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REPRODUCIBILITY OF NIRS ASSESSMENT OF MUSCLE OXIDATIVE CAPACITY IN SMOKERS WITH AND WITHOUT COPD

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RUNNING HEAD: Non-invasive assessment of muscle oxidative capacity in COPD

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ABSTRACT

Low muscle oxidative capacity contributes to exercise intolerance in chronic obstructive pulmonary disease (COPD). Near-infrared spectroscopy (NIRS) allows non-invasive determination of the muscle oxygen consumption ($m\dot{V}O_2$) recovery rate constant (*k*), which is proportional to oxidative capacity assuming two conditions are met: 1) exercise intensity is sufficient to fully-activate mitochondrial oxidative enzymes; 2) sufficient O_2 availability. We aimed to determine reproducibility (coefficient of variation, CV; intraclass correlation coefficient, ICC) of NIRS *k* assessment in the *gastrocnemius* of 64 participants with (FEV_1 64±23%predicted) or without COPD (FEV_1 98±14%predicted). 10-15s dynamic contractions preceded 6min of intermittent arterial occlusions (5-10s each, ~250mmHg) for *k* measurement. *k* was lower (P<0.05) in COPD (1.43±0.4min^{-1}; CV=9.8±5.9%, ICC=0.88) than controls (1.74±0.69min^{-1}; CV=9.9±8.4%; ICC=0.93). Poor *k* reproducibility was more common when post-contraction $m\dot{V}O_2$ and deoxygenation were low, suggesting insufficient exercise intensity for mitochondrial activation and/or the NIRS signal contained little light reflected from active muscle. The NIRS assessment was well tolerated and reproducible for muscle dysfunction evaluation in COPD.

KEY WORDS

Skeletal muscle; Mitochondria; Exercise intolerance; Oxygen consumption; Kinetics; Quality-control

1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by dyspnea on exertion, with subsequent reduced exercise tolerance and quality of life. Skeletal muscle dysfunction is a systemic consequence of COPD that also contributes to increased morbidity and mortality in this population (Agustí et al., 2003; Casaburi et al., 2001; Decramer et al., 2008; Maltais et al., 2000, 2014; Nici, 2000; Vogiatzis and Zakynthinos, 2012; Wounters et al., 2002). Morphological and structural skeletal muscle alterations in COPD are especially prevalent in the locomotor muscles, and include atrophy and weakness, loss of type I fibers, loss of muscle oxidative capacity and mitochondrial dysfunction, among others (Allaire et al., 2004; Coronell et al., 2004; Couillard and Prefaut, 2005; Engelen et al., 2000; Gosker et al., 2002, 2007; Maltais et al., 2014; Picard et al., 2008; Whittom et al., 1998). Amelioration of these muscular alterations contributes to the substantial benefits of pulmonary rehabilitation in COPD patients (Maltais et al., 2014).

The prevalence and progression of the loss of muscle oxidative phenotype in relation to disease severity is still unclear, and this is partly because measurement of muscle oxidative capacity requires an invasive biopsy or complex ^{31}P magnetic resonance spectroscopy assessments. In review, Meyer et al. (2013) showed that low muscle oxidative capacity and increased reactive oxygen species production was evident in skeletal muscle across all spirometric stages of COPD disease severity. Furthermore, Natanek et al. (2013) showed wide heterogeneity in quadriceps type I fiber expression in 114 COPD patients evenly distributed across GOLD stages 2-4. These findings demonstrate that muscle oxidative capacity appears to be highly variable across disease severity, which underscores the need for simple methods to assess changes in muscle oxidative capacity in COPD patients independent from systemic effects of the disease.

