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Relationship between muscle venous blood oxygenation and near-infrared spectroscopy: quantitative analysis of the Hb and Mb contributions

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Running Head: Relationship between muscle NIRS signals and venous oxygenation

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Abstract

A linear relationship between skeletal muscle venous (C_{ven}^{T}) and oxygenated ($\Delta HbMbO_{2,N}$) or deoxygenated ($\Delta HHbMb_N$) near-infrared spectroscopy (NIRS) signals suggest a main Hb contribution to the NIRS signal. However, experimental, and computational evidence supports a significant contribution of Mb to the NIRS. Venous and NIRS measurements from a canine model of muscle oxidative metabolism (Med. Sci. Sports Exerc. 48(10):2013-2020, 2016) were integrated into a computational model of muscle O_2 transport and utilization to evaluate whether the relationship between venous and NIRS oxygenation can be affected by a significant Mb contribution to the NIRS signals. The mathematical model predicted well the measure of the changes of C_{ven}^{T} and NIRS signals for different O_2 delivery conditions (blood flow, arterial O_2 content) in muscle at rest (T1, T2) and during contraction (T3). Furthermore, computational analysis indicates that for adequate O_2 delivery, Mb contribution to NIRS signals was significant (20-30%) even in the presence of a linear C_{ven}^{T}-NIRS relationship; for a reduced O_2 delivery the non-linearity of the C_{ven}^{T}-NIRS relationship was related to the Mb contribution (50%). In this case (T3), the deviation from linearity is observed when O_2 delivery is reduced from 1.3 to 0.7 L $kg^{-1} min^{-1}$ (C_{ven}^{T} < 10 mLO_2 $100mL^{-1}$) and Mb saturation decreased from 85 to 40% corresponding to an increase of the Mb contribution to $\Delta HHbMb_N$ from 15 to 50% and the contribution to $\Delta HbMbO_{2,N}$ from 0% to 30%. In contrast to a common assumption, our model indicates that both NIRS signals ($\Delta HHbMb_N$ *and* $\Delta HbMbO_{2,N}$) are significantly affected by Hb and Mb oxygenation changes.

New & Noteworthy

Measurements of skeletal muscle oxygenation by near-infrared spectroscopy (NIRS) are used to detect imbalances between O_2 consumption and delivery. Within the NIRS signal, the contribution from hemoglobin (Hb) is indistinguishable from that derived from myoglobin (Mb). In this study, we present a quantitative analysis indicating that Mb contribution to NIRS is significant under both normal and reduced muscle O_2 transport conditions. Also, a linear relationship between muscle venous O_2 content and NIRS signals does not necessarily indicate a negligible Mb contribution to the NIRS signal. A reduced O_2 delivery to the muscle has distinct effects on the microvascular (Hb) and extravascular (Mb) oxygenations that significantly affect the NIRS signals. The integrative approach proposed is a powerful way to assist in interpreting NIRS signals and understanding the basic elements from which the signals are derived.

78 ## Introduction

79 The assessment of skeletal muscle function in healthy and disease states by near-
80 infrared spectroscopy (NIRS) technology has received increased attention during the last
81 decades (Wilson *et al.*, 1989; Hamaoka *et al.*, 1996; McCully *et al.*, 2011; Grassi *et al.*, 2019).
82 Nevertheless, its clinical application has been limited because of the semi-quantitative
83 nature of the NIRS signal in quantifying the oxygen (O_2) level in skeletal muscle.
84 Specifically, NIRS signals reflect the O_2 saturation of hemoglobin (Hb) and myoglobin
85 (Mb) within the microvascular and extravascular compartments, respectively, (Lai *et al.*,
86 2009*b*; Masuda *et al.*, 2010; Davis & Barstow, 2013) but their contributions to the NIRS signals
87 which vary during muscle contraction (Lai *et al.*, 2009*b*; Spires *et al.*, 2011; Koirala *et al.*,
88 2021*b*), cannot be easily quantified. Although the quantitative analysis of the temporal
89 profile of muscle NIRS signals provides a valuable parameter characterizing the NIRS
90 kinetics (i.e., time constant), inferences are limited by the unknown contribution of Hb and
91 Mb to the kinetics.

92 NIRS measurement in animals have reported that the contribution of Mb could be in the
93 range of 20-50% (Nioka *et al.*, 2006; Marcinek *et al.*, 2007*a*; Masuda *et al.*, 2010) whereas
94 human studies reported a contribution close to 50-80% (Marcinek *et al.*, 2007*a*; Davis &
95 Barstow, 2013). Integrative approaches that combine experimental and computational
96 methods were used to analyze the NIRS measurement in contracting muscle during human
97 exercise (Binzoni *et al.*, 1999; Zhou *et al.*, 2008; Lai *et al.*, 2009*b*, 2009*a*; Spires *et al.*, 2011). It
98 was reported that Mb contribution to the signal varies from 20 to 80% during contraction
99 due to the O_2 delivery conditions (Lai *et al.*, 2009*b*). The significance of the Mb contribution
100 inferred by the analysis of the computational model was also confirmed by a quantitative
101 analysis reported by another group (Davis & Barstow, 2013).

102 Another quantitative and experimental approach used to evaluate the Hb/Mb
103 contribution to the NIRS is to examine the correlation between venous and muscle
104 oxygenation by NIRS. The correlation or linear relationship between these two variables
105 which is considered evidence for a major Hb contribution to the NIRS signal is not always
106 present for measurement in skeletal muscle. The presence of this relationship was reported
107 in canine gracilis muscle (Wilson *et al.*, 1989) and human forearm muscle (Mancini *et al.*,

108 1994). In other studies, the relationship was not always present and the effects of oxygen
109 delivery (Boushel *et al.*, 1998; Wüst *et al.*, 2014) in contracting muscle on the relationship
110 were not always consistent among the studies. In particular, the correlation between venous
111 and NIRS oxygenation in vastus lateralis was not present at normoxia but was significant at
112 low oxygen delivery (i.e., hypoxia) (Costes *et al.*, 1996; Macdonald *et al.*, 1999). In contrast,
113 the same correlation was weakened by lower oxygen delivery (i.e., blood flow) in forearm
114 flexor (Boushel *et al.*, 1998) and pump perfused canine muscle (Wüst *et al.*, 2014).
115 Some of the differences observed could be in part related to confounding factors such as
116 adipose tissue, skin blood flow and blood volume changes that complicate the interpretation
117 of this relationship in human studies.
118 To avoid the effects of these confounding factors, an animal model (Hernández *et al.*,
119 2010*b*) was used to quantify the effects of O_2 delivery (i.e., varying arterial O_2 content or
120 blood flow) on the relationship between venous O_2 content (C_{ven}^{T}) and NIRS signals (Sun *et*
121 *al.*, 2016) in the presence or absence of contraction. In this experimental setting, a decreased
122 O_2 delivery determined a linear decrease of venous oxygen content and an increase of the
123 amplitude of both NIRS signals; a decrease of the oxygenated and an increase of the
124 deoxygenated forms, respectively. Thus, these results appear to be consistent with a
125 predominant contribution of Hb to the NIRS signal. However, because O_2 transport and
126 utilization are intimately coupled and followed by O_2 content changes in both blood and
127 tissue compartments, it is possible that the oxygenation changes detected by the NIRS
128 signals are related to concurrent Hb and Mb oxygenation changes. These concomitant
129 changes can occur in both the animal studies as well as the human studies mentioned in the
130 previous paragraph. There has been not investigation of those experimental conditions
131 causing a deviation of the correlation between venous and NIRS oxygenation even in
132 presence of concomitant Hb and Mb oxygenation changes. We used a physiologically
133 based mathematical model of energy metabolism to quantify Hb and Mb contributions to
134 the NIRS signals (Lai *et al.*, 2009*b*). The metabolic response to contraction in human and
135 animal models has been predicted by a computational model of O_2 transport and utilization
136 in skeletal muscle (Lai *et al.*, 2007; Spires *et al.*, 2012, 2013) under different experimental
137 conditions (Grassi *et al.*, 1998*b*, 1998*a*, 2000). The capability of this model was validated and

