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Special Communications: Contrasting Perspectives

NIRS-Based Muscle Oxygenation Is Suitable for Computation of the Convective and Diffusive Components of O_2 Transport at $\dot{V}O_{2max}$

Giorgio Manferdelli[1], Thomas J. Barstow[2], and Grégoire P. Millet[1]

[1]Institute of Sport Sciences, University of Lausanne, Lausanne, SWITZERLAND; [2]Department of Kinesiology, Kansas State University, Manhattan, KS

Address for Correspondence:

Giorgio Manferdelli, Institute of Sport Sciences (ISSUL), University of Lausanne, Synathlon, 1015 Lausanne, Switzerland; E-mail: Giorgio.Manferdelli@unil.ch

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Maximal oxygen uptake ($\dot{V}O_{2max}$) is likely one of the most investigated parameters in the field of exercise physiology. The first description of a maximal rate of the cardiovascular and respiratory systems to transport and utilize oxygen (O_2) to produce physical work is dated back in early '1900s with Hill and Lupton (1). Since then, the $\dot{V}O_{2max}$ concept has been validated and extended (2, 3).

The O_2 cascade from ambient air to muscle mitochondria, aiming to support oxidative ATP production, consists of several consecutive steps, including 1) convective O_2 flow to the alveoli by pulmonary ventilation; 2) diffusive gas transfer across the alveolar-capillary membrane into the capillary blood; 3) convective transport in the blood to the peripheral tissues (i.e., skeletal muscle); and 4) diffusive movement out of the muscle capillaries to the mitochondria where O_2 is utilized (4, 5). Perturbations at any level of the O_2 cascade may significantly affect O_2 transport and utilization, thus $\dot{V}O_{2max}$ (4).

Near-infrared spectroscopy (NIRS) is a non-invasive optical tool to measure the oxygenation status of the primary heme compounds (hemoglobin and myoglobin) suppling O_2 to and within skeletal muscle, and its application in exercise physiology research has increased exponentially in recent years (6). Below, we explain why we believe that NIRS can be used as a non-invasive approach to investigate the relative convective and diffusive components of O_2 transport at $\dot{V}O_{2max}$.

A proposed model to independently evaluate convective and diffusive O_2 transport

The four steps involved in the O_2 cascade can be summarized by two main mechanisms

termed convective ($\dot{Q}O_2$) and diffusive (DO_2) O_2 transport (7). While $\dot{Q}O_2$ is determined by the bulk movement of O_2 in air or blood, DO_2 is the passive movement of O_2 down a pressure gradient across both the alveolar-capillary membrane and the muscle capillary-mitochondrion diffusion pathway (8).

Wagner has proposed a mechanistic model to graphically illustrate the integration of both $\dot{Q}O_2$ and DO_2 mechanisms into a single scheme that depicts how each component contributes to $\dot{V}O_{2max}$ (2, 5, 7). Although the validity of this model is generally accepted and used to understand the site of limitation to $\dot{V}O_{2max}$ in different populations and/or environmental conditions (9-11), this approach requires invasive collection of both arterial and muscle venous blood (by catheterization) to algebraically calculate $\dot{Q}O_2$ (by the Fick Principle of Mass Conservation, *Equation 1*) and DO_2 (by Fick's Law of Diffusion, *Equation 2*).

$$\dot{V}O_2 = \dot{Q} \times (CaO_2 - CvO_2) \qquad (1)$$

$$\dot{V}O_2 = DO_2 \times (PcapO_2 - PmitoO_2) \qquad (2)$$

Briefly, in *Equation 1*, $\dot{Q}$ represents cardiac output, CaO_2 and CvO_2 the arterial and venous O_2 content, respectively, while, in *Equation 2*, DO_2 is the diffusivity of O_2, $PcapO_2$ and $PmitoO_2$ represent mean capillary and mitochondria partial pressure of oxygen (PO_2), respectively. *Equation 2* can be simplified considering that, at maximal exercise intensity (i.e., $\dot{V}O_{2max}$), $PcapO_2$ was shown to be proportional to mean venous PO_2 ($P\bar{v}O_2$) (12) and $PmitoO_2$ is usually ~1–3 Torr (13, 14) and therefore assumed to be negligible. However, the compulsory requirement for arterial and venous blood sampling is an important limitation for widespread

application of the technique, requiring medical supervision and logistics, additional technical personnel support, significant time for subject and laboratory preparation, and the willingness of subjects to endure catheterization. In addition, invasive studies limit the number of repeated studies across days or weeks possible on a given subject/patient. While the direct measurement of arterial and venous PO_2 to determine $PcapO_2$ by Bohr integration has been historically the gold standard, a non-invasive approach with sufficient accuracy and response time to evaluate the integrative responses of the cardiopulmonary system and skeletal muscles to physical exercise would advance our understanding of the potential underlying mechanisms limiting aerobic power in a variety of subjects.

Near-Infrared Spectroscopy: a non-invasive monitoring of muscle oxygenation

NIRS has emerged as a useful and popular non-invasive optical method to monitor changes in local skeletal muscle oxygenation in response to different stimuli, including exercise. Briefly, this technique measures the absorption of near-infrared light at different wavelengths to measure concentration changes in oxygenated and deoxygenated myoglobin and hemoglobin (HbO_2 and HHb, respectively) in the investigated tissue. A primary advantage of NIRS is that the signals specifically reflect the balance between O_2 delivery and O_2 utilization at the microvascular level within working skeletal muscles (15), and thus directly relate to the conditions reflected in the Wagner diagram, in contrast to more global measures of O_2 content and pressure in arterial and venous blood. Its non-invasive nature makes it ideal for use in human participants ranging from fragile clinical populations (16, 17) to top-class athletes (6). Extensive reviews on NIRS applications in exercise science have provided mechanistic, physiological explanations which underpin this technique, as well as its advantages, limitations, and practical

applications to investigate skeletal muscle physiology (15, 17). NIRS-derived signals (including HbO_2, HHb and tissue saturation (StO_2 or Tissue Saturation Index, TSI)) accurately reflect both venous O_2 saturation (SvO_2) and content CvO_2 of the evaluated skeletal muscle tissue (18-20), in the isolated dog gastrocnemius preparation (R^2=0.69 to 0.93). These relationships between muscle NIRS variables and O_2 levels in the venous effluent were evident under different muscle blood flows, inspired O_2 fractions, and exercise intensities (18, 20). Further, a recent study on trained cyclists exercising in both normoxia and hypoxia reported a good linear correlation between regional StO_2 and femoral SvO_2 (21), thus supporting the use of NIRS-derived StO_2 to empirically estimate SvO_2.

These results suggest that NIRS StO_2 can be used as a non-invasive surrogate of venous oxygenation and thus is suitable to compute and investigate the relative contributions of $\dot{Q}O_2$ and DO_2 to O_2 transport at $\dot{V}O_{2max}$ using the Wagner approach.

A non-invasive approach to compute convective and diffusive O_2 transport mechanisms

HHb and total ($HHb+HbO_2$) hemoglobin and myoglobin have often been viewed as proxies of perfusive and diffusive O_2 transport (15), respectively, while StO_2 represents a relative measure of O_2-binding-site availability in hemoglobin and myoglobin, and, similarly to HHb and CvO_2 (18), reflects the balance between muscle O_2 supply and O_2 demand (17, 18). One advantage of StO_2 - at least in continuous wave NIRS devices - is that it does not require a 'physiological calibration' to normalize the signal for skeletal muscle maximum deoxygenation capacity since StO_2, contrarily to HHb, is based on absolute changes in tissue [heme] saturation (15). Further, while the original view computes $\dot{Q}O_2$ and DO_2 from different, but related, venous

O_2 variables (CvO_2 in *Equation 1* and $P\bar{v}O_2$ in *Equation 2*), NIRS allows the integration of StO_2 in both computations (22) to permit semi-quantitative comparison of the relative contribution of changes in $\dot{Q}O_2$ and/or DO_2 to $\dot{V}O_{2max}$. It should be noted that to generate the Wagner diagram, peak $\dot{Q}O_2$ (as $\dot{Q}$max and [Hb]) needs to be measured as well.