We aimed to address this using a non-invasive method based on near-infrared spectroscopy (NIRS; Motobe et al., 2004; Ryan et al., 2012). This technique provides measurement of the recovery rate constant (k) of muscle oxygen consumption ($m\dot{V}O_2$), isolated from influences of circulatory or pulmonary function, and which is directly related to muscle oxidative capacity in single muscle fibers (r^2=0.77; Wüst et al., 2013). Muscle k can be assessed by NIRS during ~6 minutes of recovery from brief contractions, using a series of intermittent arterial occlusions (5-10 s each); during occlusions, the rate of decline in the muscle tissue saturation index (TSI) is directly proportional to $m\dot{V}O_2$. This technique has been validated in young healthy subjects against phosphocreatine

recovery kinetics and quadriceps muscle biopsy (Ryan et al., 2013, 2014). It has also been used to assess muscle oxidative capacity in spinal cord injury (Erickson et al., 2013), amyotrophic lateral sclerosis (Ryan et al., 2014) and chronic heart failure (Southern et al., 2015), among other conditions. However, to our knowledge, this technique has not been applied in COPD where muscle morphologic adaptations such as fat infiltration, fibrosis, inflammation, increased subcutaneous adipose, loss of type I fibers and mitochondrial density (Maltais et al., 2014) may hamper NIRS measurement of muscle oxidative capacity.

The method relies on two competing assumptions: 1) that exercise is sufficiently intense to maximally activate mitochondrial oxidative enzymes and elicit a sufficient increase in $m\dot{V}O_2$ (Korzeniewski and Rossiter, 2015; Wüst et al., 2011, 2013); 2) that O_2 delivery is not limiting to k (Haseler et al., 2004). This latter condition is especially important in COPD where poor systemic O_2 delivery, muscle capillary rarefaction and brief arterial occlusions may combine to reduce TSI below some critical threshold, thereby slowing $m\dot{V}O_2$ recovery kinetics. Test-retest reliability (intraclass correlation coefficient, ICC) of k in healthy subjects ranges from 0.26 to 0.68 (Ryan et al., 2012; Southern et al., 2014), and whether reliable measurements are possible in COPD is currently unknown. This is particularly important in relation to the expected effect magnitude of oxidative capacity loss in COPD (~10-50%; Meyer et al., 2013). Therefore, we aimed to determine the reliability of NIRS assessment of *gastrocnemius* muscle oxidative capacity in smokers with and without COPD. We hypothesized that test-retest variability in k would be sufficiently low to allow NIRS estimates of oxidative capacity to a useful method to detect COPD-related loss. Secondly, we aimed to identify correlates of high variability in repeated k measurement, if it occurred. These correlates may provide a basis for quality control of the NIRS muscle assessment.

2. MATERIALS AND METHODS

2.1 Participants

Both smoking (Montes de Oca et al., 2008) and COPD (Maltais et al., 2014) have each been implicated in COPD-associated muscle dysfunction. Therefore, to account for the independent influence of smoking history, we sought current and former smokers with at least 10 pack-year smoking history to volunteer: 32 COPD patients (GOLD stage 1-4, defined by the criteria for the Global initiative for Chronic Obstructive Lung Disease) and 28 participants with normal spirometry (CON) (Table 1). This was an ancillary study of COPDGene (ClinicalsTrials.gov Identifier

NCT00608764), for which a complete list of inclusion and exclusion criteria is given in Regan et al. (2010). Participants were informed about the procedures and risks associated with the study, and gave written informed consent. The study was approved by the Institutional Review Board of Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, in accordance with the Declaration of Helsinki.

2.2 Protocol

Each participant visited the laboratory once, during which NIRS muscle oxidative capacity and a spirometry tests were performed.

2.2.1 NIRS muscle oxidative capacity test. A wireless, portable, continuous-wave, spatially-resolved spectroscopy (SRS) NIRS device (PortaMon, Artinis, The Netherlands) was used to measure relative concentrations of *deoxy*-hemoglobin and *deoxy*-myoglobin (here termed HHb for simplicity) and *oxy*-hemoglobin and *oxy*-myoglobin (HbO_2) in the tissues ~1.5 cm beneath the probe (interoptode distance was 3 cm). From these measurements relative changes in total hemoglobin and myoglobin (THb = HHb+HbO_2) and the Hb difference (Hb_{diff} = HbO_2-HHb) were calculated. In addition, the tissue saturation index (TSI, %) was measured using the SRS approach (using interoptode distances of 2-3 cm) (Ferrari et al., 2004).