138 enhanced to quantify oxygenation in the microvascular and extravascular compartments to
139 study the effects of blood flow and volume on the NIRS signals (Koirala *et al.*, 2021*a*, 2021*b*).
140 Thus, this tool can be used to quantify the Hb and Mb contribution to NIRS signals under
141 differing O_2 delivery conditions.

142 In the present study, we propose to use this computational model, validated with canine
143 data, to analyze the effects of O_2 delivery on the relationship between venous and NIRS
144 oxygenation and determine the extent of the Mb contribution to the NIRS signal.

145 **Methods**

146 *Animal Model*

147 The experimental data for the computational analysis of our study were obtained from a
148 previous investigation that used an animal model of muscle oxidative metabolism
149 (Hernández *et al.*, 2010*b*, 2010*a*; Goodwin *et al.*, 2012). In this work the experimental setting
150 and trial notation reported in Figure 1 were the same as those used in the experimental work
151 (Sun *et al.*, 2016) from which the NIRS data were used for the analysis proposed here. The
152 experimental protocol provided venous (C_{ven}^{T}) and muscle oxygenation measurements at
153 rest (T1 and T2) under different experimental conditions (i.e., blood flow, arterial O_2
154 content) and during contraction (T3) for different blood flows. Also, the T5 trial was
155 considered because the NIRS signals obtained for this condition were used to normalize the
156 NIRS signals of T1, T2 and T3 trials.

157 A continuous-wave NIRS system (Oxymon Mk III, Artinis Medical Systems BV) was
158 used to measure the muscle oxygenated ($\Delta HbMbO_{2,N}$) and deoxygenated ($\Delta HHbMb_N$)
159 heme group concentration changes relative to rest. N indicates that $\Delta HbMbO_2$ and
160 $\Delta HHbMb$ measurements were normalized to the maximal variation of the oxygenated and
161 deoxygenated NIRS signals observed for trial T5 during blood flow occlusion (Sun *et al.*,
162 2016). The light at two different wavelengths (760 and 860 nm) were emitted and received
163 by two fiber-optic bundles. The optodes were placed over the medial head of the left
164 gastrocnemius and held in place with an elastic band. Experimental control of muscle blood
165 flow was made possible by a peristaltic pump connected to the arterial inflow to the
166 gastrocnemius. The right carotid artery or the right femoral artery was cannulated to route

167 blood to the peristaltic pump (Hernández *et al.*, 2010*a*). All experimental data were averaged
168 from five measurements for each trial condition.

169 *Mathematical model*

170 A mathematical model of skeletal muscle O_2 transport and metabolism (Koirala *et al.*,
171 2021*a*) was used to quantify the Hb and Mb contribution to the NIRS measurement obtained
172 with the animal model introduced in the previous section (Sun *et al.*, 2016). The muscle
173 volume (V_{mus}) consists of extravascular tissue (cells and interstitial space, V_t) and blood
174 (V_b) which is assumed to have vascular ($V_{b,v}$) and microvascular ($V_{b,m}$) compartments. The
175 microvascular volume consists of arterioles, capillaries, and venules ($V_{b,m} = V_{art} + V_{cap} +$
176 V_{ven}). The mathematical model simulates the spatial distribution of the free O_2
177 concentration $C_{cap}^F(v)$ in the capillary and extravascular $C_t^F(v)$ tissue compartments (Koirala
178 *et al.*, 2021*a*). The concentrations depend on the tissue location as indicated by the muscle
179 volume variable (v) from the arterial input $v = 0$ to the venous output $v = V_{mus}$. Also, the
180 model quantifies the relationship between O_2 concentration dissolved ($C^F =$
181 $C_{art}^F, C_{cap}^F, C_{ven}^F$) and bound ($C^B$) either to Hb (arterioles, capillaries, venules) or Mb
182 (myocytes). These are used to quantify the oxygenated Hb (HbO_2) and Mb (MbO_2)
183 concentration in muscle as:

184 $$HbO_2 = f_{b,m}\left[C_{art}^B\ \omega_{art} + C_{cap}^B\ \omega_{cap} + C_{ven}^B\ \omega_{ven}\right]/4 = f_{b,m}\left(\Delta C_{Heme,N}^{exp}\right)C_{b,m}^B/4 \quad 1$$

185 $$MbO_2 = f_t\ C_t^B/4 \quad 2$$

186 where $f_{b,m}$ and f_t are the microvascular and extravascular tissue volume fraction in muscle,
187 $C_{b,m}^B$ is the bound O_2 concentration in the microvascular, C_{cap}^B and C_t^B are the spatial
188 average of the bound O_2 concentration in capillary and extravascular tissue, respectively.
189 $f_{b,m}$ is a function of the relative heme concentration changes ($\Delta C_{Heme,N}^{exp}$) detected by the
190 NIRS signal and used to quantify the microvascular volume changes. The general
191 expression for any volume averaged variable (Y) with a spatial profile $y(v)$ is calculated as:

192 $$Y = \frac{\int_0^{V_x} y(v)dv}{V_x} \quad V_x = V_{cap}, V_t \quad 3$$

193 where the variable y is C_{cap}^B, C_t^B, the oxygen saturation (SO_2) or the partial pressure (P_{O_2}) in
194 the capillary and myocytes compartments.

195 For blood (b) and extravascular tissue (t) compartments, the total (T) O_2 concentration
196 is calculated by the sum of the free (F) and bound (B) O_2 concentrations ($C_x^T = C_x^B + C_x^F$,
197 $x = b, t$), which are related by local equilibrium (Lai *et al.*, 2007). In blood compartments
198 (arteriole, capillary, venule) the relationship is

199 $$C_b^B = 4\, C_{b,Hb} SO_{2,b} = 4\, C_{b,Hb} \frac{(P_{O_2,b})^n}{(P_{50,Hb})^n + (P_{O_2,b})^n} \qquad \text{with } P_{O_2,b} = C_b^F / \alpha_{O_2} \qquad 4$$

200 and in the extravascular muscle (myocyte) is

201 $$C_t^B = C_{t,Mb}\, SO_{2,t} = C_{t,Mb} \frac{P_{O_2,t}}{P_{50,Mb} + P_{O_2,t}} \qquad \text{with } P_{O_2,t} = C_t^F / \alpha_{O_2} \qquad 5$$

202 where $SO_{2,b}$ and $SO_{2,t}$ are the oxygen saturation (SO_2) in the microvascular (arterioles,
203 capillaries, venules) and extravascular tissue (myocytes) compartments, α_{O_2} is the
204 coefficient of oxygen solubility, $P_{50,Hb}$, n and $P_{50,Mb}$ are the parameters of the Hill's
205 equation for Hb and Mb. The concentration of Hb in blood ($C_{b,Hb}$) and red blood cells
206 ($C_{rbc,Hb}$) are related by Hct whereas the concentration of Mb ($C_{t,Mb}$) in muscle tissue (cells
207 and interstitial space) and myocytes ($C_{mc,Mb}$) are related by W_{mc} ($C_{b,Hb} = C_{rbc,Hb}\, Hct$;
208 $C_{t,Mb} = C_{mc,Mb}\, W_{mc}$).