Given the previous discussion, we propose NIRS StO_2 as a promising surrogate of $P\bar{v}O_2$. This variable can be easily employed to noninvasively estimate $\dot{Q}O_2$ and DO_2 to understand the site of functional limitation to exercise capacity (22). *Equation 1 (23)* and *Equation* 2 (24) could therefore be rewritten as:

$$\dot{V}O_2 = \dot{Q} \times (CaO_2 - (1.34 \times StO_2 \times [Hb] + 0.003 \times StO_2)) \quad (3)$$

$$\dot{V}O_2 = DO_2 \times StO_2 \quad (4)$$

In addition to cardiopulmonary and NIRS monitoring, this approach will require one relatively small capillary blood sample from the earlobe or the fingertip to measure [Hb] to compute CaO_2 and CvO_2 in Equation 3 (9). Influence of P_{50} on the SO_2/PO_2 relationship and convective O_2 delivery needs to also be considered, and specific equations to calculate this parameter were previously proposed (23).

The NIRS StO_2 signal has already been used to semi-quantitatively illustrate the difference in contributions of convection and diffusion of O_2 during supine and upright incremental exercise to exhaustion (22). In addition, StO_2 (as TSI) has been used as a proxy of $PmvO_2$ to determine O_2 diffusion capacity (24).

Recognized general limitations to the use of NIRS need to be briefly mentioned here – the influence of adipose tissue thickness (ATT) to potentially attenuate the NIRS signal strengths, the presence of myoglobin in the NIRS signals, and the limited sample size for the examined tissue. Approaches have been developed to correct the original NIRS data for attenuation by ATT (25). The relative contributions of Mb and Hb to the NIRS signals from skeletal muscle have been previously estimated and discussed (26). The general conclusion is that during exercise, the whole diffusion pathway, including vascular Hb and intracellular Mb, would desaturate with a similar time course, and thus would not invalidate the interpretation that NIRS signals like StO_2 reflect the dynamic balance between muscle $\dot{Q}O_2$ and $\dot{V}O_2$ in the volume of tissue under consideration (15, 17). Finally, NIRS typically samples a relatively small, local region of tissue, which given the relative heterogeneity of NIRS responses (27) may not reflect an average value for the contracting muscle. We believe this potential concerned is adequately balanced by obtaining a direct estimate of $PmvO_2$ within the contracting muscle.

In conclusion, we propose that NIRS StO_2 is an appropriate variable to non-invasively investigate the relative contribution of $\dot{Q}O_2$ and DO_2 to $\dot{V}O_{2max}$ and extend our current knowledge of the factors underlying exercise intolerance, using the graphical model proposed by Wagner. This approach will be especially valuable in studies on clinical patients and/or top-class athletes, in which the application of more invasive approaches involving arterial and/or venous catheterization may not be feasible or desirable, and which limit repeated measures of the responses in those participants.

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REFERENCES

1. Hill AV, Lupton H. Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Q J Med*. 1923;16(62):135-71.
2. Wagner PD. Algebraic analysis of the determinants of VO2,Max. *Resp Physiol*. 1993;93(2):221-37.
3. Saltin B, Strange S. Maximal oxygen uptake: "old" and "new" arguments for a cardiovascular limitation. *Med Sci Sports Exerc*. 1992;24(1):30-7.
4. di Prampero PE, Ferretti G. Factors limiting maximal oxygen consumption in humans. *Respir Physiol*. 1990;80(2-3):113-27.
5. Wagner PD. An integrated view of the determinants of maximum oxygen uptake. *Adv Exp Med Biol*. 1988;227:245-56.
6. Perrey S, Ferrari M. Muscle oximetry in sports science: a systematic review. *Sports Med*. 2018;48(3):597-616.
7. Wagner PD. Central and peripheral aspects of oxygen transport and adaptations with exercise. *Sports Med*. 1991;11(3):133-42.
8. Leach RM, Treacher DF. Oxygen transport-2. Tissue hypoxia. *BMJ*. 1998;317(7169):1370-3.
9. Ade CJ, Broxterman RM, Moore AD, Barstow TJ. Decreases in maximal oxygen uptake following long-duration spaceflight: role of convective and diffusive O2 transport mechanisms. *J Appl Physiol (1985)*. 2017;122(4):968-75.
10. Porcelli S, Grassi B, Poole DC, Marzorati M. Exercise intolerance in patients with mitochondrial myopathies: perfusive and diffusive limitations in the O2 pathway. *Curr Opin Physiol*. 2019;10:202-9.

11. Poole DC, Hirai DM, Copp SW, Musch TI. Muscle oxygen transport and utilization in heart failure: implications for exercise (in)tolerance. *Am J Physiol Heart Circ Physiol*. 2012;302(5):H1050-63.

12. Roca J, Hogan MC, Story D et al. Evidence for tissue diffusion limitation of VO2max in normal humans. *J Appl Physiol (1985)*. 1989;67(1):291-9.

13. Gayeski TE, Honig CR. O2 gradients from sarcolemma to cell interior in red muscle at maximal VO2. *Am J Physiol*. 1986;251(4 Pt 2):H789-99.

14. Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, Wagner PD. Myoglobin O2 desaturation during exercise. Evidence of limited O2 transport. *J Clin Invest*. 1995;96(4):1916-26.

15. Barstow TJ. Understanding near infrared spectroscopy and its application to skeletal muscle research. *J Appl Physiol (1985)*. 2019;126(5):1360-76.

16. Willingham TB, McCully KK. In vivo assessment of mitochondrial dysfunction in clinical populations using near-infrared spectroscopy. *Front Physiol*. 2017;8:689.

17. Grassi B, Quaresima V. Near-infrared spectroscopy and skeletal muscle oxidative function in vivo in health and disease: a review from an exercise physiology perspective. *J Biomed Opt*. 2016;21(9):091313.

18. Sun YI, Ferguson BS, Rogatzki MJ, McDonald JR, Gladden LB. Muscle near-infrared spectroscopy signals versus venous blood hemoglobin oxygen saturation in skeletal muscle. *Med Sci Sports Exerc*. 2016;48(10):2013-20.

19. Wilson JR, Mancini DM, McCully K, Ferraro N, Lanoce V, Chance B. Noninvasive detection of skeletal muscle underperfusion with near-infrared spectroscopy in patients with heart failure. *Circulation*. 1989;80(6):1668-74.

20. Wust RC, McDonald JR, Sun Y, et al. Slowed muscle oxygen uptake kinetics with raised metabolism are not dependent on blood flow or recruitment dynamics. *J Physiol*. 2014;592(8):1857-71.

21. Vogiatzis I, Habazettl H, Louvaris Z, et al. A method for assessing heterogeneity of blood flow and metabolism in exercising normal human muscle by near-infrared spectroscopy. *J Appl Physiol (1985)*. 2015;118(6):783-93.

22. Goulding RP, Okushima D, Fukuoka Y, et al. Impact of supine versus upright exercise on muscle deoxygenation heterogeneity during ramp incremental cycling is site specific. *Eur J Appl Physiol*. 2021;121(5):1283-96.

23. Severinghaus JW. Simple, accurate equations for human blood O2 dissociation computations. *J Appl Physiol Respir Environ Exerc Physiol*. 1979;46(3):599-602.

24. Pilotto AM, Adami A, Mazzolari R, et al. Near-infrared spectroscopy estimation of combined skeletal muscle oxidative capacity and O2 diffusion capacity in humans. *J Physiol*. 2022;600(18):4153-68.

25. Craig JC, Broxterman RM, Wilcox SL, Chen C, Barstow TJ. Effect of adipose tissue thickness, muscle site, and sex on near-infrared spectroscopy derived total-[hemoglobin + myoglobin]. *J Appl Physiol (1985)*. 2017;123(6):1571-8.