A modified NIRS protocol based on Ryan et al. (2012) was used. The participant lay supine and the NIRS probe was wrapped in plastic film, placed longitudinally on the belly of the right medial *gastrocnemius*, and secured with an elastic bandage. A 13 x 85 cm rapid-inflation pressure-cuff (SC12D, Hokanson, USA) was placed on the proximal thigh of the same leg and attached to an electronically-controlled rapid cuff-inflator (E20, Hokanson, USA). A pad was placed under the ankle such that the lower leg and NIRS probe was suspended above the bed. During the ~30 min assessment, the participant was asked to relax and refrain from moving the leg except when instructed.

Initially, the participant was familiarized with the execution of cyclical plantar-flexion/relaxation exercise at ~1Hz, to activate the medial *gastrocnemius* against a manually applied resistance, and with the rapid-cuff inflation procedures. Repeated cuff inflations from low (~50 mmHg) to high (~250 mmHg) pressures were performed during this familiarization phase. Arterial occlusion was

147 determined from a tolerated cuff-pressure within the range of 230-300 mmHg (236±17 mmHg) that
148 resulted in HHb rise, HbO_2 fall and approximately constant THb over ~15-20 s.
149
150 The measurement protocol began after 2-3 min of rest, where baseline TSI and SpO_2 at a fingertip
151 (Rad-5 Pulse Oximeter MasimoSET®, Masimo Co., Irvine, CA) were measured over 2 min.
152 Subsequently, after having removed the pulse oximeter, the participant was instructed to execute
153 10-12 cycles of plantar-flexion exercise, followed immediately by arterial occlusion until a steady-
154 state in TSI was reached (mean duration ~90 s; Figure 1). The cuff was then instantly deflated and
155 muscle reoxygenation was recorded until a steady-state was reached (typically ~3 min). This
156 procedure (the physiologic normalization, PN) identified the functional range of TSI under resting
157 conditions from TSI*min* at the end of the sustained arterial occlusion to TSI*max* at the peak of the
158 reactive hyperemia (Figure 1). Finally, the participant performed two oxidative capacity
159 assessments. These consisted of: 1) cyclical plantar-flexion exercise to desaturate the muscle to a
160 target of 50% of the PN amplitude (typically 10-15 s of contractions) (Hamaoka et al., 2007; McKully
161 et al., 1994; Motobe et al., 2004; Ryan et al., 2012) (Figure 1); 2) a series of intermittent arterial
162 occlusions (AO; 5 occlusions for 5 s, and 10 for 10 s, each separated by 5-20 s recovery). A single
163 oxidative capacity assessment lasted ~6 minutes. The second repetition was conducted once a
164 resting steady state was re-established (typically ~1 min).
165
166 At the end of the procedure the skinfold at the NIRS site was measured to estimate adipose tissue
167 thickness (ATT, mm) (Lange Skinfold Caliper, Beta Technology Inc., Santa Cruz, CA).
168
169 *2.2.2 Spirometry*. Approximately 15 minutes before spirometric testing, participants inhaled two
170 puffs of metered dose albuterol sulfate (ProAir HFA, Teva Respiratory, Horsham, PA, USA).
171 Spirometry was performed in accordance with the American Thoracic Society guidelines (Miller et
172 al., 2005) using a dual beam Doppler ultrasound-based spirometer (EasyOne Pro, Ndd Medical,
173 Zürich, Switzerland) (Regan et al., 2010). FEV_1 and forced vital capacity (FVC) were measured from
174 the greatest FEV_1 and FVC over up to eight maximum expiratory maneuvers, where the greatest two
175 measurements were within 150 mL.
176
177 ***2.3 Analyses***

2.3.1 NIRS oxidative capacity test. During the repeated oxidative capacity tests, for each intermittent arterial occlusion the negative slope of TSI (%.s^{-1}) was fitted by a linear function to estimate relative m$\dot{V}O_2$ (Figure 2A,C). Note that during occlusion the rate of deoxygenation (the negative slope of TSI) is inversely proportional to m$\dot{V}O_2$, and is therefore reported below as a positive value (%.s^{-1}). The m$\dot{V}O_2$ exponential recovery rate constant (k, min^{-1}) was estimated using non-linear least-squares regression (Figure 2B,D) (OriginPro v8.6, OriginLab Co., Northampton, USA) (Wüst et al., 2013).