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

211 $$HHb = f_{b,m}\left(\Delta C_{Heme,N}^{exp}\right) C_{b,Hb} - HbO_2 \qquad 6$$

212 $$HMb = f_t\, C_{t,Mb}/4 - MbO_2 \qquad 7$$

213 Details of the models are reported in another computational study (Koirala *et al.*, 2021*a*).

214 *Model simulations*

215 The details of the strategy to simulate the conditions of the three trials is similar to that
216 reported in (Koirala *et al.*, 2021*a*). The model inputs (i.e., arterial O_2 content, blood flow) that
217 reflect the experimental condition of each trial reported in Figure 1 are: 1) metabolic rate
218 (VO_2) to estimate k_{ATPase} with the expression reported in Appendix (A1); 2) extravascular
219 and microvascular volume distribution at rest and for blood volume changes (See
220 Appendix).

221 The parameter values in Table 1 applies to all experiments and are independent of the
222 input conditions. The muscle volume was estimated from muscle mass measurement and

223 assuming the muscle density of 1.05 g mL^{-1}. And the microvascular volume distribution
224 was based on measurements obtained in previous studies (Bebout, 1991; Bebout *et al.*, 1993;
225 Poole *et al.*, 1995). The Hill's equation parameters for Hb and Mb equilibrium are reported
226 in Table 2 and the rest of the parameter values of this model are available from previous
227 studies (Lai *et al.*, 2007, 2008; Spires *et al.*, 2012, 2013; Koirala *et al.*, 2021*a*).

228 The experimental data compared with model simulations, are the venous O_2
229 concentration (C_{ven}^{T}), the rate of muscle O_2 uptake ($\dot{V}O_2$) and the normalized oxygenated
230 ($\Delta HbMbO_{2,N}$) and deoxygenated ($\Delta HHbMb_N$) NIRS measurements:

231 $$\Delta HbMbO_{2,N} = \frac{\Delta HbMbO_2}{\Delta HbMbO_2^{T5}}; \quad \Delta HHbMb_N = \frac{\Delta HHbMb}{\Delta HHbMb^{T5}}; \qquad 8$$

232 T5 indicates trial n.5 in (Sun *et al.*, 2016) and represents the condition during blood flow
233 occlusion. The contribution of Hb and Mb to the oxygenated ($\Delta HbMbO_2$) and
234 deoxygenated ($\Delta HHbMb$) NIRS signals under different experimental conditions are
235 quantified as:

236 $$y_{\Delta HbO_2} = \Delta HbO_2/\Delta HbMbO_2\,; \qquad y_{\Delta MbO_2} = 1 - y_{\Delta HbO_2} \qquad 9$$

237 $$y_{\Delta HHb} = \Delta HHb/\Delta HHbMb; \qquad y_{\Delta HMb} = 1 - y_{\Delta HMb} \qquad 10$$

238

239 **Results**

240 The overall strategy to analyze the Hb/Mb contribution to the relationship between 241 venous and NIRS signal oxygenations was to a) validate the mathematical model with 242 blood and NIRS experimental data (Sun *et al.*, 2016) (Fig. 2); b) relate muscle venous 243 oxygenation to Hb and Mb contribution to the NIRS signals (Fig. 3) and c) analyze the 244 nature of this relationship for differing oxygen delivery (Fig. 4, 5 and 6).

245 *Mathematical model validation*

246 The capability of the mathematical model to reproduce the experimental data was tested 247 with measurements of venous O_2 content (C_{ven}^{T}) and tissue oxygenated ($\Delta HbMbO_{2,N}$) and 248 deoxygenated ($\Delta HHbMb_{N}$) heme group concentrations obtained for different conditions 249 (Fig. 2A): at rest (T1, panel a, b) with a blood flow (Q) range of 0.01-0.4 L kg^{-1} min^{-1}; at 250 rest (T2, panel c, d) with an arterial O_2 content (P_{O_2}) range of 40-500mmHg; during 251 contraction (T3, panel e, f) with a blood flow range of 0.6-1.4 L kg^{-1} min^{-1} (Sun *et al.*, 2016). 252 For each trial simulation, the parameter (k_{ATPase}) associated with the energy demand, was 253 used to simulate the changes in $\dot{VO}_2$ (Fig. 2B, panel a, c, e). Simulations accounted for 254 microvascular blood volume fraction ($f_{b,m}$) changes detected by the sum of the oxygenated 255 and deoxygenated NIRS signals ($\Delta C_{Heme,N}^{exp}$) (Fig. 2B, panel b, d, f). k_{ATPase} and $f_{b,m}$ were 256 computed as reported in the Appendix.

257 The model predicted well the changes of C_{ven}^{T} (Fig. 2A: a, c, e), $\Delta HbMbO_{2,N}$ and 258 $\Delta HHbMb_{N}$ (Fig. 2A: b, d, f) with different O_2 delivery resulting from changes in Q and 259 arterial P_{O_2}. The higher the O_2 delivery, the higher C_{ven}^{T} and the lower the amplitude of the 260 NIRS signal changes. In T1 (Fig. 2A), for reduced O_2 delivery (Q<0.1 L kg^{-1} min^{-1}), 261 simulations predicted an abrupt nonlinear decline in C_{ven}^{T} (panel a) and $\Delta HbMbO_{2,N}$ and an 262 increase in $\Delta HHbMb_{N}$ (panel b). Similarly, in T2, for a reduced O_2 delivery obtained under 263 hypoxia, simulations predicted an abrupt nonlinear decline in C_{ven}^{T} (panel c) and 264 $\Delta HbMbO_{2,N}$ (panel d) and a rise in $\Delta HHbMb_{N}$. In T3, C_{ven}^{T} (panel e), $\Delta HbMbO_{2,N}$ and 265 $\Delta HHbMb_{N}$ (panel f) showed a quasi-linear relationship with blood flow.