26. Davis ML, Barstow TJ. Estimated contribution of hemoglobin and myoglobin to near infrared spectroscopy. *Respir Physiol Neurobiol*. 2013;186(2):180-7.

27. Okushima D, Poole DC, Rossiter HB et al. Muscle deoxygenation in the quadriceps during ramp incremental cycling: Deep vs. superficial heterogeneity. *J Appl Physiol (1985)*. 2015;119(11):1313-9.

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NIRS-Based Muscle Oxygenation Is Not Suitable to Compute Convective and Diffusive Components of O_2 Transport at $\dot{V}O_{2max}$

Simone Porcelli[1,2], A.M. Pilotto[1,3], and Harry B. Rossiter[4]

[1]Department of Molecular Medicine, University of Pavia, Pavia, ITALY; [2]Institute of Biomedical Technologies, National Research Council, Milan, ITALY; [3]Department of Medicine, University of Udine, Udine, ITALY; [4]Division of Respiratory and Critical Care Physiology and Medicine, The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA

Address for Correspondence:

Simone Porcelli, MD, PhD, Assistant Professor in Human Physiology Institute of Physiology, Department of Molecular Medicine University of Pavia, Italy, Via Forlanini 6, 27100 Pavia – Italy; Phone: +39 0382987538; E-mail: simone.porcelli@unipv.it

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GlaxoSmithKline, Novartis, AstraZeneca, Astellas, United Therapeutics, Genentech and Regeneron. He is a visiting Professor at the University of Leeds, UK. The views of the perspective are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. This perspective does not constitute endorsement by the American College of Sports Medicine.

Measurement of Convective and Diffusive Limitations to $\dot{V}O_2$max

During exercise, oxygen transport from the environment to muscle mitochondria occurs through a sequence of highly-interdependent diffusive and convective steps terminating at muscle mitochondrial oxygen consumption ($\dot{V}O_2$) (1–4). Maximum muscle oxygen uptake depends on the maximum flux of convective O_2 transport by cardiovascular system, diffusing capacity of O_2 from hemoglobin to muscle mitochondria, and muscle enzyme activities. The intersection of these processes at maximum O_2 flux ($\dot{V}O_2$max) are mathematically described in the "Wagner diagram" (3) (**Figure 1**), where Fick's principle, which states that:

$$\dot{V}O_2 = \dot{Q}m \times (CaO_2 - CvO_2) \qquad \text{(Equation1)}$$

where $\dot{Q}m$ is muscle blood flow and CaO_2 and CvO_2 are arterial and muscle venous O_2 concentrations, respectively, conflates with Fick's law:

$$\dot{V}O_2 = DmO_2 \times (PmvO_2 - PimO_2) \qquad \text{(Equation 2)}$$

where DmO_2 is muscle O_2 diffusive conductance and $PmvO_2$ and $PimO_2$ are O_2 partial pressures of the microvasculature and intramyocyte, respectively.

Application of these principals *in vivo* is simplified by the assumption that intramyocyte (or mitochondrial) PO_2 is very low at $\dot{V}O_2$max i.e., functionally negligible, such that $PmvO_2$ approximates the PO_2 driving pressure for transmembrane diffusive O_2 flow (4–6). This reduces

the unknowns in equation 2, allowing convective ($\dot{Q}m$) and diffusive (DmO_2) contributions to O_2 transport at $\dot{V}O_2max$ to be calculated by knowledge of $\dot{V}O_2$, ($CaO_2 - CvO_2$) and $PmvO_2$ (1, 7–9).

The validity of this approach has been experimentally demonstrated in humans using invasive arterial and muscle venous blood sampling and blood flow measurement across an exercising muscle group (typically the lower limb during cycling or knee-extension) and under manipulations of fractional inspired O_2 concentration (4, 5, 10). In these studies, $PmvO_2$ is estimated from mean capillary PO_2 ($\overline{Pcap}O_2$), which is algebraically calculated from knowledge of the arterial and muscle venous PO_2, providing a solution to simultaneous equations 1 and 2. It should be noted that convective ($\dot{Q}m$) and diffusive (DmO_2) contributions to O_2 transport can be plotted on the same graph by substituting $PmvO_2$ with $PvO_2 \times k$, where k represents a constant related to the difference in O_2 partial pressure in the microvasculature compared to the venous compartment. Thus, the Wagner diagram represents the current gold standard to solve for convective and diffusive O_2 flows at $\dot{V}O_2max$. Although this approach requires invasive measurements, it has provided seminal insight into the locus of limitations to $\dot{V}O_2max$ under varying environmental conditions, state of training or in chronic disease (11, 12).

The ability to quantify convective and diffusive O_2 flow by a non-invasive method could provide a major advance in our ability to diagnose and treat exercise intolerance, and thus has a strong rationale for investigation. The Wagner diagram has been reconstructed using non-invasive ^{31}P-magnetic resonance spectroscopy under conditions of altered O_2 availability (e.g. 13), to investigate the role of O_2 availability in limiting isolated-muscle $\dot{V}O_2max$ and to determine O_2 diffusive conductance. This approach, however, cannot estimate the convective O_2 flow and, currently, is limited to exercise in an isolated muscle group. Thus, other non-invasive

approaches are needed to quantify both convective and diffusive O_2 processes during whole-body exercise.

Using Near-Infrared Spectroscopy (NIRS) to Solve the Wagner Diagram

Near-infrared spectroscopy (NIRS) of human muscle (18, 19) provides a non-invasive window into tissue oxygenation at the interface between muscle microvasculature and the myocyte. Because absorption of NIR light is high in large blood vessels, exercise-induced reductions in the concentrations of deoxygenated hemoglobin (Hb) and myoglobin (Mb) (Δ[deoxy(Hb+Mb)]) by NIRS are assumed to reflect changes in oxygenation of the capillary, venular and muscle compartments (20, 21). As such, NIRS may provide a non-invasive surrogate for O_2 extraction; conceptually substituting for ($CaO_2 – CvO_2$) in equation 1 (22). If so, it may be possible to solve for $\dot{Q}m$ in equation 1, using Δ[deoxy(Hb+Mb)] and by simultaneously estimating muscle $\dot{V}O_2$ from NIRS using a brief arterial occlusion (24).

Spatially resolved, frequency domain or time-resolved NIRS provides an index of microvascular (Hb) and muscle (Mb) oxygen saturation e.g., tissue saturation index (TSI), the fraction of [Hb+Mb] bound to oxygen. Thus, it may also be possible to solve for DmO_2 in equation 2 using NIRS, by estimating muscle $\dot{V}O_2$ (as above) and using TSI to calculate $PmvO_2$ (N.B. $PimO_2$ is assumed negligible). Calculating $PmvO_2$ from TSI requires knowledge of the instantaneous relationship between [Hb+Mb] O_2 saturation and partial pressure.

The question at hand is whether such a non-invasive approach is valid. First, we consider available evidence and then we address the rigor of the key assumptions.

Does the Literature Support a Non-Invasive Solution to the Wagner Diagram?

Here we compare invasively-measured data during maximal exercise in 10 mitochondrial myopathy (MM) patients from Jeppesen et al. (26) with unpublished non-invasive NIRS data from our lab obtained from 4 MM patients. Both groups of patients performed the same maximal knee-extension exercise, and had similar $\dot{V}O_2$max during cycling of 1.00 $L \cdot min^{-1}$.

At maximal knee-extension exercise, participants in Jeppesen et al. (26) had a single-leg $\dot{Q}m$ = 4.13 $L \cdot min^{-1}$ and ($CaO_2 - CvO_2$) = 87 $mlO_2 \cdot L^{-1}$, resulting in a muscle $\dot{V}O_2$ = 0.36 $L \cdot min^{-1}$ (equation 1; Figure 1). The diffusive component was estimated from PaO_2 (120 mmHg) and PvO_2 (34 mmHg), which, at a $\dot{V}O_2$ of 0.36 $L \cdot min^{-1}$, results in a DmO_2 of 10.6 $ml \cdot min^{-1} \cdot mmHg^{-1}$ (equation 2; Figure 1). Diffusive conductance in these MM patients is 2-3 times less than that expected from healthy participants (27), most likely as a result of the impaired mitochondrial O_2 utilization that limits the demand for capillary to myocyte O_2 flow, rather than due to a direct diffusion limitation, as previously reported by our group (28). This analysis highlights the utility of the Wagner diagram, to diagnose the locus of impairment in O_2 transport and could be used guide to clinical decision making.