2.3.2 NIRS quality control. Low test-retest variability (>1 SD) was used as the quality control criterion. For those tests with reproducibility outside 1 SD, the potential for limitations in O_2 delivery and/or contraction-induced activation of mitochondrial oxidative phosphorylation during the oxidative capacity test were investigated to assess for physiologic contributors to test-retest variability. To determine a value of TSI during the sustained occlusion in the PN phase where the decline in TSI began to slow (a deflection in TSI; Figure 1B), a linear regression was applied from the onset of the sustained AO up to a point just before TSI deviated from linearity. This was investigated as a potential marker for the onset of O_2 delivery limitation to m$\dot{V}O_2$ during arterial occlusion. The lowest TSI (TSI_{LOW}) reached during each oxidative capacity test was recorded (both as an absolute muscle saturation and relative to the PN) and compared with the TSI deflection point (Figure 1). The increase in m$\dot{V}O_2$ during contractions was estimated from the greatest m$\dot{V}O_2$ recorded during the oxidative capacity test, and expressed in absolute units (%.s^{-1}) and as a fold-change above the steady-state resting m$\dot{V}O_2$ (measured at the end of the oxidative capacity test): a small increase or fold-change in m$\dot{V}O_2$ may indicate insufficient contractile stimulus for mitochondrial oxidative phosphorylation and result in a low k.

2.3.3 Statistics. A Student's paired t test was used to identify differences between COPD and CON. A Bland-Altman analysis for repeated measurements was used to assess the agreement between the two m$\dot{V}O_2$ recovery k assessments (Bland and Altman, 1999). Coefficient of variation (CV) and intraclass correlation coefficient (ICC) were used to assess within-subject test-retest reproducibility. Variables correlated with the difference between repeated-measures of k (Δk) were sought by Spearman univariate linear regression analysis. Significant differences were accepted at $P \leq 0.05$. Results are presented as mean ± SD, unless otherwise specified. A Shapiro-Wilk's test ($P \geq 0.05$) and visual inspection of the histograms, Q-Q plots and box plots were performed to determine normal distribution of k values for both COPD and CON groups (COPD, $P > 0.45$; CON, $P > 0.06$). Statistical

analyses were performed using Prism v6.0f (GraphPad, San Diego, CA, USA) and SPSS v20 (IBM, Chicago, IL, USA).

3. RESULTS

3.1 Participant characteristics

Four COPD patients were unable to successfully complete the NIRS muscle protocol: two could not tolerate the sustained arterial occlusion for the PN, and the $m\dot{V}O_2$ *k* could not be confidently resolved in one repeat of two other COPD patients. These 4 COPD patients were excluded from further analysis. Results are reported from 28 COPD and 28 normal spirometry CON participants. Two COPD patients required nasal cannula O_2 during the visit (at 3-4 $L.min^{-1}$). Participant characteristics are shown in Table 1. CON were younger than COPD (60 ± 7 *vs*. 65 ± 8 years, $P < 0.05$).

3.2 Muscle near-infrared spectroscopy

3.2.1 Resting muscle. Resting muscle TSI and ATT did not differ between COPD patients and CON (Table 1).

3.2.2 Physiologic normalization (PN). In all COPD patients, PN ranged from a minimum of 22 % TSI to a maximum of 77 % TSI, with a mean range (max - min) of 32 ± 9 %. This was not different ($P > 0.05$) than CON: PN ranged 19 to 81 %, with a mean of 32 ± 11 %.

