_{PGM} - \phi_{ENOL} \]  
B.15

\[ \frac{\partial C_{pep}}{\partial t} = \phi_{ENOL} - \phi_{PK} \]  
B.16

\[ \frac{\partial C_{i}}{\partial t} = -\psi_{GP} - \phi_{GAPDH} + \phi_{ATPase} - 6\phi_{OxPhos} \]  
B.17

\[ \frac{\partial C_{ADP}}{\partial t} = \phi_{PFK} - \phi_{PGK} - \phi_{PK} + 2\phi_{ADK} + \phi_{CK} + \phi_{ATPase} - 6\phi_{OxPhos} \]  
B.18

\[ \frac{\partial C_{AMP}}{\partial t} = -\phi_{ADK} \]  
B.19

\[ \frac{\partial C_{Cr}}{\partial t} = \phi_{CK} \]  
B.20

\[ \frac{\partial C_{NADH}}{\partial t} = \phi_{GAPDH} - \phi_{LDH} \]  
B.21
Oxygen Equilibrium

O$_2$ can be either free or bound to hemoglobin or myoglobin. The sum of free and bound O$_2$ is total O$_2$ concentration:

$$C^{T}_{O_2,x} = C^{B}_{O_2,x} + C^{F}_{O_2,x}, \ x=b, c \quad \text{C.1}$$

Free and bound O$_2$ concentrations are related by local chemical equilibria. In blood, the relationship is:

$$C^{B}_{O_2,b} = 4Hct \ C_{rbc,Hb} \frac{K_{HB}(C^{F}_{O_2,b})^{nH}}{1+K_{HB}(C^{F}_{O_2,b})^{nH}} \quad \text{C.2}$$

and in tissue the relationship is:

$$C^{B}_{O_2,c} = W_mC_{mc,Mb} \frac{K_{Mb}C^{F}_{O_2,b}}{1+K_{Mb}C^{F}_{O_2,b}} \quad \text{C.3}$$

Transport Fluxes

The axial fluxes for the substrates present in both the blood and tissue compartments: O$_2$, lactate, and pyruvate.

$$J^{b,c}_{O_2} = PS(C^{F}_{O_2,b} - C^{F}_{O_2,c}) \quad \text{C.4}$$

$$J^{b,c}_{lac} = T_{lac} \left( \frac{c_{lac,b}}{M_{lac} + c_{lac,b}} - \frac{c_{lac,c}}{M_{lac} + c_{lac,c}} \right) \quad \text{C.5}$$

$$J^{b,c}_{pyr} = T_{pyr} \left( \frac{c_{pyr,b}}{M_{pyr} + c_{pyr,b}} - \frac{c_{pyr,c}}{M_{pyr} + c_{pyr,c}} \right) \quad \text{C.6}$$

Metabolic Fluxes

Most of these fluxes are calculated using ordered bi-bi Michaelis-Menten kinetics. The exceptions are the creatine kinase flux, the oxidative phosphorylation flux, and the ATPase flux.

The metabolic flux expression for each reaction is reported as follows:
Glycogen Phosphorylase:

\[
\phi_{GP} = \frac{V_{\text{max},f,GP}c_{\text{glyc}}c_{\text{Pyruvate}}}{K_{\text{glyc}}K_{f}} - \frac{V_{\text{max},r,GP}c_{\text{glyc}}c_{\text{Pyruvate}}}{K_{\text{glyc}}K_{r}}
\]

\[
\phi_{GP} = \frac{V_{\text{max},f,GP}c_{\text{glyc}}c_{\text{Pyruvate}}}{1 + \frac{c_{\text{glyc}}}{K_{\text{glyc}}} + \frac{c_{\text{Pyruvate}}}{K_{f}}}
\]

C.7

C.8

where glycogen phosphorylase flux \(\Psi_{GP}\) accounts for isozyme form A and B with \(\omega_a\) and \(\omega_b\) fractions as follows:

\[
\Psi_{GP} = \omega_a \phi_{GP} + \omega_b \phi_{GP} = (1 - \omega_b) \phi_{GP} + \omega_b \phi_{GP}
\]

C.9

It is assumed that there is no phosphorylase isozyme conversion from one form (i.e., a, b) to the other during contraction.