A similar approach was applied using NIRS. In our 4 MM patients, we substituted Δ[deoxy(Hb+Mb)]) for ($CaO_2 - CvO_2$) in equation 1 and TSI (converted to a partial pressure) for ($PmvO_2 - PimO_2$) in equation 2, using a standard Hb-O_2 dissociation curve (REF). We assumed that $\dot{V}O_2$ was 0.36 $L \cdot min^{-1}$ (as in 26), but note that NIRS may also be used to calculate absolute $\dot{V}O_2$ from the rate of deoxygenation during arterial occlusion (24,25). NIRS-based extraction was 35% at maximal exercise, which corresponds to ($CaO_2 - CvO_2$) = 71 $mlO_2 \cdot L^{-1}$. Substituting in equation 1 gives $\dot{Q}m$ = 5.07 $L \cdot min^{-1}$; ~25% greater than the invasively-determined value of 4.13

$L \cdot min^{-1}$ (26). TSI was 66% at maximum exercise in our 4 MM patients, which corresponds to $PmvO_2$ = 34 mmHg, using a standard Hb-O_2 dissociation curve (REF). Substituting in equation 2, results in DmO_2 = 10.6 $ml \cdot min^{-1} \cdot mmHg^{-1}$; the same value as the invasive estimate.

Although these estimates show how NIRS may be applied to solve the Wagner diagram, they also highlight the inherent assumptions and many potential sources of inaccuracy of a quantitative NIRS-based solution. Those assumptions that we feel are most significant, are discussed below.

Assumptions Required for a Non-Invasive Solution to the Wagner Diagram

1. ***Δ[deoxy(Hb+Mb)] cannot substitute muscle CvO_2 or ($CaO_2 - CvO_2$).***

Using NIRS to solve equation 1 requires a NIRS-based surrogate for muscle CvO_2 or (CaO_2 – CvO_2). However, NIRS signals carry no information about the spatial location of oxygenation changes within the arterial and venous compartments. Using Δ[deoxy(Hb+Mb)] for ($CaO_2 - CvO_2$) assumes that arterial oxygen concentration is constant. A reduction in CaO_2 is common at maximal exercise in healthy humans and some patients. Alternatively, substituting Δ[deoxy(Hb+Mb)] for CvO_2 alone, requires knowledge of CaO_2. This could be measured directly from an arterial blood sample, or perhaps estimated from pulse oximetry (SpO_2) coupled with prediction equations for hemoglobin concentration. However, estimating hemoglobin concentration carries a wide range of error, even in healthy humans, and does not account for effects of chronic disease.

In addition, the degree to which different NIRS-visible compartments (arteriolar, capillary, venular, muscular) may contribute to the NIRS signals at maximal exercise is

unknown. Experiments using progressive head-up tilt or perfused muscle models demonstrate that these compartments can change their heme concentration independently (30-32). Δ[Hb+Mb] can markedly influence the Δ[deoxy(Hb+Mb)], causing a dissociation of unknown magnitude between changes in whole tissue heme concentration and muscle deoxygenation measured by NIRS from microvascular muscle O_2 extraction (30). This limitation is present even when absolute [Hb+Mb] is measured by time-resolved or spatially-resolved NIRS equipment.

Finally, skin and adipose tissue thickness (ATT) strongly affects NIRS signals and may cause an overestimation of muscle oxygenation (35, 36). Δ[deoxy(Hb+Mb)] is not proportional to the change in CvO_2 among individuals varying ATT (34). Attempting to address this by normalizing to maximal Δ[deoxy(Hb+Mb)] (e.g. via cuff occlusion or sustained isometric contraction), assumes that all individuals have a similar muscle CvO_2 at $\dot{V}O_2$max, which is not the case (34).

2. NIRS-based protocols cannot measure absolute values of muscle $\dot{V}O_2$.

NIRS can provide a relative estimate of muscle oxidative capacity (22, 37) from the recovery rate constant of m$\dot{V}O_2$ (*k*) established using serial, intermittent, arterial occlusions after exercise (38-40). However, this approach does not provide absolute values for muscle $\dot{V}O_2$. A single-limb occlusion delivered at $\dot{V}O_2$max could be used to estimate the relative rate of deoxygenation of the muscle under the NIRS probe at $\dot{V}O_2$max, in the absence of convective O_2 delivery. However, to convert a relative deoxygenation rate to an absolute $\dot{V}O_2$ requires knowledge of the regional [Hb+Mb], which is typically unknown.

3. *TSI cannot be used to estimate $PmvO_2$*

With knowledge of $\dot{V}O_2max$, DmO_2 can be solved if ($PmvO_2$ – $PimO_2$) is known. As discussed, $PimO_2$ is typically considered negligible, such that a NIRS-based estimate of $PmvO_2$ is needed. TSI represents a relative measure of O_2-binding-site availability in hemoglobin and myoglobin within the NIRS field of view (22). Thus, using TSI as a surrogate for muscle microvascular O_2 saturation ($SmvO_2$) seems a reasonable approach. However, calculating $PmvO_2$ from $SmvO_2$ using the Hb-O_2 dissociation curve requires knowledge of the local (muscle microvascular) temperature, PCO_2, and pH. The significance of the effect of these variables on the shape of Hb-O_2 dissociation at maximal exercise curve should not be understated. For example, muscle venous saturation (SvO_2) may reduce from approximately 30% at the gas exchange threshold to approximately 15% at maximal exercise in healthy individuals, despite $PmvO_2$ remaining relatively constant over this range (41). Continued muscle O_2 extraction above approximately 50% $\dot{V}O_2max$ is essentially possible only due to the actions of temperature, PCO_2, and, primarily, pH shifting the Hb-O_2 dissociation curve to the right (the Bohr shift). Therefore, without knowledge of local pH, at least, it is unlikely that a reasonable estimate of $PmvO_2$ can be derived from $SmvO_2$ or TSI.

4. NIRS signals do not account for spatial heterogeneity of muscle properties

NIRS investigates only a small superficial portion of muscle. Invasive methods, on the other hand, samples blood from a large vein draining the entire limb. Although venous blood sampling does not provide knowledge of the extent of the distribution of $PmvO_2$ throughout the exercising muscle, and are subject to flow weighted averaging, unlike NIRS O_2 extraction from the entire active and inactive musculature is represented in the $PmvO_2$ sample. Muscle deoxygenation and microvascular hemodynamics have different profiles between e.g. the

superficial and deep *rectus femoris*, and the superficial *vastus lateralis* (42). Thus, a clear contribution of convective and diffusive O_2 transport at $\dot{V}O_2$max may not be obtained from NIRS signals of superficial muscle, due to heterogeneity of muscle properties.

CONCLUSIONS

In conclusion, the seminal mechanistic model proposed by Wagner to integrate both $\dot{Q}$m and DmO_2 factors involved in the O_2 cascade from ambient air to muscle mitochondria is an essential step to understand the physiologic mechanisms underlying $\dot{V}O_2$max. However, inherent limitations in NIRS prevent its application for quantitative measurement of convective and diffusive components of O_2 transport at $\dot{V}O_2$max.

Conflict of Interest

The authors have no conflicts of interest to disclose. The views of the perspective are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. This perspective does not constitute endorsement by the American College of Sports Medicine.