3.2.3 Muscle oxidative capacity ($m\dot{V}O_2$ k). A total of 112 $m\dot{V}O_2$ recovery kinetics assessments were performed for the study. On average, there was no difference between repeated *k* measurements within COPD or CON participants (Table 2). The individual test-retest reliability was not different between COPD (CV = 9.9%, ICC = 0.88) and CON (CV = 9.9%, ICC = 0.93) (Table 2, Figure 3). Power analyses (G*Power 3.1; Faul et al, 2007) revealed a 1- β = 0.81 for comparison of *k* between groups (the primary outcome). In all participants, Bland-Altman limit of agreement analysis revealed low mean bias ($-0.03 min^{-1}$), and 95% confidence intervals of -0.58, 0.64 min^{-1} (Figure 4). We could detect no order effect between repeats of *k* measurement ($P = 0.24$; 1-tailed t-test). On average, $m\dot{V}O_2$ *k* was ~25% lower in COPD than CON (Table 2) and was diminished at all GOLD stages: CON, 1.74 ± 0.71 min^{-1} (n=28); GOLD 1, 1.45 ± 0.36 min^{-1} (n=7); GOLD 2, 1.48 ± 0.37 min^{-1} (n=13); GOLD 3, 1.22 ± 0.32 min^{-1} (n=5); and GOLD 4, 1.54 ± 0.41 min^{-1} (n=3).

242

243 *3.2.4 NIRS test quality control*. During the oxidative capacity test, the lowest TSI (TSI_{LOW}) in both
244 repeats was typically achieved within the first or second AO (e.g., see Figure 1). In both COPD and
245 CON, TSI_{LOW} averaged ~47% absolute (Table 3), equivalent to ~32% and ~29% of the PN range
246 respectively. On average, the TSI deflection point occurred at 46 ± 9 % in COPD and 46 ± 11 % in
247 CON. This meant that, typically (66% of tests), TSI_{LOW} was greater than TSI deflection point. In 38
248 tests (27%) TSI_{LOW} was below TSI deflection point.
249
250 In COPD, the peak $m\dot{V}O_2$ during the oxidative capacity test was 1.38±0.59 $\%.s^{-1}$ and 1.51±0.88 $\%.s^{-1}$
251 respectively for the first and second repetitions (equivalent to a 14±7 and 16±13 fold increase
252 above the recovery steady-state, 0.12±0.12 $\%.s^{-1}$). Peak $m\dot{V}O_2$ values during the oxidative capacity
253 test in CON were 1.71±1.89 $\%.s^{-1}$ and 1.49±1.17 $\%.s^{-1}$ respectively for the first and second repeat,
254 equivalent to a 18±12 and 16±11 fold increase above resting (0.10±0.05 $\%.s^{-1}$), and were not
255 different compared with COPD (P = 0.68).
256
257 Variables predictive of poor reproducibility were sought as potential quality control indices for the
258 NIRS oxidative capacity test. Univariate linear regression analysis revealed that variability in
259 repeated k measurements, assessed from the difference between the two k values (Δk), was
260 positively correlated with k (r^2 = 0.17; $P \leq 0.001$): meaning faster $m\dot{V}O_2$ kinetics were related to
261 greater variably of measurement. However, other variables hypothesized to explain variability in k,
262 including those expected to contribute to limitations in $m\dot{V}O_2$ activation, O_2 delivery, or NIRS signal
263 sensitivity, such as minimum TSI during PN and TSI_{LOW}, age and resting TSI, PN maximum value and
264 $m\dot{V}O_2$ fold change, did not show a strong association with Δk ($P > 0.10$).
265
266 We investigated the characteristics of poorly-reproducible tests where Δk exceeded 1 SD (equal to
267 the mean effect size for COPD; $\Delta k > 0.3$ min^{-1}): thirteen participants (5 COPD, 8 CON) exceeded this
268 variability threshold. These unreliable tests were characterized by a low $m\dot{V}O_2$ (TSI = 1.15 ±
269 0.44 $\%.s^{-1}$) and poor exercise deoxygenation (e.g. a high TSI_{LOW} value of 53.5 ± 5.8 %). Six of these
270 participants had large adipose layer (ATT = 8.3 ± 3.0 mm) and six had high skin melanin, each likely
271 limiting the volume of the muscle interrogated by the NIRS probe. In all these 13 participants, the
272 lowest TSI during the test (TSI_{LOW}) was below the TSI deflection point, suggesting that low O_2
273 availability was not associated with muscle oxidative capacity assessment reliability. Excluding tests

