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VIEWPOINT

Commentaries on Viewpoint: Principles, insights, and potential pitfalls of the noninvasive determination of muscle oxidative capacity by near-infrared spectroscopy

COMMENTARY ON VIEWPOINT

TO THE EDITOR: In their Viewpoint, Adami and Rossiter (1) eloquently explore the strengths and weaknesses of combining near-infrared spectroscopy (NIRS) with repetitive postexercise arterial cuff occlusions to measure skeletal muscle oxidative capacity in vivo. Before the advent of this technique, the assessment of mitochondrial function was limited to invasive muscle biopsies and/or expensive and time-consuming magnetic resonance spectroscopy (MRS) techniques. Now, clinicians and researchers alike have a robust, high-throughput clinical platform to noninvasively assess muscle oxidative capacity across a wide range of muscles and disease states. That this approach is easily transportable and relatively low cost opens new possibilities for bedside medicine, clinical decision making, and incorporation into large multicenter clinical trials. However, this increased emphasis on clinical populations also emphasizes the need for future technology development to overcome the well-established limitations of NIRS with regards to limb adiposity (2). While Adami and Rossiter appropriately highlight the relatively large test-retest variability with this technique compared with the differences often observed between health and disease, it is important to emphasize that this variability is similar to that achieved with MRS (5) or muscle biopsies (4). This does highlight the importance of careful experimental/clinical design, however. Indeed, over 40% of the investigations referenced in this Viewpoint studied locomotor muscle groups, which are highly dependent on physical activity level (3). Targeting nonlocomotor muscle groups should help limit within-group variability. With these considerations in mind, we believe NIRS-derived muscle oxidative capacity assessments hold great promise in clinical translational medicine.

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APPLICATION OF IN VITRO OXIDATIVE PHOSPHORYLATION ASSAY TO HUMAN LIMBS USING NEAR-INFRARED SPECTROSCOPY

TO THE EDITOR: Adami and Rossiter (1) provide a contemporary viewpoint on the use of near-infrared spectroscopy (NIRS) to determine skeletal muscle oxidative capacity. There are numerous approaches and applications of NIRS to study skeletal muscle (4). The approach to evaluate muscle oxidative capacity evolved from observations of changes in muscle oxygenation in response to ischemia. Because continuous wavelength NIRS does not provide the absolute value of muscle oxygenation due to undetermined optical path lengths, a physiological calibration was adopted using ischemia to define a scale oxygen levels from near zero to near 100% (3). It was observed that the initial rate of deoxygenation after muscle contractions was greater than that of resting conditions, which reminded me (Hamaoka) of the in vitro assays of mitochondrial oxidative phosphorylation reported by Chance and Williams (2). They showed oxygen tension declined more rapidly under the conditions of higher (state 3) mitochondrial respiration (2). It was felt that the results in vitro should apply in vivo. This hypothesis was validated using brief arterial occlusions and comparing rates of muscle deoxygenation with NIRS to rates of change of ADP and phosphocreatine (PCr) using ^{31}phosphorus magnetic resonance spectroscopy (3). Later, consecutive brief arterial occlusions were applied during the recovery phase after muscle contractions. The rate of recovery for muscle oxygen consumption was validated with comparisons to the rate of recovery for PCr (5). It is an honor to witness the growth of the original concept and methodology for measuring muscle oxidative rate or capacity, and its application to clinical settings.

The author acknowledges Dr. Kevin McCully for the stimulating discussions that contributed to the ideas presented in this commentary.

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VALIDATION OF NEAR-INFRARED SPECTROSCOPY METHODOLOGY TO ASSESS SKELETAL MUSCLE OXIDATIVE CAPACITY TO THAT OF HIGH-RESOLUTION RESPIROMETRIC METHODOLOGY SHOULD EASE SOME CONCERNS