Phosphoglucomutase:

\[
\phi_{PGLM} = \frac{V_{\text{max},f,PGLM}c_{\text{glyc}}}{K_{\text{glyc}}} - \frac{V_{\text{max},r,PGLM}c_{\text{glyc}}}{K_{r}}
\]

C.10

Phosphoglucoisomerase:

\[
\phi_{PGI} = \frac{V_{\text{max},f,PGI}c_{\text{glyc}}}{K_{\text{glyc}}} - \frac{V_{\text{max},r,PGI}c_{\text{glyc}}}{K_{r}}
\]

C.11
Phosphofructokinase:

\[
\phi_{PFK} = \frac{V_{\text{max,f,PFK}}C_{\text{ATP}}C_{\text{f6p}} \cdot V_{\text{max,r,PFK}}C_{\text{ADP}}C_{\text{f6p}}}{K_{\text{ATP}}K_{\text{f6p}}K_{\text{ADP}}K_{\text{f6p}}} \cdot \left(1 + \frac{K_{\text{ATP}}K_{\text{f6p}}(\frac{\Delta}{\Delta'})^3}{1+L(\frac{\Delta'}{\Delta})^4}\right)
\]

where \(\Delta, \Delta'\) and \(L\) are computed with the following expressions:

\[
\Delta = \left(1 + \frac{C_{\text{f6p}}}{K_{\text{f6p}}}(1 + \frac{C_{\text{ATP}}}{K_{\text{ATP}}}) + \frac{C_{\text{ADP}}}{K_{\text{ADP}}} + \frac{C_{\text{f6p}}}{K_{\text{f6p}}} \left(1 + \frac{C_{\text{ADP}}}{K_{\text{ADP}}}\right)\right)
\]

\[
\Delta' = \left(1 + \frac{C_{\text{f6p}}}{K_{\text{f6p}}}(1 + \frac{C_{\text{ATP}}}{K'_{\text{ATP}}}) + \frac{C_{\text{ADP}}}{K'_{\text{ADP}}} + \frac{C_{\text{f6p}}}{K'_{\text{f6p}}} \left(1 + \frac{C_{\text{ADP}}}{K'_{\text{ADP}}}\right)\right)
\]

\[
L = L_o \left[\left(1 + \frac{C_{\text{ATP}}}{K_{\text{ATP}}}\right) \left(1 + \frac{C_{\text{AMP}}}{K_{\text{AMP}}}\right) \left(1 + \frac{C_{\text{AMP}}}{K_{\text{AMP}}}\right)^4\right]
\]

Aldolase:

\[
\phi_{ALD} = \frac{V_{\text{max,f,ALD}}C_{\text{f6p}} \cdot V_{\text{max,r,ALD}}C_{\text{dhap}}C_{\text{gap}}}{K_{\text{f6p}}K_{\text{dhap}}K_{\text{gap}}} \cdot \frac{1}{1 + \frac{C_{\text{dhap}} + C_{\text{gap}}}{K_{\text{dhap}} + K_{\text{gap}}}}
\]

Triose Phosphate Isomerase:

\[
\phi_{TPI} = \frac{V_{\text{max,f,TPI}}C_{\text{gap}} \cdot V_{\text{max,r,TPI}}C_{\text{dhap}}}{K_{\text{gap}}K_{\text{dhap}}} \cdot \frac{1}{1 + \frac{C_{\text{gap}} + C_{\text{dhap}}}{K_{\text{gap}} + K_{\text{dhap}}}}
\]

Glyceraldehyde-3-Phosphate Dehydrogenase:

\[
\phi_{GAPDH} = \frac{V_{\text{max,f,GAPDH}}C_{\text{gap}}C_{\text{NAD}}C_{\text{Pi}} \cdot V_{\text{max,r,GAPDH}}C_{\text{13bpg}}C_{\text{NADH}}}{K_{\text{gap}}K_{\text{NAD}}K_{\text{Pi}}K_{\text{13bpg}}K_{\text{NADH}}} \cdot \frac{1}{D}
\]

C.12

C.13

C.14

C.15

C.16

C.17

C.18
where $D$ is calculated as

\[
D = 1 + \frac{C_{gap}}{K_{gap}} + \frac{C_{NAD}}{K_{NAD}} + \frac{C_{Pi}}{K_{Pi}} + \frac{C_{gap}}{K_{gap}} \frac{C_{NAD}}{K_{NAD}} + \frac{C_{Pi}}{K_{Pi}} + \frac{C_{13bpg}}{K_{13bpg}} + \frac{C_{NADH}}{K_{NADH}} + \frac{C_{13bpg}}{K_{13bpg}} \frac{C_{NADH}}{K_{NADH}}
\]

C.19

Phosphoglycerate Kinase:

\[
\phi_{PGK} = \frac{V_{max,f,PK} C_{3bpg} C_{ADP} V_{max,r,PK} C_{3pg} C_{ATP}}{K_{3bpg} K_{ADP} K_{3pg} K_{ATP}} \left( 1 + \frac{C_{13bpg}}{K_{13bpg}} \frac{C_{ADP}}{K_{ADP}} + \frac{C_{13bpg} C_{ADP}}{K_{13bpg} K_{ADP}} + \frac{C_{3pg} C_{ATP}}{K_{3pg} K_{ATP}} + \frac{C_{3pg} C_{ATP}}{K_{3pg} K_{ATP}} \right)
\]

C.20

Phosphoglyceromutase:

\[
\phi_{PGM} = \frac{V_{max,f,PGM} C_{3pg} C_{2pg} V_{max,r,PGM} C_{2pg}}{K_{3pg} K_{2pg}} \left( 1 + \frac{C_{3pg}}{K_{3pg}} + \frac{C_{2pg}}{K_{2pg}} \right)
\]