on the basis of $\Delta k > 0.3$ min^{-1} improved k measurement reliability (CV = 7.0±4.3%, ICC = 0.98, n = 43).

4. DISCUSSION

This is the first study to measure locomotor muscle oxidative capacity (from m$\dot{V}O_2$ recovery rate constant, k) in a large group of smokers with or without COPD, using a non-invasive, relatively simple, short-duration assessment by NIRS. Fifty-six out of 60 participants (93%) tolerated the NIRS assessment and returned interpretable results. Overall these data showed: there was no mean bias between test-retest repeats of *gastrocnemius* k measurement by NIRS in both COPD patients and age-similar smokers without airflow obstruction; that individual test-retest reproducibility was high (CV = 9.9%, ICC = 0.9); and that k averaged 25 % less in COPD compared to smokers with normal spirometry. Despite known muscle morphologic adaptations including increased fat and fibrotic infiltration, inflammation, loss of type I fibers and mitochondrial density (Maltais et al., 2014), our findings support that the NIRS assessment is a reliable method to detect COPD-related loss of muscle oxidative capacity.

We also aimed to identify correlates of tests with reproducibility that lay outside 1 SD of the distribution of all tests, as potential features for quality control. 13 of 56 participants (5 COPD, 8 CON) showed a high variability in k ($\Delta k > 0.3$ min^{-1}, which was the mean effect size of COPD). Poor reproducibly was not associated with presence of COPD. Poor reproducibility was explained principally by a small increase in m$\dot{V}O_2$ and only modest deoxygenation during contractions. This suggests that insufficient contractile stimulus for mitochondrial activation and/or that the NIRS signal contained little light reflected from active muscle (large adipose layer in n=6, and high skin melanin content in n=6), may contribute to poor test quality. These findings indicate that a poor quality tests are related to an insufficient increase in m$\dot{V}O_2$ during contractions, and not to the presence of an O_2 delivery limitation, as might be anticipated in COPD.

4.1 Muscle oxidative capacity in COPD

We found that k in the *gastrocnemius* skeletal muscle was, on average, 25 % less (range ~12-30%) in COPD than smokers of a similar age but without pulmonary obstruction. This average is consistent with the ~10-50% lower muscle oxidative enzyme activity (e.g. citrate synthase) or oxidative capacity observed in *quadriceps* biopsy samples from COPD patients compared with

controls (Meyer et al., 2013), suggesting that the NIRS test provides a relevant non-invasive alternative to invasive biopsy assessments (Ryan et al., 2014). It is of note that *k* in our control group was 25% lower than non-smoking control participants in other studies using the same NIRS methods (non-smoker *gastrocnemius* k = ~2 min^{-1} equivalent to an oxidative capacity of ~250 $pmol.s^{-1}.mg\ dry\ weight^{-1}$; Ryan et al., 2014). However, those studies almost exclusively included young participants aged 24-27 years, while our smokers ranged 49-77 years. Whether the apparently low muscle oxidative capacity in our study relates to the effects of long-term smoking, or alternatively to age or physical inactivity remains to be determined.

