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Principles, Insights and Potential Pitfalls of the Non-Invasive Determination of Muscle Oxidative Capacity by Near-Infrared Spectroscopy

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Skeletal muscle oxidative capacity is highly plastic, strongly associated with whole-body aerobic capacity (16,18) and state of health. Loss of muscle oxidative capacity is associated with physical inactivity, aging and chronic disease (17), and has been implicated in the pathophysiology of obesity and diabetes (21). Evaluating these changes has traditionally been limited to invasive or costly assessments (biopsy or ^{31}P MRS). To address this, Hamaoka and colleagues developed an innovative, non-invasive approach using near-infrared spectroscopy (NIRS) to quantitatively measure muscle oxygen consumption ($m\dot{V}O_2$;12) and use this to infer muscle oxidative capacity based on the $m\dot{V}O_2$ recovery rate constant (*k*) (23; later modified 26). This technique has been subsequently used to interpret relative differences in oxidative capacity across a wide range of muscles, ages and disease states (Figure 1C). The purpose of this Viewpoint is to open a discussion on the principles, insights and potential pitfalls of using NIRS to measure *k* and infer muscle oxidative capacity.

Principles

First order Michaelis-Menten enzyme kinetics dictates that $m\dot{V}O_2$ kinetics are directly proportional to muscle oxidative capacity (6,20,22). This concept is broadly supported when comparing across species during whole-body exercise (24); and was specifically identified in the recovery *k* of single frog muscle fibers (r^2=0.77; 33) (20). Such observations form the basis to infer muscle oxidative capacity from *k* in humans. Of note, this is distinct from the recovery *k* of pulmonary $\dot{V}O_2$ following exercise, which is dependent on both muscle and circulatory function. Isolated muscle cellular $\dot{V}O_2$ can be measured by NIRS during arterial occlusion from the changes in concentration of *oxy*- and *deoxy*-hemoglobin and myoglobin (10,13) i.e. in the absence of blood flow, muscle deoxygenation occurs solely by O_2 consumption. For this method, brief light-intensity muscle contractions are used to elicit an increase in $m\dot{V}O_2$, after which recovery *k* is assessed using a series of intermittent arterial occlusions (each 5-10 s, separated by 5-20 s of reperfusion; Figure 1A, 1B). Recovery *k* by NIRS has been experimentally validated against ^{31}P MRS (r^2=0.77-0.90; 29) and muscle biopsy (r^2=0.46; 25); the 'gold-standard' techniques for muscle oxidative capacity measurement.

Insights

The major advantage of NIRS-based muscle oxidative capacity estimation is its relative ease of application compared with muscle biopsy or ^{31}P MRS. It is non-invasive, relatively inexpensive, short duration and well tolerated. The isolated nature of the brief muscle contractions allows even functionally limited patients to perform the test. Assessment of different superficial limb muscle groups

(plantar flexors, knee extenders, wrist flexors), or between limbs (e.g. for unilateral impairments), is highly feasible. The technique is particularly useful for assessing longitudinal change or interventional efficacy, such as following the response to training (7,28,30).

In the past five years the technique has found wide application in health (5,28) and clinical populations (1-4,8,9,27,30,34). Figure 1C shows *k* values across a wide range of muscle groups, age and health status. These data reveal the extreme plasticity of relative muscle oxidative capacity (c.f. 16), with a ~5-fold difference between muscles in motor-complete spinal cord injured patients and endurance athletes. Evidence of the well-established age-associated decline in muscle oxidative capacity is seen among these cross-sectional studies in both upper and lower limb muscles. Also observed is the somewhat lower oxidative capacity of the wrist flexors compared with the *vastus lateralis* or *gastrocnemius* muscles across comparable groups, presumably reflecting the lower expression of oxidative type I muscle fibers in the forearm. Loss of muscle oxidative capacity (~25-45% vs. similar aged controls) is seen in COPD (GOLD class 3-4) and CHF (NYHA class I-III), a loss that appears consistent between upper and lower limbs.

Potential Pitfalls

As a major advantage of the NIRS approach is that it relies on $m\dot{V}O_2$ *kinetics* to estimate oxidative capacity, quantification of absolute $m\dot{V}O_2$ (which is complex by NIRS) is not necessary; only relative change in $m\dot{V}O_2$ over time is required. However, method relies on at least two competing assumptions and some technical limitations.

Two key assumptions are: 1) that mitochondrial oxidative enzymes are maximally activated by the brief contractions, ratifying the assumption of 'functionally' first order enzyme kinetics (21,32,33); and 2) that O_2 concentration is not limiting to *k* (15,33). Recent studies suggest that control of oxidative phosphorylation in human muscle is not first order (19). However, exercise rapidly activates mitochondrial enzymes (11,19) and the recovery of this activation process is slow in relation to *k* (19). The NIRS approach relies upon brief contractions to release inhibition of mitochondrial enzyme activity such that linear proportionality exists between cellular oxidative capacity and *k* (33). An insufficient contraction-related stimulus could result in a low *k* that misrepresents the 'true' oxidative capacity. Low activation may also reduce the confidence of the fitted curve and the modeled *k*. While there appears to be no ordering effect of repeated measurements made during the same visit (1,9,27), we caution that poor test-retest reproducibility of *k* is found in participants with a low contraction-induced increase in $m\dot{V}O_2$ (1).