266 *NIRS signal, venous O_2 content and Hb/Mb contributions*

267 The model predicted well $\Delta HbMbO_{2,N}$ and $\Delta HHbMb_N$ changes with C_{ven}^T that
268 correspond with the experimental observations of $\Delta HHbMb_N$ and $\Delta HbMbO_{2,N}$ (Fig. 3a
269 and 3b). The sets of experimental data for T1, T2 and T3 trials were obtained with ranges
270 of oxygen delivery (T1: 0.1-0.3 and T3:0.8-1.25 L kg^{-1} min^{-1}) that covered different regions
271 of the C_{ven}^T-NIRS signal relationship. The model simulations of trial T1 and T3 were
272 performed for a larger range of blood flow than that investigated by Sun et al. (Sun *et al.*,
273 2016) to analyze the C_{ven}^T-NIRS signal relationship in the entire experimental range of C_{ven}^T
274 (5-25 mLO_2 $100mL^{-1}$). Specifically, to cover this C_{ven}^T range, the blood flow range used for
275 T1 and T3 simulations was 0.01-0.4 and 0.6-1.4 L kg^{-1} min^{-1}, respectively. An overall
276 linear relationship is notable between C_{ven}^T and $\Delta HHbMb_N$ or $\Delta HbMbO_{2,N}$ when all sets
277 (T1, T2, T3) of data are considered. Nevertheless, when the sets of data are independently
278 considered at low oxygen delivery (C_{ven}^T < 7-9 mLO_2 $100mL^{-1}$), the model predictions
279 showed that the relationship between the venous and muscle oxygenation ($\Delta HHbMb_N$) was
280 still linear for T1 (red line) but was not linear for T3 (green line) (Fig. 3a, 3b). A similar
281 difference between T1 and T3 was also observed for the relationship between C_{ven}^T and
282 $\Delta HbMbO_{2,N}$.

283 The $\Delta HbMbO_{2,N}$ and $\Delta HHbMb_N$ were further analyzed to relate the changes of the Hb
284 contribution to the $\Delta HbMbO_{2,N}$ ($y_{\Delta HbO_2}$) or $\Delta HHbMb_N$ ($y_{\Delta HHb}$) signal with C_{ven}^T (Fig. 3c,
285 3d). At high oxygen delivery (i.e., high C_{ven}^T), the Hb contribution to both signals were
286 above 80% for all trials (red, blue and green lines), reaching a plateau close to 85% for
287 $y_{\Delta HHb}$ and to 100% for $y_{\Delta HbO_2}$. Under normal oxygen delivery during contraction (green
288 line), $y_{\Delta HHb}$ and $y_{\Delta HbO_2}$ decreased to approximatively 60% and 73%, respectively. When
289 the oxygen delivery decreased, C_{ven}^T decreased (C_{ven}^T < 9 mLO_2 $100mL^{-1}$), and the Hb
290 contribution to both signals decreased linearly for T1 (red line) and non-linearly for T3
291 (green line).

292 *Hb and Mb contribution to the signals*

293 To investigate nature of the nonlinearity between C_{ven}^T and Hb contribution to the NIRS
294 signals, the components of the deoxygenated ($\Delta HHbMb$) or oxygenated ($\Delta HbMbO_2$) forms

295 within the microvascular (ΔHHb or ΔHbO_2: capillary, venule) and extravascular (ΔHMb or
296 ΔMbO_2) compartments were analyzed in relationship to C_{ven}^T changes in T1 (Fig. 4a, 4b)
297 and T3 (Fig. 4c, 4d). For both T1 and T3 trials, with a decrease of C_{ven}^T, the amplitude of
298 both deoxygenated (Fig. 4a, c) and oxygenated (Fig. 4b, d) forms in the capillaries (green
299 line) and venules (blue line) changed linearly whereas those in myocytes changed
300 nonlinearly (black line) with a biphasic pattern. Also, the change of slope of ΔMbO_2 and
301 ΔHMb occurred when C_{ven}^T reached 7 mLO_2 $100mL^{-1}$ in T1 (Fig. 4a, b) and 9 mLO_2
302 $100mL^{-1}$ in T3 (Fig. 4c, d), respectively. For both trials, the slope change occurred for
303 blood flow - O_2 consumption ratio (Q/VO_2) approximatively of 7 and PO_2 values of
304 approximately 45, 25 and 20 mmHg in capillary, venule, and myocyte, respectively. This
305 change of slope indicates a higher sensitivity of Mb than Hb to oxygen delivery changes.
306 Thus, the Hb contribution to the NIRS signals (Fig. 3c, d) decreased because capillary
307 ΔHbO_2 and ΔHHb amplitude increases were lower than extravascular tissue ΔMbO_2 and
308 ΔMHb amplitude increases for lower C_{ven}^T. In T3, the effect of blood flow (i.e., decrease of
309 C_{ven}^T) on the amplitude of both deoxygenated and oxygenated forms in myocytes was even
310 larger than that observed in T1 and led to a non-linear increase of the Mb contribution to
311 the NIRS signal.

312 The slope change observed for the NIRS signals in Fig. 3 and for their components in
313 Fig. 4 are related to the microvascular and extravascular oxygen saturation (SO_2) changes
314 with PO_2 in blood and tissue compartments, respectively. In both compartments, the
315 simulated PO_2 decreased with a decrease in oxygen delivery as observed for C_{ven}^T.
316 However, because the oxygen delivery effect on oxygenation of the microvascular
317 compartments differed from that on the extravascular compartment the SO_2 changes in the
318 capillaries, venules (microvascular) and myocytes (extravascular) compartments were
319 analyzed relative to C_{ven}^T changes. The volume averaged Hb and Mb saturation changes
320 were plotted against C_{ven}^T (Fig. 5a and b). For both T1 and T3, a decrease of C_{ven}^T with a
321 reduction of O_2 delivery determined a linear decrease of the SO_2 of Hb in venules and
322 capillary compartments and a non-linear decrease of the SO_2 in the myocytes. SO_2 in
323 capillary and venules is more sensitive than SO_2 in myocytes to C_{ven}^T changes for C_{ven}^T> 7-9

324 mLO_2 100 mL^{-1}. For both T1 and T3, the slope of the C_{ven}^T-SO_2 relationship changes for the
325 same Q/VO_2 (i.e., 7) value observed for Fig. 4c and 4d. Thus, for further C_{ven}^T decrease
326 followed by SO_2 decrease of Mb below 80-85%, SO_2 of Mb changes become more
327 sensitive than those observed for capillary and venule. This sensitivity in T3 is even larger
328 than T1: a C_{ven}^T decrease of 1 mLO_2 100mL^{-1} from 7 (T1) or 9 (T3) mLO_2 100mL^{-1} was
329 followed by a decrement of 4% (81 to 77%) and 13% (80 to 67%) of the Mb saturation
330 (myocytes compartment) in T1 and T3, respectively. The SO_2 changes in venules and
331 capillaries in T1 were comparable to those observed for T3.

332 Another analysis was performed on the role of Mb versus Hb. Specifically, the
333 following question was considered: how could there be a higher sensitivity of the NIRS
334 signals to the volume averaged Mb saturation ($SO_{2,Mb}$, myocytes) than to the Hb saturation
335 ($SO_{2,Hb}$) in venules and capillaries during O_2 delivery changes when Mb saturation has a
336 P_{50} of 2.4 mmHg versus a P_{50} of 31.2 mmHg for Hb? The analysis focused only on
337 contracting muscle (T3) because this phenomenon was more evident in this condition than
338 that observed for T1. For the values of volume averaged $SO_{2,Hb}$ in capillaries, indicated
339 with short dash lines in Fig. 5b (57, 64, and 67%, the corresponding SO_2 spatial profile
340 obtained with a blood flow of 0.7 (red line), 1.1 (green line) and 1.3 (blue line) L kg^{-1} min^{-1}
341 was reported in Fig. 6a. Also, for the same blood flow values used for capillary simulations
342 in Fig. 5b, the $SO_{2,Mb}$ spatial profile corresponding to the volume averaged SO_2 in
343 myocytes is reported in Fig. 6c. In addition, the spatial profile of the partial pressure of
344 oxygen (PO_2) in capillaries and myocytes was reported in Fig. 6b and 6d, respectively. The
345 legend also reports for each spatial profile of SO_2 and PO_2 the corresponding volume
346 averaged of SO_2 and PO_2. It should be noted that Eq. (3) was used to calculate the volume
347 average of each variable.