TO THE EDITOR: Adami and Rossiter (1) highlight important considerations when assessing skeletal muscle oxidative capacity ($OXPHOS_{SM}$) with near-infrared spectroscopy (NIRS). Their two primary concerns *1*) achieving maximal activation of oxidative enzymes and *2*) potential limitations of oxygen delivery to muscle, were addressed when NIRS-derived $OXPHOS_{SM}$ was validated to in situ measures of $OXPHOS_{SM}$ via high-resolution respirometry (HRR) (5). The HRR protocol utilized saturating substrate concentrations and reported strong correlations (Pearson's $r = 0.61–0.74$, $P < 0.01$) between HRR- and NIRS-derived $OXPHOS_{SM}$ (5). Cellular respiration coordinates and integrates the interaction of many enzymes to function collectively within a system, often below their individual maximal enzymatic velocities; i.e., HRR derived cytochrome c oxidase activity, alone, exceeds complementary measures of $OXPHOS_{SM}$ (4). Thus valid measures of $OXPHOS_{SM}$ require maximal activation of respiratory pathways opposed to individual enzymes. Additionally, HRR uses saturating concentrations of oxygen such that observed variations across measurements reflect differences in mitochondrial function per se versus inconsistent fiber permeabilization and attendant diffusive disparities. Correlation between NIRS- and HRR-derived $OXPHOS_{SM}$ suggests that oxygen availability is not problematic when assessing $OXPHOS_{SM}$ via NIRS in healthy subjects. Moreover, oxygen delivery is a component of in vivo respiratory capacity. If oxygen availability becomes limiting, as observed in certain diseased states such as COPD, then delivery dictates $OXPHOS_{SM}$ and respiratory capacity adapts accordingly (3). We appreciate the primary concerns raised by Adami and Rossiter; however, we contend that controlling the influence of skin blood flow and subcutaneous adipose thickness are more significant considerations when assessing $OXPHOS_{SM}$ via NIRS (2).

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COULD NIRS BE AS UBIQUITOUS AS THE METABOLIC CART IN EXERCISE PHYSIOLOGY LABORATORIES?

TO THE EDITOR: Until about the 1970s, the routine measurement of oxygen uptake ($\dot{V}O_2$) was limited to a small field of niche experts who specialized in work physiology. This was likely attributable to the complicated nature of the methodology; although elegant in its underlying principles, the Douglas bag technique (2) is cumbersome in its execution and highly subject to user expertise. Gradually, through technological advances and methodological simplifications (5), it has become routine for exercise physiology laboratories to have the ability to measure $\dot{V}O_{2max}$. Although speculative, it seems possible that an analogous evolution is underway for the assessment of muscle oxidative capacity. Due to the limitations of traditional methods (biopsy and ^{31}P MRS) (1), mitochondrial capacity assessment has been traditionally confined to laboratories that specialize almost exclusively in muscle mitochondrial biology. But recent advances (3) in near-infrared spectroscopy (NIRS)-based methods could enable more investigators to incorporate assessments of mitochondrial capacity into their studies in a complementary manner to their primary focus. For example, our recent study (4) included a NIRS-based assessment of cycling training-induced changes in quadriceps oxidative capacity. Our NIRS measurement served as a validation of our training stimulus; i.e., was the training sufficient to increase muscle oxidative capacity, when other novel (vascular) markers may or may not have changed in response to training. Although further advances in the NIRS method are still needed, it is exciting to consider the novelty and innovation that could result from the collective field's expanded ability to include assessments of mitochondrial capacity into integrative research projects. The author acknowledges Dr. Kevin McCully for the stimulating discussions that contributed to the ideas presented in this commentary.

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COMMENTARY ON VIEWPOINT

TO THE EDITOR: Muscle oxidative capacity can be defined as the tissue's maximum rate for oxidative phosphorylation, a process that takes place in the mitochondria and is supported by the delivery of oxygen and substrate. In their Viewpoint, Adami and Rossiter (1) discuss the potential for near-infrared spectroscopy (NIRS) to measure muscle oxidative capacity in vivo. Support for this viewpoint includes the associations between NIRS variables and both respirometry and 31-phosphorus magnetic resonance spectroscopy (MRS; 4, 5) measures, the current gold standards for in vitro and in vivo assessment of oxidative capacity, respectively (2). Indeed, with the recent development of frequency-domain NIRS systems, many of the technical problems related to this methodology appear to have been addressed. It is worth bearing in mind, however, that each of these methods differs in the physiological compartment being sampled. In the case of respirometry, oxidative capacity can be determined directly in isolated mitochondria, single muscle fibers, and fiber bundles (2, 3), but in the absence of full physiological conditions. In contrast, the in vivo measures obtained by MRS and NIRS reflect changes in the cytosol and vasculature, respectively. Furthermore, MRS is used to quantify oxidative ATP production in the volume of interest, whereas NIRS evaluates changes in oxygen saturation of hemoglobin (and to some extent, myoglobin) and therefore reflects oxygen consumption rather than oxidative ATP production. Thus, selection of the appropriate tool for evaluating muscle oxidative capacity will depend on instrumentation quality, the research question to be addressed, and an understanding of all methodological assumptions and constraints.