Recovery *k* only reflects oxidative capacity when [O_2] is abundant (33). As exercise and the imposed arterial occlusions required by the method reduce muscle PO_2, care is required that [O_2] does not become limiting. Haseler et al. (15) showed that PCr recovery was slowed during hypoxia compared with normoxia. For this reason it is recommended that NIRS estimation of oxidative capacity be preceded by a ~5 min arterial occlusion, to identify the functional range of tissue O_2 saturation (StO_2). Subsequently, brief contractions and occlusions are metered such that StO_2 remains high (1). Little data exists to determine whether or not this 'ischemic preconditioning' acutely alters mitochondrial function or recovery *k*. Nevertheless, as StO_2 is measured by NIRS itself, the assessor can administer the test so as to ensure that recovery *k* remains a reflection of the intrinsic intramuscular capacity for oxidation, and independent of vascular function.

There exist technical challenges with the NIRS assessment that also require consideration. Early attempts at NIRS-based $m\dot{V}O_2$ measurement identified that tissue hemoglobin often varies during arterial occlusion. This was attributed to residual pressure gradients causing movement of heme chromophores in and/or out of the NIRS field of view, even during arterial occlusion (26). Thus, if total hemoglobin is not constant, changes in *deoxy*-hemoglobin and myoglobin may result from not only O_2 consumption but also hemo-concentration/dilution. To address this, Ryan et al. (26) developed a correction method for hemoglobin volume change, based on the instantaneous relative oxygenation. Other studies have used spatially resolved spectroscopy (10) to estimate StO_2, producing similar results (1). Nevertheless, failure to adequately control for hemoglobin changes during the brief arterial occlusions will influence the measured *k*.

The technique relies upon complete occlusion of blood flow, such that changes in oxygenation reflect only $m\dot{V}O_2$: should partial occlusion occur (particularly relevant to measurements of the *vastus lateralis* in well-muscled or obese individuals), the result becomes misleading. This requirement effectively limits the application to limb muscles, as respiratory or abdominal muscles cannot be easily subject to arterial occlusion.

Other considerations for valid and reproducible application of the technique include that the skin and adipose tissue thickness be low enough that the diffused NIRS light can reach muscle, and sufficient intensity of light is received at the NIRS detector. Poor probe placement, large skinfold or high skin melanin content can obfuscate these requirements.

Overall, the test-retest reliability of *k* assessment by NIRS is good (coefficient of variation, ~10%; intraclass correlation coefficient range, 0.26-0.93; 1,26,31), and is typically non-inferior to biopsy or ^{31}P MRS methods. Both NIRS and ^{31}P MRS have the added advantage that they sample a larger volume of

(albeit superficially-weighted) muscle than biopsy. But test-retest variability is somewhat large compared to the typical effect size of oxidative capacity loss observed in disease (Figure 1C). For this reason it is recommended to average 2-3 repeat *k* measurements in the same individual to minimize variability and increase sensitivity (1,9,27).

By meeting each of these conditions, a reliable estimate of relative muscle oxidative capacity, independent of macro- or microvascular (dys)function, can be inferred from *k*.

Conclusion

Test-retest reliability is sufficient across several labs for muscle *k* assessment to be used as a non-invasive tool to assess the efficacy of interventions designed to ameliorate muscle mitochondrial impairment in patients with chronic disease. The ease of application of the method is a major benefit, but quality control procedures are needed to ensure measurement validity and to minimize error. Overall, the NIRS-based assessment of muscle *k*, originally developed by Hamaoka and colleagues, offers promise to simplify identification of relative changes in muscle oxidative capacity in both research and clinical settings.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: A.A. analyzed the data and made the first draft of the manuscript; A.A. and H.B.R. edited, revised and approved final version of manuscript.