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REFERENCES

1. Wagner PD. Diffusive resistance to O2 transport in muscle. *Acta Physiol Scand*. 2000;168(4):609–14.

3. Wagner PD. Gas exchange and peripheral diffusion limitation. *Med Sci Sports Exerc*. 1992;24(1):54–8.

3. Richardson RS, Knight DR, Poole DC, et al. Determinants of maximal exercise VO2 during single leg knee-extensor exercise in humans. *Am J Physiol*. 1995;268(4 Pt 2):H1453-61.

4. Knight DR, Schaffartzik W, Poole DC, Hogan MC, Bebout DE, Wagner PD. Effects of hyperoxia on maximal leg O2 supply and utilization in men. *J Appl Physiol (1985)*. 1993;75(6):2586–94.

5. Hogan MC, Roca J, West JB, Wagner PD. Dissociation of maximal O2 uptake from O2 delivery in canine gastrocnemius in situ. *J Appl Physiol (1985)*. 1989;66(3):1219–26.

6. Poole DC, Musch TI, Colburn TD. Oxygen flux from capillary to mitochondria: integration of contemporary discoveries. *Eur J Appl Physiol*. 2022;122(1):7–28.

7. Roca J, Hogan MC, Story D, et al. Evidence for tissue diffusion limitation of VO2max in normal humans. *J Appl Physiol (1985)*. 1989;67(1):291–9.

8. Richardson RS, Grassi B, Gavin TP, et al. Evidence of O2 supply-dependent V̇O2max in the exercise-trained human quadriceps. *J Appl Physiol (1985)*. 1999;86(3):1048–53.

9. Wagner PD. Systemic oxygen transport and utilization. *J Breath Res*. 2008;2(2):024001.

10. Hirai DM, Musch TI, Poole DC. Exercise training in chronic heart failure: improving skeletal muscle O2 transport and utilization. *Am J Physiol Heart Circ Physiol*. 2015;309(9):H1419–39.

11. Haseler LJ, Lin AP, Richardson RS. Skeletal muscle oxidative metabolism in sedentary humans: 31P-MRS assessment of O2 supply and demand limitations. *J Appl Physiol (1985)*. 2004;97(3):1077–81.

12. Barstow TJ. Understanding near infrared spectroscopy and its application to skeletal muscle research. *J Appl Physiol (1985)*. 2019;126(5):1360–76.

13. Adami A, Rossiter HB. Principles, insights, and potential pitfalls of the noninvasive determination of muscle oxidative capacity by near-infrared spectroscopy. *J Appl Physiol (1985)*. 2018;124(1):245–8.

14. Sun Y, Ferguson BS, Rogatzki MJ, McDonald JR, Gladden LB. muscle near-infrared spectroscopy signals versus venous blood hemoglobin oxygen saturation in skeletal muscle. *Med Sci Sports Exerc*. 2016;48(10):2013–20.

15. Wilson JR, Mancini DM, McCully K, Ferraro N, Lanoce V, Chance B. Noninvasive detection of skeletal muscle underperfusion with near-infrared spectroscopy in patients with heart failure. *Circulation*. 1989;80(6):1668–74.

16. Grassi B, Quaresima V. Near-infrared spectroscopy and skeletal muscle oxidative function in vivo in health and disease: a review from an exercise physiology perspective. *J Biomed Opt*. 2016;21(9):091313.

17. De Blasi RA, Cope M, Elwell C, Safoue F, Ferrari M. Noninvasive measurement of human forearm oxygen consumption by near infrared spectroscopy. *Eur J Appl Physiol Occup Physiol.* 1993;67(1):20-5.

18. Jeppesen TD, Vissing J, González-Alonso J. Influence of erythrocyte oxygenation and intravascular ATP on resting and exercising skeletal muscle blood flow in humans with mitochondrial myopathy. *Mitochondrion*. 2012;12(3):414-22.

19. Richardson RS, Grassi B, Gavin TP, et al. Evidence of O2 supply-dependent VO2 max in the exercise-trained human quadriceps. *J Appl Physiol (1985).* 1999;86(3):1048-53.

20. Porcelli S, Grassi B, Poole DC, Marzorati M. Exercise intolerance in patients with mitochondrial myopathies: perfusive and diffusive limitations in the O2 pathway. *Curr Opin Physiol.* 2019;10:202-9.

21. Severinghaus JW. Simple, accurate equations for human blood O2 dissociation computations. *J Appl Physiol Respir Environ Exerc Physiol.* 1979;46(3):599–602.

22. Adami A, Koga S, Kondo N, et al. Changes in whole tissue heme concentration dissociates muscle deoxygenation from muscle oxygen extraction during passive head-up tilt. *J Appl Physiol (1985)*. 2015;118(9):1091–9.

23. Truijen J, Kim YS, Krediet CTP, et al. Orthostatic leg blood volume changes assessed by near-infrared spectroscopy. *Exp Physiol*. 2012;97(3):353–61.

24. Koirala B, Concas A, Sun Y, Gladden LB, Lai N. Blood volume versus deoxygenated NIRS signal: computational analysis of the effects muscle O 2 delivery and blood volume on the NIRS signals. *J Appl Physiol (1985).* 2021;131(5):1418–31.

25. Niemeijer VM, Jansen JP, Van Dijk T, et al. The influence of adipose tissue on spatially resolved near-infrared spectroscopy derived skeletal muscle oxygenation: the extent of the problem. *Physiol Meas*. 2017;38(3):539–54.

26. Pirovano I, Porcelli S, Re R, et al. Effect of adipose tissue thickness and tissue optical properties on the differential pathlength factor estimation for NIRS studies on human skeletal muscle. *Biomed Opt Express*. 2020;12(1):571-87.

27. Taivassalo T, Jensen TD, Kennaway N, DiMauro S, Vissing J, Haller RG. The spectrum of exercise tolerance in mitochondrial myopathies: a study of 40 patients. *Brain*. 2003;126(2):413–23.

28. Hamaoka T, McCully KK. Review of early development of near-infrared spectroscopy and recent advancement of studies on muscle oxygenation and oxidative metabolism. *J Physiol Sci*. 2019;69(6):799–811.

29. Adami A, Cao R, Porszasz J, Casaburi R, Rossiter HB. Reproducibility of NIRS assessment of muscle oxidative capacity in smokers with and without COPD. *Respir Physiol Neurobiol*. 2017;235:18–26.

30. Ryan TE, Southern WM, Reynolds MA, McCully KK. A cross-validation of near-infrared spectroscopy measurements of skeletal muscle oxidative capacity with phosphorus magnetic resonance spectroscopy. *J Appl Physiol (1985)*. 2013;115(12):1757–66.

31. Pilotto AM, Adami A, Mazzolari R, et al. Near-infrared spectroscopy estimation of combined skeletal muscle oxidative capacity and O2 diffusion capacity in humans. *J Physiol.* 2022;600(18):4153-68.