348 In the capillaries, the spatial profile of SO_2 (Fig. 6a) and PO_2 (Fig. 6b) along the muscle
349 volume was linear and nonlinear, respectively. The linear trend of the SO_2 spatial profile
350 was related to the non-linear relationship of SO_2 to PO_2. The SO_2 spatial profile decreased
351 with a decrease of the oxygen delivery (from 1.3 to 0.7 L kg^{-1} min^{-1}) although this variation
352 was modest (Fig. 6a) due to the minor effect of the oxygen delivery on PO_2 spatial profile

353 (Fig. 6b). In the myocytes, a significant drop of the $SO_{2,Mb}$ spatial profile with a decrease of
354 the oxygen delivery (Fig. 6c) was noticeable. Specifically, when blood flow was decreased
355 from 1.3 to 1.1 L kg^{-1} min^{-1}, the volume averaged $SO_{2,Mb}$ decreased from 85% (blue line) to
356 74% (green line) even though the volume averaged PO_2 declined only from 22 to 17
357 mmHg (Fig. 6d) which were values above $P_{50,Mb}$. This was caused by a significant volume
358 fraction of the muscle (approximately 30%) with myocytes at $PO_2 < 10$ mmHg. The effect
359 of the PO_2 spatial distribution on the $SO_{2,Mb}$ was even more evident for a lower blood flow
360 0.7 L kg^{-1} min^{-1} (red line) that caused a further decrease of the volume averaged PO_2 to 6
361 mmHg. In this condition, the volume averaged SO_2 decreased to 38% because more than
362 60% of the muscle myocytes had a PO_2 less than 1 mmHg (Fig. 6d).

363 **Discussion**

364 A computational model of muscle O_2 transport and metabolism was validated with 365 experimental data to analyze how Hb and Mb contributions to the NIRS signal are affected 366 by O_2 delivery. The primary finding of this study was that for adequate O_2 delivery (1.1 L 367 kg^{-1} min^{-1}), Mb contribution to the NIRS signals was approximatively 40% for $\Delta HHbMb$ 368 and 27% for $\Delta HbMbO_2$ even in the presence of a linear C_{ven}^{T}-NIRS relationship. For 369 reduced O_2 delivery, the non-linearity of the C_{ven}^{T}-NIRS (oxygenated or deoxygenated) 370 relationship was mainly related to a Mb desaturation of a large portion of the extravascular 371 tissue. With a reduced O_2 delivery, Mb saturation varied from 85 to 40%, corresponding to 372 a variation of the Mb contribution to the NIRS from 15 to 50%.

373 *Venous O_2 concentration vs. NIRS signals relationship*

374 The linearity between the venous O_2 concentration and tissue oxygenation measured by 375 NIRS has been reported in animal (Wüst *et al.*, 2014; Sun *et al.*, 2016) and human (Mancini *et* 376 *al.*, 1994; Vogiatzis *et al.*, 2015) studies. This evidence supports the idea that the NIRS signal 377 is affected by oxygenation of Hb within the venous compartment but does not exclude Mb 378 contribution. Our model simulations indicate that the Mb contribution to the NIRS signal 379 depends on the balance between O_2 delivery and utilization (Fig. 4) and quantifies the Mb 380 contribution responsible for a deviation from a linear relationship between venous O_2 381 concentration and tissue oxygenation. These aspects are discussed in this section. Other 382 studies suggested a tight interplay between O_2 convection, diffusion and utilization 383 kinetics; thus, both capillary/venous and tissue O_2 content change simultaneously (Lai *et al.*, 384 2007; Takakura *et al.*, 2015) with blood flow during muscle contraction. Thus, it appears that 385 NIRS changes reflect not only Hb, but also Mb oxygenation adjustments.

386 The concurrent change in Hb and Mb oxygenation predicted by the simulations is 387 supported by a human study with simultaneous measurement of the Mb signal by ^{1}H NMR 388 and the Hb/Mb signal by NIRS (Bendahan *et al.*, 2017). Another human study (Boushel *et al.*, 389 1998) reported that muscle oxygenation changes by NIRS did not follow those of the 390 venous O_2 saturation, thus suggesting that Mb contribution to the NIRS could have been in 391 part responsible for the differences.

392 The results of the mathematical model indicate that the relationship between NIRS
393 signals and C_{ven}^{T} is linear for high blood flow – oxygen consumption ratio (Q/VO_2) and
394 becomes non-linear for $Q/VO_2 < 7$. To analyze this aspect the model was used to simulate
395 experimental conditions characterized by different oxygen delivery rates for a similar
396 metabolic rate under a pseudo-steady state resting or contracting muscle. Thus, the
397 microvascular and extravascular O_2 content resulting from the interplay between O_2
398 delivery and utilization simulated, is not related to the onset of muscle contraction.

399 Model simulations suggest that a linear relationship between NIRS signals and C_{ven}^{T} can
400 coexist with a Mb contribution to the NIRS signal smaller than 35% observed in resting
401 muscle (Fig. 3, T1 - red line and T2 - blue line). This is possible because both NIRS signals
402 are mainly affected by the microvascular oxygenation (Fig. 4) which is linearly related to
403 O_2 delivery. The nonlinear decrease of Mb saturation (Fig. 5a) observed with a reduced O_2
404 delivery in T1 has only a minor effect on the amplitude of the NIRS signals (Fig. 4a and
405 4b). During contractions (T3), with a more pronounced effect of the imbalance between O_2
406 delivery and utilization on C_{ven}^{T} than that observed at rest (T1), the simulated relationship
407 between NIRS signals and C_{ven}^{T} becomes non-linear with Mb contribution to the NIRS
408 signal greater than 35% (Fig. 3c, green line). In contrast, the experimental data obtained for
409 T3 condition showed a linear relationship between venous and NIRS signal oxygenations.
410 Two aspects can be considered to interpret these apparent conflicting results: a) model
411 simulations deviated from the linearity, but they are still within the range of the
412 experimental data standard deviation; and b) the oxygen delivery range used by the
413 computational model was larger (0.6-1.4 L kg^{-1} min^{-1}) than that investigated in the
414 experimental study (0.8-1.2 L kg^{-1} min^{-1}), thus the model offered the opportunity to explore
415 the C_{ven}^{T}-NIRS signal relationship for oxygen delivery lower than that investigated (<0.8 L
416 kg^{-1} min^{-1}) in the experimental study (Sun *et al.*, 2016). The nonlinear relationship between
417 NIRS signals and C_{ven}^{T} simulated by the computational model and characterized by Mb
418 contribution within the range of 35-50% is consistent with a similar Mb contribution to the
419 NIRS signal observed in a rat muscle perfused with a hemoglobin free buffer (Masuda *et al.*,
420 2010) and in other animal and human studies (Nioka *et al.*, 2006; Marcinek *et al.*, 2007*b*).
421

422 *Q effects on SO_2: Hb and Mb contributions to the NIRS signal*

423 The SO_2 in the microvascular (i.e., Hb) and extravascular (i.e., Mb) compartments 424 appear to be key factors in determining the O_2 content redistribution within the 425 microvascular and tissue compartments and thus the Hb/Mb contribution to the NIRS 426 signals ($y_{\Delta HbO_2}$, $y_{\Delta MbO_2}$) with differing O_2 delivery. It has been suggested that the 427 difference of O_2 desaturation between Hb and Mb during muscle contraction could in part 428 explain a slower muscle deoxygenated NIRS kinetics than that of the microvascular O_2 429 content by phosphorescence-quenching (Koga *et al.*, 2012). This finding was related to a 430 faster O_2 desaturation of Hb than that of Mb during contraction. In comparison to this study 431 (Koga *et al.*, 2012), our results offer a different perspective in studying the effect of SO_2 on 432 Hb/Mb contribution because our simulations were obtained for steady-state conditions with 433 O_2 in equilibrium with either Hb or Mb. Thus, any Mb contribution to the NIRS signal 434 related to a slower O_2 desaturation kinetics of Mb in comparison to that of Hb were not 435 present. Nevertheless, an important effect of blood flow on SO_2 of Hb and Mb affecting the 436 NIRS signals was still present; this finding is discussed in the following section.