While our study was not sufficiently powered to detect differences across disease severity, one outlier of the three GOLD 4 patients (k = 2.12 min^{-1}) likely skewed a general trend for a progressive decline in oxidative capacity across GOLD severity classifications 2-4. Even so, the distribution of *k* values among the 28 COPD patients suggests that low *k* may occur at any GOLD stage, and therefore muscle dysfunction may not be solely associated with the disease severity in COPD (Maltais et al., 2014; Wagner, 2006). The precise etiologies resulting in loss of muscle oxidative capacity in COPD awaits further research. Nevertheless, the 93% tolerability and good test-retest agreement of the NIRS test in COPD prove the feasibility and reliability of this approach for discriminating patients with poor muscle function i.e. *gastrocnemius k* value lower than the 2 min^{-1} reference for a healthy adult. Because the NIRS test is non-invasive, it may provide the opportunity for muscle assessment in large-cohort studies, which are needed to better identify the complex multifactorial etiology of muscle oxidative dysfunction in COPD.

4.2 Variables influencing reproducibility of the NIRS muscle oxidative capacity test

The NIRS assessment of muscle oxidative capacity relies on the observed linear proportionality between the recovery rate constant (k) of cellular $\dot{V}O_2$ and cellular oxidative capacity i.e. the $\dot{V}O_{2max}$ of the muscle cells investigated (Wüst et al., 2013). The primary predictor in our study of poor *k* reproducibility was a high value for k (r^2 = 0.17; $P \leq 0.001$). This is likely a simple reflection of the limited ability to accurately model the recovery rate constant with a limited number of $m\dot{V}O_2$ measurements when $m\dot{V}O_2$ kinetics are rapid. Nevertheless, this does not limit the ability of the test to detect abnormally low muscle oxidative capacity, which is the primary aim for studies of COPD patients or other conditions of chronic inactivity or disease.

The relationship between cellular recovery *k* and oxidative capacity in muscle is predictable based on first order rate reaction kinetics (Voet and Voet, 2004), as long as O_2 concentration in muscle mitochondria remains non-limiting. However, recent studies suggest that control of oxidative phosphorylation in skeletal muscle in humans is not first order, and that allosteric or 'each step' activation of mitochondrial oxidative pathways is required to activate the enzymes limiting cellular $\dot{V}O_{2max}$ (Korzeniewski and Rossiter, 2015; Wüst et al., 2011). Thus, accurate measurement of oxidative capacity by NIRS relies on competing demands to achieve a sufficiently high level of muscle activity and $m\dot{V}O_2$ to release mitochondrial oxidative enzyme regulation, but to limit muscle activity to a sufficiently low level such that muscle mitochondrial O_2 delivery does not become a limiting variable. Failure to meet either of these conditions would result in an erroneously low measurement of muscle oxidative capacity by NIRS.

Using single muscle fibers from the frog suspended in a medium containing a high O_2 concentration, Wüst et al. (2013) observed that, unlike poorly-oxidative fibers, $m\dot{V}O_2$ recovery *k* of highly-oxidative fibers was dependent on the frequency of stimulation of the preceding contractions. A contractile protocol sufficient to elicit ~50% $\dot{V}O_{2max}$ was required to release oxidative enzyme regulation to allow recovery *k* to become proportional to cellular oxidative capacity. The duration required of this contractile protocol was not assessed. The human medial *gastrocnemius,* the site of NIRS probes in our study, expresses a mixed fiber type distribution and contains both poorly and highly-oxidative muscle fibers. Therefore, a low $m\dot{V}O_2$ response during muscle contractions would be predictive of a poor quality assessment and result in an erroneously low oxidative capacity. Consistent with this notion, in 13 of our participants the NIRS test results were poorly reproducible and in these there was a tendency (P = 0.20) for a low exercise-induced increase in $m\dot{V}O_2$. This highlights the importance of ensuring high-intensity muscle contractions during the NIRS oxidative capacity assessment for validity of the test.