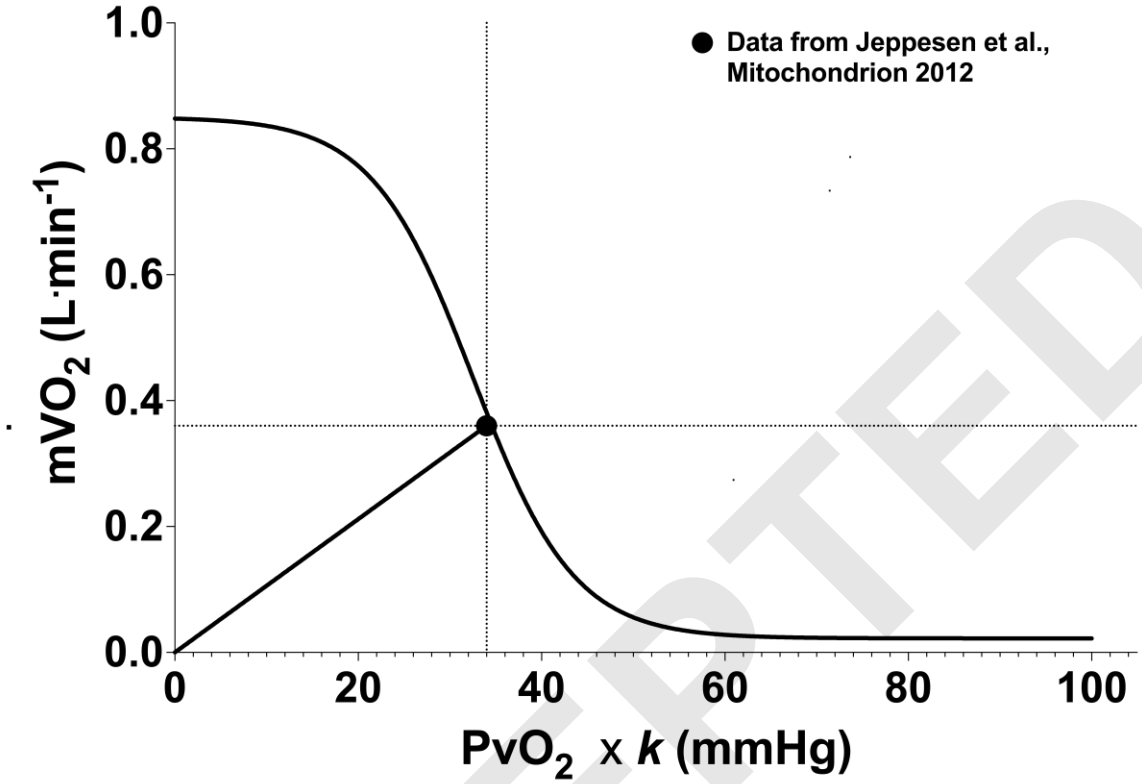
32. Stringer W, Wasserman K, Casaburi R, Porszasz J, Maehara K, French W. Lactic acidosis as a facilitator of oxyhemoglobin dissociation during exercise. *J Appl Physiol (1985).* 1994;76(4):1462–7.

33. Koga S, Okushima D, Barstow TJ, Rossiter HB, Kondo N, Poole DC. Near-infrared spectroscopy of superficial and deep rectus femoris reveals markedly different exercise response to superficial vastus lateralis. *Physiol Rep*. 2017;5(17):e13402.

FIGURE LEGEND

Figure 1. The Wagner diagram produced for data from 10 mitochondrial myopathy (MM) patients during knee extension reported in Jeppesen et al (26). Single-leg VO_2 at maximal exercise is 0.36 $L.min^{-1}$, represented as the intersection of convective O_2 flux (solid curve) and diffusive O_2 conductance (solid line). mVO_2, muscle oxygen consumption; PvO_2, partial pressure of oxygen in the venous blood; k, constant related to changes in O_2 partial pressure from microvasculature to venous compartment.

Figure 1



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Special Communications: Contrasting Perspectives

NIRS-Based Muscle Oxygenation Is Suitable for Computation of the Convective and Diffusive Components of O_2 Transport at $\dot{V}O_{2max}$: Response to Porcelli, Pilotto, and Rossiter

Giorgio Manferdelli[1], Thomas J. Barstow[2], and Grégoire P. Millet[1]

[1]Institute of Sport Sciences, University of Lausanne, Lausanne, SWITZERLAND; [2]Department of Kinesiology, Kansas State University, Manhattan, KS

Address for Correspondence:

Giorgio Manferdelli, Institute of Sport Sciences (ISSUL), University of Lausanne, Synathlon, 1015 Lausanne, Switzerland; E-mail: Giorgio.Manferdelli@unil.ch

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Response to Porcelli, Pilotto, and Rossiter

Prof. Porcelli and colleagues' elegant perspective contrasts our initial proposal on the feasibility of using NIRS to non-invasively compute convective and diffusive components involved in the O_2 transport. Their position was primarily based on two points: (i) NIRS overestimates convective, but not diffusive, O_2 transport; and (ii) well-known limitations of the NIRS technique hamper its application for a quantitative non-invasive solution to the Wagner model.

First, we would like to congratulate our opponents for comparing previous invasive results in the literature to preliminary unpublished data collected by their group using NIRS in patients with mitochondrial myopathies. In their calculations, Porcelli and colleagues estimated convective and diffusive O_2 transport mechanisms by substituting Δ[deoxy(Hb+Mb)]) for (CaO_2 – CvO_2) in equation 1 and StO_2 for ($PmvO_2$ – $PimO_2$) in equation 2, respectively. While the diffusion values determined invasively or by NIRS StO_2 were similar, convection was ~25% greater when derived by NIRS Δ[deoxy(Hb+Mb)] than from femoral artery catheterization. Though we understand the use of Δ[deoxy(Hb+Mb)] as surrogate of muscle O_2 extraction (1), we support StO_2 as a proxy of venous SO_2 to estimate CvO_2. At this regard, a good linear correlation was described between StO_2 and CvO_2 under different muscle blood flows and inspired O_2 fractions (2, 3). Porcelli and colleagues agreed that StO_2 may represent a reasonable surrogate of venous SO_2 but not of $PmvO_2$. Their view is based on the importance of exercise-induced changes in microvascular temperature, PCO_2, and, in particular, pH which elicit a right-shift of the Hb-O_2 dissociation curve (Bohr effect) (4). On average, femoral venous PO_2, reaches a value between 15 and 25 mmHg at peak leg exercise (5-7), with a variability likely due to

differences in study methodology and training status of the investigated groups. In the study of Stringer and colleagues (4), the decreases in femoral vein PO_2 (down to ~20 mmHg) remained similar across increasing exercise intensities, despite a continuous fall in femoral venous SO_2. Several previous investigators considered femoral vein PO_2 measured during incremental cycling exercise as a surrogate of mean capillary PO_2 (7, 8). Roca and colleagues also demonstrated a strong linear relationship between femoral vein PO_2 and mean capillary PO_2 at maximal cycling exercise (9). Of note, in the paper of Roca and colleagues, mean capillary PO_2 was not measured directly but estimated from venous PO_2 in the femoral compartment (9). Therefore, microvascular PO_2 can be estimated from microvascular SO_2, thus StO_2; since - as agreed by our opponents - NIRS StO_2 is a surrogate of microvascular SO_2. To conclude, as recently performed (10, 11), we encourage our opponents to recalculate convective O_2 delivery by interpolating StO_2, rather than Δ[deoxy(Hb+Mb)], as a surrogate of CvO_2.

A strong point of agreement with our opponents relies on the well-recognized limitations of the NIRS technique, including the influence of adipose tissue thickness (ATT) on signal response and the small muscle fraction investigated by the NIRS probe. ATT is known to affect NIRS signal strength by reducing the relative contribution of the underlying skeletal muscle tissue to the NIRS signals (12). Despite this, recent satisfying results were collected in obese (BMI = 33.9 ± 1.1 $kg \cdot m^{-2}$) individuals (13). Therefore, although we cannot exclude a possible impact of ATT on NIRS-derived parameters, we are confident that the light penetration is deep enough to investigate the underlying capillary bed with no major influences from ATT. However, further work is recommended to clarify the influence of ATT in obese individuals.

We also obviously agree that NIRS does not account for spatial heterogeneity of large muscle microvascular bed response during exercise (14), as the investigated muscle portion by the NIRS probe is relatively small (~3-10 mg of tissue). On the other hand, it is also true that the direct Fick method integrates the entire limb response, possible sampling venous effluent from inactive muscle(s). The resulting estimate of $PmvO_2$ using Bohr integration, therefore, may not reflect any specific muscle or site. For example, a single global estimate of $PmvO_2$ would not have revealed the varied responses of the vastus lateralis, medialis and rectus femoris during incremental exercise (15), leading to possibly misleading conclusions regarding perfusive and diffusive O_2 delivery. In contrast, the ability to place a NIRS probe over specific muscles can provide unique insight not available with a global measure based on blood gases (15). Further, many of the criticisms raised by our opponents are resolved, including potential influence of adipose tissue thickness and multiple muscle heterogeneity, when NIRS is used to examine primarily single muscle activation during forearm handgrip exercise (16). That being said, we recommend that potential muscle heterogeneity be considered as a limitation when a single probe is used when investigating large muscle groups.