C.21

Enolase:

\[
\phi_{ENOL} = \frac{V_{max,f,ENOL} C_{2pg} V_{max,r,ENOL} C_{pep}}{K_{2pg} K_{pep}} \left( 1 + \frac{C_{2pg}}{K_{2pg}} + \frac{C_{pep}}{K_{pep}} \right)
\]

C.22

Pyruvate Kinase:

\[
\phi_{PK} = \frac{V_{max,f,PK} C_{pep} C_{ADP} V_{max,r,PK} C_{pyr,t} C_{ATP}}{K_{pep} K_{ADP} K_{pyr} K_{ATP}} \left( 1 + \frac{C_{pep} C_{ADP}}{K_{pep} K_{ADP}} + \frac{C_{pep} C_{ADP}}{K_{pep} K_{ADP}} + \frac{C_{pyr,t} C_{ATP}}{K_{pyr} K_{ATP}} + \frac{C_{pyr,t} C_{ATP}}{K_{pyr} K_{ATP}} \right)
\]

C.23

Lactate Dehydrogenase:

\[
\phi_{LDH} = \frac{V_{max,f,LDH} C_{pyr,t} C_{NADH} V_{max,r,LDH} C_{lac,c} C_{NAD}}{K_{pyr K_{NADH}} K_{lac K_{NAD}}} \left( 1 + \frac{C_{pyr,t} C_{NADH}}{K_{pyr} K_{ADP}} \frac{C_{pyr,t} C_{NADH}}{K_{pyr} K_{ADP}} + \frac{C_{lac,c} C_{NAD}}{K_{lac} K_{NAD}} + \frac{C_{lac,c} C_{NAD}}{K_{lac} K_{NAD}} \right)
\]

C.24
Adenylate Kinase:

\[
\phi_{ADK} = \frac{V_{\text{max},f,ADK} C_{ATP} C_{AMP} - V_{\text{max},r,ADK} C_{ADP}^2}{K_{ATP} K_{AMP} K_{ADP}^2}
\]

C.25
APPENDIX D

Appendix D.1 Maximal metabolic fluxes (mM min⁻¹)

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Adenosine Kinase - ADK

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APPENDIX E

Enzymes

ADK adenylate kinase
ALD fructose-bisphosphate aldolase
ATPase myosin ATPase
CK creatine kinase
ENOL phosphopyruvate hydratase
GAPDH glyceraldehyde-3-phosphate dehydrogenase
GP glycogen phosphorylase
LDH L-lactate dehydrogenase
PFK 6-phosphofructokinase
PGI phosphoglucone isomerase
PGK phosphoglycerate kinase
PGLM phosphoglucomutase
PGM phosphoglycerate mutase
PK pyruvate kinase
TPI triose-phosphate isomerase

Metabolites

ADP adenosine diphosphate
AMP adenosine monophosphate
ATP adenosine triphosphate
13BPG glycerate-1,3-biphosphate
2PG glycerate-2-phosphate
3PG glycerate-3-phosphate
Cr creatine
DHAP 1,3-dihydroxyacetone phosphate
F6P fructose-6-phosphate
F6P fructose-1,6-biphosphate
G1P glucose-1-phosphate
G6P glucose-6-phosphate
GAP glyceraldehyde-3-phosphate
GLY glycogen
LAC L-lactate
NAD nicotinamide adenine dinucleotide (oxidized)
NADH nicotinamide adenine dinucleotide (reduced)
PCr phosphocreatine
PEP phosphoenolpyruvate
Pi inorganic phosphate
PYR pyruvate
APPENDIX F

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Best wishes on your dissertation. Please let me know if I may be of further assistance.

Best
Katie Sinclair
Editorial Assistant, Publications
SPIE – the international society for optics and photonics
katie@spie.org
1 360 685 5400
SPIE.

---

Wang, Lihong <lhhong@caltech.edu>

to me

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Best regards,

Lihong.

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Andrew and Peggy Cheng Department of Medical Engineering
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Division of Engineering and Applied Science
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Fax: 626-395-1347
Email: LW@Caltech.edu
Web: http://COILab.Caltech.edu

Assistant:
Catherine (Katie) L. Pichotta (pichotta@caltech.edu)
Work: 626-395-1970

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EDUCATION

Ph.D. in Biomedical Engineering, Old Dominion University, Norfolk VA, 2021

M. Sc. in Biotechnology, Instituto Superior Técnico, Portugal, 2013

M. Sc. tech. in Computational and systems biology, Aalto University, Finland, 2012

B. tech. in in Biotechnology, Kathmandu University, Nepal, 2010

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Graduate Teaching Assistant, Old Dominion University, 2018-2020

Graduate Research Assistant, Old Dominion University, 2017-2021

Lecturer, Kathmandu university, 2014-2017

Research Investigator, Instituto Superior Técnico, 2012-2013