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USE OF AUTOMATION SOFTWARE FOR INCREASED STANDARDIZATION, ACCURACY, AND PRECISION

TO THE EDITOR: Near infrared spectroscopy (NIRS) permits the assessment of skeletal muscle mitochondrial oxidative capacity in situ. In situ, mitochondrial respiration is dependent on several physiological systems operating within a closed environment, i.e., microvascular perfusion of blood, tissue oxygen extraction, and terminal respiratory chain oxidative coupling. Therefore, NIRS, which has been validated against phosphorus-magnetic resonance spectroscopy based assessments (2), measures a composite of mitochondrial respiratory capacity. However, NIRS procedures require substantial operator expertise, and further technological innovation is necessary to improve measurement precision (reliability) and accuracy (validity). First, with respect to the former, we have found that rapid ($<$1.0 s) occlusion improves reliability (3). Second, our custom automated analysis software enables immediate outcome feedback and retesting as required. Lastly, we plan to develop automation software to control arterial occlusions based on real-time NIRS tissue oxygen saturation (StO_2) feedback. Accurate assessment of mitochondrial oxidative capacity is dependent on maximal activation of mitochondrial oxidative enzymes while maintaining adequate StO_2 so as to not alter phosphocreatine recovery kinetics (1). This can be achieved by using a 5-min arterial occlusion to determine the minimum and subsequent maximum StO_2. Then, during the oxidative capacity test, the automation software will prevent full StO_2 depletion during the occlusion, then ensure adequate recovery following occlusion based on real-time StO_2 feedback. In summary, automation software, for arterial occlusion control and outcome analysis, will limit operator bias and assist with measurement standardization, thereby enhancing measurement accuracy and precision.

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MITOCHONDRIAL CAPACITY: WHAT DOES IT MEAN?

TO THE EDITOR: Adami and Rossiter (1) present an emerging method for evaluating skeletal muscle mitochondrial capacity. A key to these measurements is the understanding of what mitochondrial capacity means and how it can be used to evaluate skeletal muscle. The near-infrared derived recovery rate constant reflects the metabolic rate with maximal substrate availability analogous to Michaelis-Menten enzyme kinetics. This can be inferred to be the maximal metabolic rate of the muscle (5). Because the NIRS measurements are performed on intact skeletal muscle in the body, the rate constant reflects structurally intact skeletal muscle. This is important because of the increasing realization that structure of mitochondrial in intact skeletal muscle is both dynamic and important in determining its function (2). The key question then is how to interpret the measurements of NIRS-derived mitochondrial capacity. A simplified approach is to consider mitochondrial capacity to be a combination of mitochondrial function and mitochondrial volume. Recent studies employing innovative approaches to gain mechanistic insight have shown that exercise training can increase mitochondrial volume independent of biogenesis (3) and describe the pathology associated with aging leading to decreases in mitochondrial capacity (4). Considering this, the advantage of the NIRS measured mitochondrial capacity is that it reflects the oxidative metabolic capacity of the intact skeletal muscle under the operating conditions in the intact organism. The limitation is that NIRS measured mitochondrial capacity does not provide mechanistic interpretations for the changes that underlie changes in mitochondrial capacity.

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COMMENTARY ON VIEWPOINT

TO THE EDITOR: Near-infrared spectroscopy (NIRS) represents an inexpensive, noninvasive, and reproducible technique offering great insight of in vivo mitochondrial metabolism. We previously used NIRS to show disparity in skeletal muscle oxidative capacity (SMOC) in patients with cystic fibrosis (2) compared with controls; however, this technique has some potential factors that should be controlled for accuracy.

As addressed in this Viewpoint (1), the evaluation of adipose tissue thickness (ATT) represents a key factor to consider using NIRS due to limitations by most commercial instruments reaching certain depths. Ultrasound imaging is an interesting methodology for the assessment of ATT with higher accuracy than traditional devices like skin fold calipers. Nonetheless, certain technical aspects like body site, compression through the transducer, or scanning orientation should also be contemplated for its correct application (5).