Concluding Statement

Are there any benefits to estimate $\dot{Q}O2$ and DO2 factors at peak exercise without invasive techniques? This contrasting perspective discussed a non-invasive approach utilizing NIRS to the integration of $\dot{Q}O2$ and DO2 mechanisms determining $\dot{V}O2max$ in order to understand the site of functional limitation to exercise capacity. We have pointed out that there are certain advantages to this non-invasive approach, including application to a variety of different populations such as elite athletes and patients, and the ability to make repeated

measurements not readily available to invasive studies. We also noted a potential limitation to the invasive measure of blood sampling of venous blood draining multiple muscles with varying contribution to the exercise task, where the resulting use of a singular common estimate of $PmvO_2$ may not reflect the microvascular condition in any individual muscle. Given the existing evidence supporting our view, NIRS StO_2 may represent a valid substitute to invasively derived blood gases to semi-quantitatively estimate convective and diffusive factors of O_2 transport from ambient air to muscle mitochondria at $\dot{V}O_{2max}$ as illustrated using the Wagner diagram.

Conflict of Interest

The authors have no conflicts of interest to disclose. The views of the perspective are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. This perspective does not constitute endorsement by the American College of Sports Medicine

REFERENCES

1. Grassi B, Quaresima V. Near-infrared spectroscopy and skeletal muscle oxidative function in vivo in health and disease: a review from an exercise physiology perspective. *J Biomed Opt*. 2016;21(9):091313.
2. Sun YI, Ferguson BS, Rogatzki MJ, McDonald JR, Gladden LB. Muscle near-infrared spectroscopy signals versus venous blood hemoglobin oxygen saturation in skeletal muscle. *Med Sci Sports Exerc*. 2016;48(10):2013-20.
3. Wilson JR, Mancini DM, McCully K, Ferraro N, Lanoce V, Chance B. Noninvasive detection of skeletal muscle underperfusion with near-infrared spectroscopy in patients with heart failure. *Circulation*. 1989;80(6):1668-74.
4. Stringer W, Wasserman K, Casaburi R, Porszasz J, Maehara K, French W. Lactic acidosis as a facilitator of oxyhemoglobin dissociation during exercise. *J Appl Physiol (1985)*. 1994;76(4):1462-7.
5. Carlson LA, Pernow B. Studies on the peripheral circulation and metabolism in man. 1. Oxygen utilization and lactate-pyruvate formation in the legs at rest and during exercise in healthy subjects. *Acta Physiol Scand*. 1961;52:328-42.
6. Donald KW, Wormald PN, Taylor SH, Bishop JM. Changes in the oxygen content of femoral venous blood and leg blood flow during leg exercise in relation to cardiac output response. *Clin Sci*. 1957;16(3):567-91.
7. Pirnay F, Lamy M, Dujardin J, Doroanne R, Petit JM. Analysis of femoral venous blood during maximum muscular exercise. *J Appl Physiol*. 1972;33(3):289-92.

8. Saltin B, Blomqvist G, Mitchell JH, Johnson RL, Jr., Wildenthal K, Chapman CB. Response to exercise after bed rest and after training. *Circulation*. 1968;38(5 Suppl):VII1-78.

9. Roca J, Hogan MC, Story D, et al. Evidence for tissue diffusion limitation of VO2max in normal humans. *J Appl Physiol (1985)*. 1989;67(1):291-9.

10. Goulding RP, Okushima D, Fukuoka Y, et al. Impact of supine versus upright exercise on muscle deoxygenation heterogeneity during ramp incremental cycling is site specific. *Eur J Appl Physiol*. 2021;121(5):1283-96.

11. Manferdelli G, Narang BJ, Bourdillon N, Debevec T, Millet GP. Physiological responses to exercise in hypoxia in preterm adults: convective and diffusive limitations in the O2 transport. *Med Sci Sports Exerc*. 2023;55(3):482-96.

12. Rosenberry R, Nelson MD. Reactive hyperemia: a review of methods, mechanisms, and considerations. *Am J Physiol Regul Integr Comp Physiol*. 2020;318(3):R605-R18.

13. Soares RN, Murias JM. Near-infrared spectroscopy assessment of microvasculature detects difference in lower limb vascular responsiveness in obese compared to lean individuals. *Microvasc Res*. 2018;118:31-5.

14. Barstow TJ. Understanding near infrared spectroscopy and its application to skeletal muscle research. *J Appl Physiol (1985)*. 2019;126(5):1360-76.

15. Chin LM, Kowalchuk JM, Barstow TJ, et al. The relationship between muscle deoxygenation and activation in different muscles of the quadriceps during cycle ramp exercise. *J Appl Physiol (1985)*. 2011;111(5):1259-65.

16. Broxterman RM, Ade CJ, Wilcox SL, Schlup SJ, Craig JC, Barstow TJ. Influence of duty cycle on the power-duration relationship: observations and potential mechanisms. *Respir Physiol Neurobiol*. 2014;192:102-11.

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NIRS-Based Muscle Oxygenation Is Not Suitable to Compute Convective and Diffusive Components of O_2 Transport at $\dot{V}O_{2max}$: Response to Manferdelli, Barstow, and Millet

Simone Porcelli[1,2], A.M. Pilotto[1,3], and Harry B. Rossiter[4]

[1]Department of Molecular Medicine, University of Pavia, Pavia, ITALY; [2]Institute of Biomedical Technologies, National Research Council, Milan, ITALY; [3]Department of Medicine, University of Udine, Udine, ITALY; [4]Division of Respiratory and Critical Care Physiology and Medicine, The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA

Address for Correspondence:

Simone Porcelli, MD, PhD, Assistant Professor in Human Physiology Institute of Physiology, Department of Molecular Medicine University of Pavia, Italy, Via Forlanini 6, 27100 Pavia – Italy; Phone: +39 0382987538; E-mail: simone.porcelli@unipv.it

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Regeneron. He is a visiting Professor at the University of Leeds, UK. The views of the perspective are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. This perspective does not constitute endorsement by the American College of Sports Medicine.

RESPONSE TO MANFERDELLI, BARSTOW, AND MILLET

Between these contrasting perspectives, there appears to be much on which the two positions agree. For example, we agree with Dr. Manferdelli et al. (1) that the Wagner model is generally accepted to quantify perfusive and diffusive O_2 flow at $\dot{V}O_{2max}$, but that the widespread or longitudinal application of this technique is limited by its invasive nature. We agree that NIRS is a highly accessible technique that provides data reflecting the balance between O_2 delivery and utilization at the interface of the muscle capillary and myocyte. We also agree that new data using NIRS, under specific protocols administered at rest, may provide insight on *relative* limitation to diffusive O_2 flow in skeletal muscle (2). Therefore, the ability of NIRS to provide information on the physiology of the capillary-myocyte interface in common units (e.g. tissue hemoglobin+myoglobin saturation; StO_2) that would facilitate mathematical substitution in both Fick equations (see equations 1 and 2 in [1]) is highly attractive. However, we do not agree with the proposal that current NIRS applications are able to *quantify*, i.e. mathematically solve, the convective and diffusive components of O_2 transport at $\dot{V}O_{2max}$.

We challenge two key aspects of the position taken by Manferdelli et al., which are: 1) the proposal to use NIRS-derived StO_2 as a surrogate for muscle venous O_2 concentration (C_VO_2) in the Fick Principle of Mass Conservation (equation 1 in [1]); and 2) the proposal to use StO_2 to estimate mean muscle capillary PO_2 ($\overline{Pcap}O_2$) in Fick's Law of Diffusion (equation 2 in [1]). The substitutions in points 1 and 2 would be used to non-invasively solve for the relative contributions of convective and diffusive O_2 transport at $\dot{V}O_2$max during exercise in humans (3). However, we believe that assumption weighs so heavy in these three proposals as to render them

functionally unable to discriminate differences in perfusive and diffusive O_2 transport among individuals or environmental conditions.