437 Our results suggest that an SO_2 characteristic difference between blood and tissue 438 contribute to determine the distinct effects of Hb and Mb contributions to the NIRS signals 439 with O_2 delivery changes. Under resting or contracting conditions when the O_2 delivery was 440 sufficient or in excess (C_{ven}^T> 7-9 mLO_2 100 mL^{-1}), the SO_2 changes in the microvascular 441 compartment (i.e. Hb) were more sensitive to O_2 delivery changes than those in the 442 extravascular compartment (i.e. Mb) whereas for reduced O_2 delivery the SO_2 changes in 443 the extravascular compartment became more sensitive to O_2 delivery changes than those in 444 the microvascular compartments (Fig. 5). The SO_2 difference between microvascular and 445 extravascular compartments can be attributed to two factors: a higher affinity of Mb than 446 Hb for O_2 and a lower PO_2 spatial distribution of the extravascular compartment 447 (myocytes) than that of the microvascular compartment (capillaries). Although the partial 448 pressure of O_2 required to achieve 50% Hb and Mb saturation (P_{50}) is 31.2 and 2.4 mmHg, 449 respectively (Lai *et al.*, 2007; Feher, 2017), the effects of blood flow on the microvascular and 450 extravascular SO_2 are also significant at higher PO_2 than P_{50}. Specifically, for the oxygen 451 saturation curve, the SO_2-PO_2 slope changes approximately at 55 and 20 mmHg for Hb and

452 Mb, respectively (Dash & Bassingthwaighte, 2004; Feher, 2017). Consistent with the Hb-SO_2
453 and Mb-SO_2 characteristics, the variation of the Mb contribution to the NIRS signal
454 became more sensitive than that of Hb (Fig. 5) when capillary PO_2 was 45 mmHg (Fig. 6b)
455 and that in myocyte was 22 mmHg (Fig. 6d). Our interpretation is consistent with the
456 finding of the NIRS kinetics study (Koga *et al.*, 2012) that suggested an earlier O_2
457 desaturation of Hb than Mb during contraction. Further, our analysis supports the idea that
458 the higher affinity of Mb than Hb for O_2 contributes to the initial and preferential O_2
459 desaturation of Hb during contraction. Also, it should be noted that in a human study, Mb
460 desaturation was observed at submaximal work rates (Richardson *et al.*, 1995). Our results do
461 not exclude this phenomenon and indicate its occurrence in the presence of an imbalance
462 between oxygen delivery and utilization. In our simulations, an important contribution of
463 the Mb desaturation (Fig. 5a and 5b) occurs for Q/VO_2 ratio lower than 7 which is similar
464 to the Q/VO_2 range observed in the human study (7-9.5).
465 The second factor responsible for SO_2 differences between microvascular and
466 extravascular compartments of contracting muscle (T3) is the differential effect of oxygen
467 delivery on the PO_2 spatial profile in capillaries and myocytes (Fig.6b and 6d). Thus, Hb
468 saturation varied from 70 to 60% and Mb saturation from 85 to 40% (Fig. 5b). With a
469 reduced oxygen delivery (0.7 L kg^{-1} min^{-1}), the entire capillary compartment had a PO_2
470 above 20 mmHg (Fig. 6b) while 60% of the myocytes compartment had a PO_2 less than 1
471 mmHg (Fig. 6d). Thus, even though the volume averaged PO_2 (6 mmHg) was above P_{50},
472 the SO_2 decline in myocytes was much larger than that observed for capillaries.
473 Nevertheless, it is possible that the 60% of the myocytes below 1 mmHg was an
474 overestimate. The cause for an overestimation of the myocyte fraction under the hypoxic
475 condition could be related to an underestimation of the simulated O_2 diffusion rate that
476 depends on the permeability surface area of the capillary membrane to oxygen.
477 Uncertainties exist about the value of this physiological parameter that quantifies O_2
478 diffusion through the capillary-tissue membrane. The estimated fraction below 1 mmHg is
479 high for the following reasons: 1) the blood flow was decreased by 37% of the control
480 value in the model analysis, and 2) there was no rapid fatigue during the low flow period.
481 On the other hand, the muscle fraction with low oxygen that corresponds to fatigue is

482 unknown. Even in the presence of uncertainties about the permeability surface area, the
483 model offers a plausible physiological mechanism for a deviation from the linear
484 relationship between NIRS signals and C^T_{ven}. With lowered oxygen delivery, even if
485 myocyte PO_2 is not as low as 1 mmHg, there would be a lower myocyte PO_2 that
486 contribute to increase the Mb contribution to the NIRS as suggested by our model findings.

487 Our findings are consistent with the notion that the determination of the mean muscle
488 PO_2 can be underestimated when it is derived from volume averaged Mb saturation by
489 MRS (Jürgens *et al.*, 2000). Our computational work indicates that the estimate of a volume
490 averaged PO_2 determined from an averaged SO_2 using PO_2–Mb relationship was smaller
491 than the volume averaged PO_2 calculated from a PO_2 spatial profile because of the
492 nonlinear relationship between PO_2 and SO_2. Specifically, the intracellular mean PO_2
493 corresponding to a 50% Mb saturation (volume averaged) measured in skeletal muscle by
494 MRS, was 3 mmHg (Richardson *et al.*, 2001). In contrast, our model for the same 50%
495 volume averaged Mb saturation, predicted a 10 mmHg volume averaged PO_2 in tissue and
496 a 40 mmHg volume averaged PO_2 in capillaries. Thus, our findings indicate a less
497 pronounced myocyte PO_2 decline with a decrease of Mb saturation than that observed in
498 the MRS study for work rate close to 50-60% of the maximal value. Our results are
499 consistent with our computational work on the relationship between myocyte PO_2 and work
500 rate (Spires *et al.*, 2012) and with the experimental work on simultaneous measurements of
501 microvascular (28.4±5.3 mmHg) and intracellular (10.6±5.2 mmHg) PO_2 in rat skeletal
502 muscle (Hirai *et al.*, 2018).