Another factor to consider is the heterogeneity of muscle. Muscle blood flow has an uneven spatial distribution within the muscles that has the potential to disturb O_2 transport and, hence, unbalance O_2 muscle delivery and O_2 mitochondrial utilization (3). Moreover, fiber type distribution is nonhomogenous within the muscle, with a more predominant content of type II fibers at the surface of the muscle and type I in deeper regions that may also impact the interpretation of results (4).

Overall and despite some technical considerations, the assessment of SMOC by NIRS can provide very valuable information about in vivo muscle metabolism through a noninvasive, accessible technique.

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ESTIMATION OF MUSCLE OXIDATIVE CAPACITY BY NEAR-INFRARED SPECTROSCOPY: DOES EXERCISE INTENSITY MATTER?

TO THE EDITOR: The Viewpoint by Adami and Rossiter (1) elegantly discussed advantages and potential drawbacks of the innovative noninvasive approach to estimate muscle oxidative capacity using near-infrared spectroscopy (NIRS). As stated by the authors (1), one assumption of this approach is that brief muscle contractions activate mitochondrial oxidative enzymes at maximal level, according to the first-order relationship between PCr dynamics and ATP production by oxidative phosphorylation. To satisfy this assumption, the level of muscle activation appears critical. Indeed, experiments on isolated single muscle fibers have shown that maximal activation of

mitochondrial enzymes may not be achieved when contraction frequency is too low (5). On the other hand, higher-order PCr recovery kinetics, indicating a nonoxidative contribution to ATP production, have been reported when a very high-intensity exercise is performed in vivo (2). Moreover, some studies have hypothesized a more complex control of mitochondrial respiration rate, including calcium accumulation and reactive oxygen species as potential regulator of mitochondrial enzymes (2, 4). Finally, a strong activation of muscle fibers may limit oxygen availability and induce a slower PCr recovery rate (3). Thus the intensity of exercise should be considered with caution for the assessment of muscle oxidative capacity by NIRS. Force output signals or muscle tissue oxygenation could serve as a benchmark for achieving a maximal activation of mitochondrial oxidative enzymes without impairing oxygen delivery (convective and diffusive) or inducing an excessive intramuscular metabolic perturbation.

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COMMENTARY ON VIEWPOINT

TO THE EDITOR: In their interesting Viewpoint (1), the authors wanted to emphasize the use of noninvasive and repeatable NIRS measurements of localized muscle oxygenation state to get information indirectly about skeletal muscle oxidative capacity in healthy subjects and patients. However, as underlined in their conclusion, the validity of the local muscle O_2 consumption ($m\dot{V}O_2$) recovery rate constant (see Fig. 1*C*) is strictly associated with the appropriate use of NIRS approach. Once verified that skin/fat thickness is reasonable for performing muscle NIRS measurements (4) and optical probes are correctly re-placed on a given portion muscle group (for repeated measurements), $m\dot{V}O_2$ at rest and during exercise can be reliably assessed using NIRS combined with the arterial occlusion method [first described in the early 90s (2, 3), then widely applied in McCully and Hamaoka laboratories (1)]. So far, $m\dot{V}O_2$ has been mostly calculated as the slope of changes in O_2Hb, or (O_2Hb − HHb), and expressed in "% calibration" using a 3–5-min ischemia. The reported $m\dot{V}O_2$ values (see Fig. 1*B*) were calculated as the slope of TSI changes. This latter parameter (expressed in % and named also as rSO_2/SO_2m/StO_2/TOI by different companies of tissue oximeters) reflects the dynamic balance between O_2 supply and consumption and it is unaltered by blood volume (O_2Hb + HHb) changes that could occur even during arterial occlusions. Therefore, I would recommend to use TSI as the only NIRS parameter for calculating more accurately $m\dot{V}O_2$ and avoiding either the correction for blood volume changes (5) or the discomfort of a 5-min ischemia used as calibration procedure.