To the first point, Manferdelli et al. suggest that NIRS-derived muscle StO_2 or [HHbMb] accurately reflects muscle venous O_2 saturation (SvO_2) or concentration (CvO_2) during contractions with r^2 values ranging from 0.69 to 0.93. It should be clarified that the r^2 between [HHbMb] and SvO_2 of 0.93 comes from exposed dog muscle (without covering skin and adipose) during pump perfusion to hold blood flow constant and maximal (4). During spontaneous perfusion in the same experiments, the best scenario leaves 31% of the variance between [HHbMb] and SvO_2 unexplained. Human data from a homogenous healthy participant group (n=6) are highly variable, providing wide individual-subject regressions (r^2 = 0.29 – 0.82) between NIRS-based StO_2 and SvO_2 (5) and the individual slopes of these regressions ranging from 1.02 – 2.21; thus, the ability for NIRS to be used to provide an absolute value for SvO_2 has at least a 54% difference among individuals.

Further, even without considering the unknown contributions of myoglobin (Mb) to the NIRS signal and the higher affinity of Mb for O_2 compared with that of Hb (6), to estimate C_VO_2 from S_VO_2 requires knowledge of muscle [Hb]. To achieve this Dr. Manferdelli et al. propose to use small capillary blood sample to determine [Hb]. However, capillary and venous [Hb] also have ~30% variation in their association at rest (r^2 = 0.69 – 0.74), have systematic bias i.e., capillary [Hb] is consistently greater than [Hb] from a peripheral vein, and capillary [Hb] is more variable than venous [Hb] on repeat testing (7). Add to this that the capillary [Hb] and tissue [Hb] in the region of muscle sampled by NIRS are unknown. Using time resolved NIRS (TRS-

NIRS), the association between tissue [Hb] (uM) and peripheral blood [Hb] (g/dL) is poor e.g., r^2=0.16 (8). Also using TRS-NIRS on muscle, it is known that tissue [HbMb] typically increases with exercise intensity from ~190 uM at rest to ~230 uM at $\dot{V}O_2$max (~20% increase); the magnitude of this increase appears to vary among individuals, muscles and with state of training (9). Continuous wave NIRS (CW-NIRS) devices, which measure a relative change in tissue [HbMb] from an unknown baseline, would be unable to determine the absolute magnitude of any exercise-induced changes. Therefore, the potential errors in the several assumptions that are needed to convert StO_2 to CvO_2 for use in the Fick Principle of Mass Conservation are so wide that establishing a reliable quantitative solution for convective O_2 transport using NIRS is not currently feasible.

To the second point, Manferdelli et al. suggest that StO_2 may be used to estimate $P\overline{cap}O_2$ (or at least mean muscle venous PO_2, $P\bar{v}O_2$, which can be related to $P\overline{cap}O_2$ by a constant; [3]), and solve Fick's Law of Diffusion for D_mO_2. They propose to use equations of Severinghaus (10) to estimate $P\overline{cap}O_2$ from NIRS derived StO_2. These equations were developed to investigate "determinants of transcutaneous PO_2 under heated skin surface electrodes" at rest, and provide relatively accurate estimates of PO_2 (i.e., within ±1 mmHg) at 37°C and pH=7.4 for SO_2 values ≤96%. However, the shape of the Hb-O_2 dissociation curve in muscle is modified by exercise due to changes in temperature, pH and PCO_2. Errors in estimating PO_2 from SO_2 increase as PO_2 falls from 1.3%/°C at high PO_2 to 7.4%/°C at low PO_2. Given that muscle temperature may reach ~41°C at $\dot{V}O_2$max (11), errors of up to ~30% are possible if muscle temperature is unknown. Errors in PO_2 estimation from S_VO_2 also increase as pH falls and PCO_2 rises. Severinghaus (10) proposed to correct for the PCO_2 component of this error using the base excess measured from a

blood sample. Therefore, appropriate correction for these confounders at $\dot{V}O_2max$ would require knowledge of base excess and pH in the region muscle under the NIRS probe; each of which could vary widely among subjects and environmental conditions. Thus, the potential errors in the several assumptions that are needed to estimate $P\overline{cap}O_2$ using StO_2 also significantly impact the reliability of the proposed approach.

Finally, it should be also highlighted that Manferdelli et al. state that StO_2 does not require 'physiological calibration' to normalize the signal for maximum deoxygenation among individuals because the measurement itself (whether using CW-NIRS or other NIRS methods) is normalized. However, skin and adipose tissue thickness (ATT) have a significant effect on NIRS data and the reduction in StO_2 at peak exercise is less as ATT increases, because exercise reduces StO_2 predominantly in the muscle compartment (and not the skin and adipose). Advanced NIRS devices show that StO_2 at rest and peak exercise is largely related to ATT (12, 13). Differences in ATT may underlie some of the 2-fold differences among individuals in the slope of the association between StO_2 and SvO_2 (discussed above; [5]).

Concluding Statement

For these reasons, we argue that the methods proposed by Manferdelli et al. do not have sufficient accuracy or reproducibility to compute the convective and diffusive components of O_2 transport at $\dot{V}O_{2max}$.

Conflict of Interest

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REFERENCES

1. Manferdelli G, Barstow TJ, Millet GP. NIRS muscle oxygenation is suitable for computation of the convective and diffusive components of O2 transport at VO2max. *Med Sci Sports Exerc.* 2023
2. Pilotto AM, Adami A, Mazzolari R, et al. Near-infrared spectroscopy estimation of combined skeletal muscle oxidative capacity and O2 diffusion capacity in humans. *J Physiol*. 2022;600(18):4153-68.
3. Wagner PD. Gas exchange and peripheral diffusion limitation. *Med Sci Sports Exerc.* 1992;24(1):54–8.
4. Wust RC, McDonald JR, Sun Y, et al. Slowed muscle oxygen uptake kinetics with raised metabolism are not dependent on blood flow or recruitment dynamics. *J Physiol*. 2014;592(8):1857-71.
5. Vogiatzis I, Habazettl H, Louvaris Z, et al. A method for assessing heterogeneity of blood flow and metabolism in exercising normal human muscle by near-infrared spectroscopy. *J Appl Physiol (1985).* 2015;118(6):783-93.
6. Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, Wagner PD. Myoglobin O2 desaturation during exercise. Evidence of limited O2 transport. *J Clin Invest*. 1995;96(4):1916-26.
7. Royal JT, Fisher JT, Mlinar T, Mekjavic IB, McDonnell AC. Validity and reliability of capillary vs. Venous blood for the assessment of haemoglobin mass and intravascular volumes. *Front Physiol*. 2022;13:1021588.
8. Lanks CW, Kim CB, Fu J, et al. A pilot study of cortical oxygenation in septic shock by time-resolved near-infrared spectroscopy. *Am J Respir Crit Care Med.* 2017;195:A1803

9. Okushima D, Poole DC, Barstow TJ, et al. Greater V˙O2peak is correlated with greater skeletal muscle deoxygenation amplitude and hemoglobin concentration within individual muscles during ramp-incremental cycle exercise. *Physiol Rep*. 2016;4(23):e13065.
10. Severinghaus JW. Simple, accurate equations for human blood O2 dissociation computations. *J Appl Physiol Respir Environ Exerc Physiol*. 1979;46(3):599-602.
11. González-Alonso J, Teller C, Andersen SL, Jensen FB, Hyldig T, Nielsen B. Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J Appl Physiol (1985)*. 1999;86(3):1032-9.
12. Pirovano I, Porcelli S, Re R, et al. Effect of adipose tissue thickness and tissue optical properties on the differential pathlength factor estimation for NIRS studies on human skeletal muscle. *Biomed Opt Express.* 2020;12(1):571-87.
13. Chiu AS, Rossiter HB, Radom-Aizik S, et al. Muscle deoxygenation during incremental exercise is delayed in children compared to young adults. *Med Sci Sports Exerc.* 2017;49(5S):640-1.