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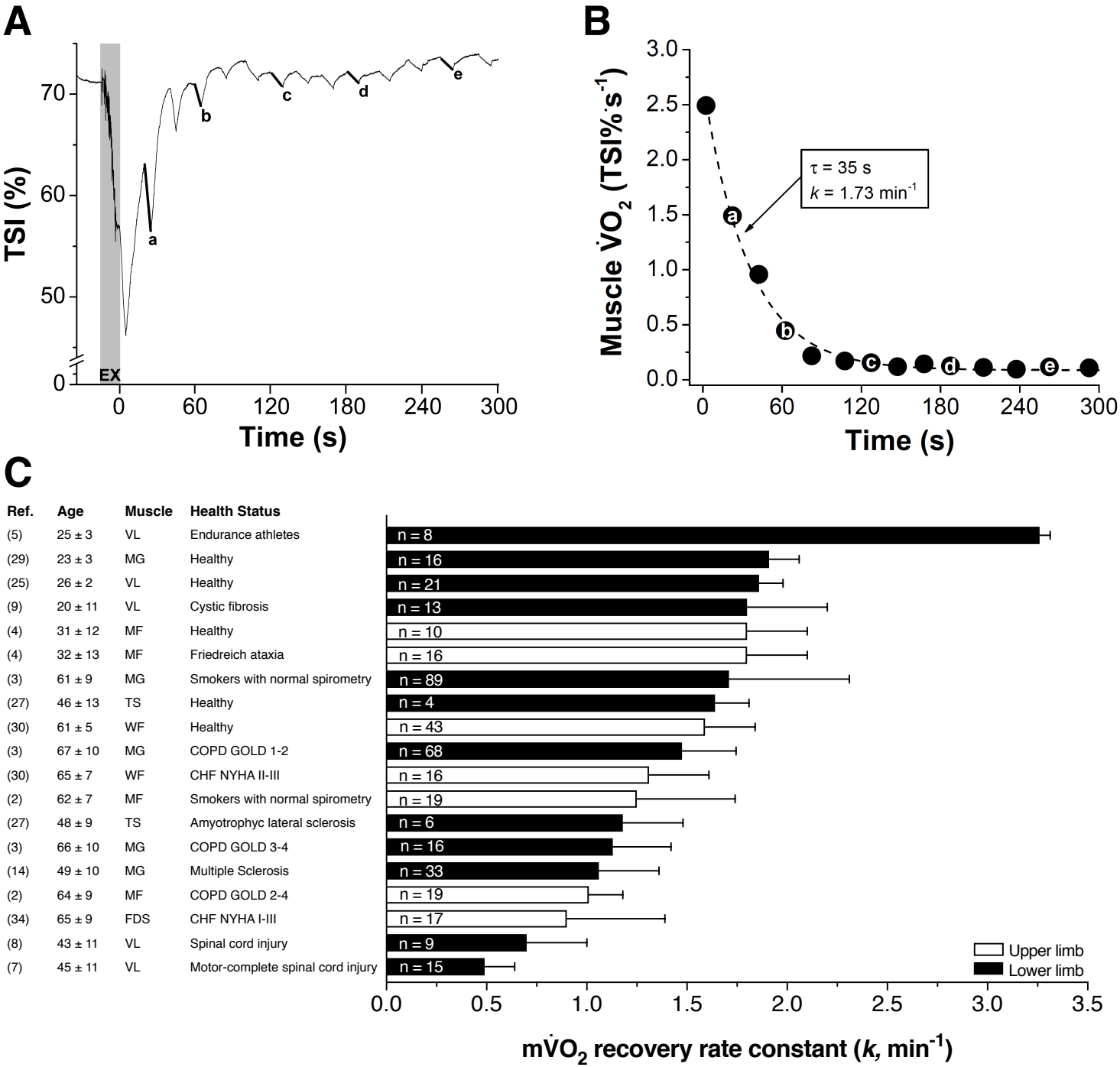
FIGURE

Figure 1. Muscle oxygen consumption ($m\dot{V}O_2$) recovery rate constant (*k*) by near-infrared spectroscopy. Panels A and B show an example of the oxidative capacity test by NIRS in the medial *gastrocnemius* of a 54 year-old female. Panel A shows the changes in the tissue saturation index (TSI) during dynamic exercise (EX, grey area) and subsequent intermittent arterial occlusions at rest. Panel B shows the $m\dot{V}O_2$ recovery kinetics derived from the rate of change of TSI during intermittent arterial occlusions measured from panel A. The $m\dot{V}O_2$ recovery data are fit to an exponential (dashed line) to estimate the recovery *k*. The time constant (τ) is the reciprocal of the rate constant k ($\tau = 1/k$). Panel C summaries current reports of the $m\dot{V}O_2$ recovery rate constant (*k*), which is proportional to oxidative capacity, in upper and lower limbs of adults in health and disease. Panel A and B are redrawn with permission from (1).



Data are mean ± SD.

Muscles: FDS, *flexor digitorum superficialis* (dominant arm); MF, medial forearm (non-dominant arm); MG, medial *gastrocnemius*; TS, *triceps surae*; WF, wrist-flexors (non-dominant arm); VL, *vastus lateralis*.

Abbreviations: CHF, chronic heart failure; COPD, chronic obstructive pulmonary disease; GOLD, Global Initiative for Obstructive Lung Disease functional class; *k*, $m\dot{V}O_2$ rate constant; $m\dot{V}O_2$, muscle oxygen consumption; NYHA, New York Heart Association functional class; Ref., reference list number.