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COMMENTARY ON VIEWPOINT

TO THE EDITOR: I read with great interest this Viewpoint by Adami and Rossiter (1) that discusses a method for evaluating skeletal muscle oxidative capacity using near-infrared spectroscopy. The authors very nicely describe the kinetic model used, key assumptions, and potential limitations of this methodology. Although the authors indicate that insufficient amounts exercise (that do not increase $m\dot{V}O_2$ much) impairs measurement accuracy and reliability, a key advantage of this method is that exercise intensity or type do not need to be strictly controlled so long as $m\dot{V}O_2$ increases sufficiently (4). This is a critical advantage for patients who have reduced muscle mass and/or activation and negates the need for sophisticated ergometers. Given the reproducibility and low cost of NIRS devices, NIRS can facilitate the study of skeletal muscle metabolic function in both large cohort studies and longitudinal studies including clinical drug trials. Furthermore, the results from NIRS can be obtained immediately after data collection with automated analysis programs. This eliminates user bias in analysis and means that results could be obtained in "real time". To increase the utility of this method in clinical research settings, we must consider the interpretation of the NIRS rate constant (k). When interpreting this NIRS method, it is important to note that changes/differences in k are predicted to be cause by several physiological adaptations including altered mitochondrial content or total creatine levels as well as altered flux of the oxidative phosphorylation system, including the creatine/adenine shuttle systems (2, 3, 5).

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INFERENCE OF SKELETAL MUSCLE OXIDATIVE CAPACITY IN ULTRA-ENDURANCE PERFORMANCE: AN OPEN RESEARCH QUESTION

TO THE EDITOR: Oxidative metabolism is the primary pathway of energy production in skeletal muscle. The analysis of its changes can give insights into understanding the muscle function in healthy and clinical conditions (2). Adami and Rossiter (1) highlighted a core set of attributes that warrant consideration when inferring skeletal muscle oxidative capacity by means of near-infrared spectroscopy (NIRS). The authors point out that quality control procedures would help in the identification of muscle oxidative capacity in both health and clinical settings. However, NIRS can also be considered a powerful tool in sport sciences since it can objectively evaluate muscle oxidative metabolism and its modifications during exercise (3), including extreme exercises such as the increasingly popular ultra-endurance events.

We recently employed NIRS to assess changes in skeletal muscle oxygenation during 4-h cycling (4) and after 330-km running (5). However, the obtained values only reflected the O_2 delivery/consumption ratio in the working muscles since the postexercise reoxygenation recovery rate was not measured. Therefore, muscle O_2 consumption ($m\dot{V}O_2$) could not be inferred. Consequently, understanding the muscle oxidative capacity based on $m\dot{V}O_2$ recovery rate constant as proposed (1) is still an open research question in ultra-endurance. We strongly suggest this approach to quantitatively measure $m\dot{V}O_2$ so as to improve our understanding of the role of muscle oxidative capacity in ultra-endurance performance. The possibility to noninvasively study local muscle oxidative metabolism might also provide important information regarding the localized muscle adaptive responses during and following these extreme exercises.

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POTENTIAL EFFECTS OF MITOCHONDRIAL IMPAIRMENT IN EVALUATING MUSCLE OXIDATIVE CAPACITY USING NIRS

TO THE EDITOR: The main advantages of near-infrared spectroscopy (NIRS) over standard muscle oxidative capacity assessments such as biopsy and ^{31}P MRS are its noninvasiveness, cost-effectiveness, and ease of operation (1). Despite the promising application of NIRS in measuring relative changes of muscle oxygen consumption ($m\dot{V}O_2$), it remains debatable whether NIRS-determined $m\dot{V}O_2$ recovery kinetics k truly represents muscle oxidative capacity (1).

Several factors including methodological shortcomings may affect the accuracy of muscle k measurement using NIRS. For example, as discussed by Adami and Rossiter (1), insufficient activation of mitochondrial oxidative enzymes can result in the misinterpretation of muscle oxidative capacity due to low k values. It is important to consider the abundance and the oxidative phosphorylation activity of mitochondria in the muscle as both are known to contribute substantially to oxidative capacity (2). The latter has further been shown to limit skeletal muscle oxidative metabolism to a greater extent than oxygen availability (2, 3). In chronic diseases or conditions (e.g., aging) where mitochondrial impairment is evidenced, NIRS-based k is not likely to be useful to infer muscle oxidative capacity since the value alters in response to decreased mitochondrial biogenesis and oxidative protein expression (2, 4). Furthermore, NIRS approach involves a series of brief arterial occlusion and reperfusion cycles, which presumably stimulates ischemic preconditioning (5) and influences mitochondrial function.

The use of NIRS in determining muscle oxidative capacity will be more reliable with the consideration of aforementioned points, and the establishment of k references in different health conditions and ages will prompt broad utilization.

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