503 Our work indicates that a decrease of O_2 delivery increases the Mb contribution to the
504 NIRS signal. Also, a human NIRS study suggested a possible Mb contribution to the NIRS
505 signal caused by reduced O_2 delivery obtained with supine exercise (Goulding *et al.*, 2020).
506 Nevertheless, the human study analyzed NIRS kinetics whereas our simulations referred to
507 steady state conditions. In this study, both blood flow and volume kinetics could have
508 affected the NIRS kinetics. Nevertheless, quantifying the extent of their contributions to the
509 NIRS signal is challenging with the current experimental settings. The mathematical model
510 proposed could be used to overcome this limitation in quantifying the effect of blood flow
511 and volume changes on the NIRS kinetics observed for supine exercise.

512 *Microvascular volume*

513 The capillary-fiber geometry data reported in the literature is consistent with the
514 microvascular volume fraction of 6% used in our study. It was determined assuming a
515 capillary volume density in canine gastrocnemius muscle of 5% (Bebout, 1991; Bebout *et al.*,
516 1993) and that the capillary volume fraction was 80% of the microvascular volume (Poole *et*
517 *al.*, 1995). Other canine skeletal muscle studies reported lower values than those reported for
518 gastrocnemius: capillary volume density was 1.5-2.5 % (Conley *et al.*, 1987) and active
519 vascular volume was 4-5% in gracilis (Baker *et al.*, 1976). If a microvascular volume fraction
520 lower than 6% were considered in our simulation, the contribution of Mb could have been
521 greater than that estimated for the conditions investigated (Fig. 3-5).

522 With regard to the microvascular volume distribution, the 10% arterial volume fraction
523 used in our work is similar to that reported for human (Ruotsalainen *et al.*, 1997) and animal
524 skeletal muscle (Poole *et al.*, 1995) whereas the capillary volume fraction of 80% was similar
525 to that reported for rat skeletal muscle (Poole *et al.*, 1995). A different microvascular volume
526 distribution with 80% venous fraction was used in the past (Kocsis *et al.*, 2006; Lai *et al.*,
527 2009*a*) and based on anatomical studies of the brain (van Lieshout *et al.*, 2003).

528 Another factor affecting the Hb contribution to the NIRS signal is the hematocrit in
529 blood vessels. In our work, we assumed that the microvascular compartment hematocrit
530 was like that in the systemic circulation. Nevertheless, the microvascular hematocrit is
531 smaller than that observed in the large vessel due to the Fåhraeus's effect (Fåhraeus, 1929).
532 Hematocrit in the microvascular volume was suggested to be 22% (Davis & Barstow, 2013) or
533 60-90% (Pries *et al.*, 1996; Fantini, 2002) of that in large vessels. In our study, because NIRS
534 signals reflect the small blood vessel, Hb contribution to the NIRS signal was corrected
535 using microvascular volume fraction rather than the systemic fraction although hematocrit
536 was assumed to be the same (45-50%). Thus, the model prediction of Hb contribution to the
537 NIRS signal would decrease by 50% if a microvascular hematocrit of 25% was considered.
538 Nevertheless, our simulations are referred to a steady state during muscle contraction in
539 which the Fåhraeus effect should be minimal because the microvascular hematocrit reaches
540 a value comparable to that of the systemic hematocrit (Davis & Barstow, 2013; Poole *et al.*,
541 2013).

542 *Limitations*

543 The inferences of our work were obtained from a mathematical model validated with
544 data of an animal model of oxidative metabolism in muscle electrically stimulated. The
545 canine muscle preparation is almost exclusively made up of oxidative and
546 oxidative/glycolytic fibers (Sun *et al.*, 2016). Thus, the findings of the present study might be
547 different in muscle with a greater percentage of glycolytic, lowly oxidative fibers.

548 The electrically stimulated muscle contraction of the animal model presents some
549 differences (Sun *et al.*, 2016) in comparison to voluntary muscle contraction in humans.
550 Nevertheless, it is expected that the inferences on the Hb and Mb contribution to the NIRS
551 signals are also applicable to humans. Any change in muscle perfusion and metabolic
552 intensity are simulated with a variation of blood flow and oxygen consumption, thus, the
553 mathematical model does not include any specific features related to the muscle electrical
554 stimulation of the animal model. It is possible that the Mb contribution to the NIRS signal
555 in human contracting muscle could be less than that quantified in the muscle of the animal
556 model because the human muscle blood flow perfusion is higher than that observed in
557 canine muscle preparation. In particular, the Q/VO_2 ratio in the exercising leg was 7 (Grassi
558 *et al.*, 1996) whereas that of the gastrocnemius was in the range of 5.6-7.

559 The reliability of the computational analysis to deconvolute the NIRS signal and
560 quantify the non-measurable O_2 content in the microvascular and extravascular
561 compartments, relies on the knowledge of blood flow, hematocrit, permeability surface area
562 and microvascular volume distribution that differs among species and individuals. Whereas
563 the model predictions proposed in our work were validated with several sets of
564 experimental data in our canine muscle preparation, validation in human studies requires
565 knowledge of muscle blood flow kinetics which cannot be easily measured and other
566 physiological and biochemical parameters less accessible in comparison to the animal
567 muscle preparation (Koirala *et al.*, 2022). Although the results of our study were obtained for
568 a pseudo steady state condition it could be inferred that Mb contribution to NIRS
569 measurements is also important at the onset of muscle contraction. Therefore, future studies
570 may focus on how the interplay of convection and diffusion of oxygen affects the kinetics
571 of Mb and Hb saturations at the onset of muscle contraction.

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 O_2 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 state. The computational analysis indicates that the NIRS and venous oxygenation relationship deviates from linearity when O_2 saturation in myocytes declines below 80% with a Mb contribution to the NIRS signal greater than 35%. This decline was associated with a large portion of the extravascular tissue with low partial pressure of oxygen due to a reduced oxygen delivery that can occur under physiological and pathophysiological conditions.

Our findings support the notion that the NIRS signal results from a combination of microvascular and extravascular oxygenation in skeletal muscle especially under pathophysiological conditions with reduced O_2 delivery either by convection and or diffusion. Thus, the determination of the oxygen consumption in contracting muscle by NIRS kinetics is challenging because of the complex nature of the NIRS signal that provides only an index of the local O_2 extraction from microvascular and extravascular compartments. Another challenge is the assessment of the factors affecting the oxygen consumption determined by NIRS kinetics. With both Hb and Mb saturations affecting the NIRS signal, it is crucial to quantify the relative importance of convective vs. diffusive transport effects on the NIRS signal. The mathematical model can quantify the extent of the convection and diffusion processes as well as the blood volume changes in the region of the NIRS measurement. Our proposed method combines the mathematical model and experimental data of blood flow and has the potential to quantify muscle oxygen consumption from NIRS measurements.

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601 Skin Diseases (NIH-NIAMS) under award number K25AR057206.

602 **Conflicts of Interest**

603 No conflicts of interest, financial or otherwise, are declared by the authors.

604

605 **Figure captions**

606 **Figure 1**. Experimental conditions simulated in this work and previously investigated by
607 (Sun *et al.*, 2016): Effect of blood flow at rest (T1) or during contraction (T3, 0.5Hz) with
608 constant arterial partial pressure of oxygen (P_{O2}=100 mmHg); Effect of P_{O2} (T2) due to
609 breathing gases of differing O_2 concentration with constant blood flow 0.2 L kg^{-1} min^{-1}.

610 **Figure 2A**. Model validation and predictions of blood and tissue oxygenation for different
611 blood flow (Q) at rest (T1, a, b), arterial oxygen partial pressure (T2, c, d) and different Q
612 during contraction (T3, e, f): comparison between model prediction (solid line) and
613 experimental data (open circle) of C_{ven}^{T} (panel a–T1; panel c–T2; panel e–T3) and (open
614 circle) $\Delta HHbMb_N$ and (filled circle) $\Delta HbMbO_{2,N}$ (panel b–T1; panel d–T2; panel f–T3;).

615 **Figure 2B**. Model inputs: simulated (black line) and experimental data (open circle) of
616 muscle oxygen uptake ($\dot{VO}_2$) and relative heme group changes of Hb/Mb concentration
617 ($\Delta C_{Heme,N}$) for (a, b) T1, (c, d) T2, and (e, f) T3 trials; rate coefficient (k_{ATPase}, grey line)
618 associated to the metabolic rate to simulate $\dot{VO}_2$ for (a) T1, (c) T2, and (e) T3 trials;
619 microvascular volume fraction ($f_{b,m}$) changes with (b, T1) Q or (d, T2) P_{O2} at rest, or (f,
620 T3) Q during contraction.

621 **Figure 3**. (a) Relationship between venous O_2 content (C_{ven}^{T}) and $\Delta HHbMb_N$, (b)
622 relationship between C_{ven}^{T} and $\Delta HbMbO_{2,N}$: comparison between model prediction (solid
623 lines: red - T1; blue - T2; green - T3) and experimental data (symbol: open circle - T1;
624 filled circle - T2; filled square - T3) of (a) $\Delta HHbMb_N$, and (b) $\Delta HbMbO_{2,N}$ obtained for
625 different C_{ven}^{T} (i.e. oxygen delivery). Simulation of the (c) relationship between C_{ven}^{T} and
626 Hb contribution to the $\Delta HHbMb_N$ NIRS signal ($y_{\Delta HHb}$); simulation of the (d) relationship
627 between C_{ven}^{T} and Hb contribution to the $\Delta HbMbO_{2,N}$ NIRS signal ($y_{\Delta HbO_2}$): (solid lines:
628 red - T1; blue - T2; green - T3). It should be noted that the Mb contribution to the
629 oxygenated ($y_{\Delta MbO_2}$) or deoxygenated ($y_{\Delta HMb}$) signals is related to that of Hb by the
630 expressions $y_{\Delta MbO_2} = 1 - y_{\Delta HbO_2}$, and $y_{\Delta HMb} = 1 - y_{\Delta HHb}$.

631 **Figure 4.** Relationship between venous O_2 content (C_{ven}^{T}) and the relative changes of
632 deoxygenated (ΔHHb, ΔHMb – dashed line, a) T1; c) T3) and oxygenated (ΔHbO_2, ΔMbO_2

633 – solid line b) T1; d) T3) Hb and Mb from rest obtained for different blood flows. Green,
634 blue and black refer to capillary, venule, and myocyte compartments.
635 **Figure 5**. Relationship between venous O_2 content (C_{ven}^T) and O_2 saturation (SO_2) in the
636 microvascular and myocyte compartments for different blood flow of (a) T1 and (b) T3;
637 green, blue, and black refer to capillary, venule, and myocyte compartments.
638 **Figure 6**. Spatial profiles of the volume averaged SO_2 and PO_2 for capillary (a, b) and
639 myocyte (c, d) compartments obtained with blood flow of 0.7 (red line), 1.1 (green line),
640 and 1.3 (blue line) L kg^{-1} min^{-1} for T3. For these blood flow value, the corresponding
641 Q/VO_2 are approximatively 5.6 (blue line), 5.7 (green line) and 7 (red line). The SO_2
642 spatial profile obtained for capillaries and myocytes correspond to the volume averaged
643 SO_2 indicated in Fig. 5 with short lines.
644

645 **APPENDIX A**

646 The reaction rate coefficient of the ATPase flux, k_{ATPase}, is a model parameter that must be
647 determined to simulate the NIRS signal for each muscle blood flow. The reaction rate
648 coefficient can be computed as

649 $$k_{ATPase} = \frac{5.6\ VO_2}{f_t\ C_{ATP}} \quad \text{(A1)}$$

650 where f_t is the extravascular tissue volume fraction in muscle and C_{ATP} is the concentration
651 of ATP in muscle. Because the muscle oxygen uptake (VO_2) are available for only four
652 muscle blood flow data a regression line of the VO_2-Q data was used in A1 to calculate
653 k_{ATPase} for each muscle blood flow of the simulations.

654 Microvascular volume fraction ($f_{b,m}$): it is a function of the relative heme concentration
655 changes ($\Delta C_{Heme,N}^{exp}$) detected by the NIRS signals as

656 $$f_{b,m} = f_{b,m}\left(\Delta C_{Heme,N}^{exp}\right) = f_{b,m}^{R} + \frac{\Delta C_{Heme,N}^{exp}\ \Delta HbMbO_{2,}^{R}}{C_{b,Hb} - C_{t,Mb}/4} \quad \text{(A2)}$$

657 $\Delta C_{Heme,N}^{exp}$ is calculated from the oxygenated and deoxygenated NIRS measurements as:

658 $$\Delta C_{Heme,N}^{exp} = \Delta HbMbO_{2,N} + \Delta HHbMb_N \quad \text{(A3)}$$

659 The blood volume changes are attributed to the capillary compartment (V_{cap}), whereas
660 arteriole and venule volumes are the same as those at rest (Table 1). $f_{b,m}$ is used to quantify
661 V_{cap} and the arteriole, capillary, and venule volume fractions (ω_{art}, ω_{cap}, and ω_{ven}).
662 Details to calculate the arteriole, capillary, and venule contributions to the NIRS signals are
663 reported in our previous studies (Koirala *et al.*, 2021*a*).

664

665 Table 1. Vascular, extravascular and microvascular volume distribution as well as
666 oxygenated and deoxygenated heme group concentrations of Hb and Mb at rest and
667 ischemia.

Notation	Unit	Rest (R)	Ischemia (I)
	Volume distribution		
V_{mus}	[g]	62.5	62.5
f_b	[%]	7	3
f_t	[%]	93	97
f_m	[%]	85	63.4
$f_{b,m}$	[%]	5.95	1.9
ω_{art}	[%]	10	32.7
ω_{cap}	[%]	80	34.6
ω_{ven}	[%]	10	32.7
	Heme group concentrations (Hb/Mb)		
HbO_2	[mM]	167.1	0
MbO_2	[mM]	72.3	0
HHb	[mM]	4.2	54.7
HMb	[mM]	2.7	78.2
$HbMb$	[mM]	246.3	132.9

668

669 Table 2. Parameters of the Hb and Mb saturation relationship for blood and tissue,
670 respectively.

Notation	Value	Unit	Reference
α_{O_2}	$1.34 \cdot 10^{-3}$	[mM mmHg^{-1}]	(Hogan *et al.*, 1992)
$P_{50,Hb}$	31	[mmHg]	(Lai *et al.*, 2007)
$P_{50,Mb}$	2.4	[mmHg]	(Lai *et al.*, 2007)
n	2.8	[-]	(Richardson *et al.*, 1998)
$C_{c,Mb}$	0.32	[mM]	(Lai *et al.*, 2007)
$C_{b,Hb}$	2.6-2.9	[mM]	(Koirala *et al.*, 2021*a*)

671

672 **Reference**

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