As stated previously, another condition that must be satisfied for validity of the NIRS test is non-limiting mitochondrial O_2 delivery. Using ^{31}P magnetic resonance spectroscopy to determine gastrocnemius PCr recovery kinetics from plantar flexion exercise (a proxy for $m\dot{V}O_2$ *k*), Haseler et al. (2004) showed in young sedentary subjects that PCr recovery was not limited by O_2 delivery under normoxic conditions. However, during hypoxic gas breathing, PCr recovery kinetics were slowed. It should be pointed out that the duration of exercise was 6 min, and therefore $m\dot{V}O_2$ and

muscle deoxygenation were likely far greater than those observed in our study where exercise was limited to ~15 s. Nevertheless, were TSI to be driven below some limiting value, $m\dot{V}O_2$ recovery *k* may become limited by O_2 delivery *in vivo,* which would result in an erroneously low *k*. While the brief exercise in the NIRS test does not strain central cardiac or pulmonary limits for O_2 delivery, age- or disease-related chronic adaptations, such as muscle capillary rarefaction, inflammation, anemia or reduced muscle myoglobin (Maltais et al., 2014), have the potential to limit muscle mitochondrial O_2 concentration in COPD and therefore invalidate the NIRS assessment. However, we found that the lowest value of TSI measured during the NIRS assessment (TSI_{LOW}; typically reached during the 1st or 2nd post-exercise arterial occlusion) was not related to poor test-retest reliability of *k*. This suggests that outlying low *k* values are not consequent to O_2 delivery limitation, at least down to TSI_{LOW} values of ~30% of the physiologic range.

4.3 Quality control of the muscle NIRS oxidative capacity assessment

In part, the beauty of the NIRS test of muscle oxidative capacity is that it relies on $m\dot{V}O_2$ kinetics, and therefore does not require calibrated measurements. For this reason, one aim of this study was to identify variables that could be used as markers of quality control. We proposed to identify features within any tests that showed poor reproducibility. The strongest correlate of variability in repeated *k* measurements (Δk) was the value of *k* itself, which does not provide a basis for quality control. To our surprise, however, we found no correlation between Δk and proposed quality assessments (e.g. increase in $m\dot{V}O_2$ increase during contractions or low TSI during occlusions). This may reflect the overall strong test-retest reproducibility in COPD patients and controls. We therefore identified 13 participants in whom variability exceeded 1 SD (Δk > 0.3 min^{-1}). Of these 13, there was a high prevalence of large ATT, high skin melanin content, low increase in $m\dot{V}O_2$ and a small exercise-induced deoxygenation. While these features alone do not form the basis of quality control, they highlight that patient physical characteristics limiting reflected light from active muscle tissue are likely partly responsible for reducing reproducibility of *k* measurements. Alternative NIRS systems, such as high-power time-resolved (TRS) NIRS, allow deeper penetration into muscle during rest and exercise (Okushima et al., 2015), and therefore may increase the reliability of $m\dot{V}O_2$ recovery kinetic assessment in these patients.

Based on our findings and experience, a few considerations emerge to inform quality control of the NIRS oxidative capacity test. First, the current best method of quality control is to perform the

measurement twice in the same visit. We propose that poorly-reproducible tests, where Δk > 0.3 min^{-1}, be repeated to reduce the influence of outlying results, and repeated measurements averaged. Careful attention should be made to the NIRS probe placement, to ensure that a muscle region is chosen that both minimizes the skinfold under the probe and maximizes the sampling of active muscle during contractions and recovery. Doppler ultrasound, skinfold calipers, muscle palpation during contraction and/or surface EMG may help to identify optimal NIRS probe placement.

Exercise stimuli that result in a small increase $m\dot{V}O_2$ and small reduction in saturation were associated with poor test reliability. Therefore, our data suggest that the risk to NIRS test validity of under-stimulating the muscle during dynamic contractions is greater than the risk of O_2 limiting deoxygenation caused by contractions that are too intense or sustained. Thus, ensuring that the exercise-induced desaturation reaches a value of ~30-50% of the physiologic range (PN) helps in test quality assurance. This can be confirmed in real time by monitoring the TSI response to contractions and adjusting the intensity and/or duration of exercise (i.e. extending the duration beyond the ~10-15 s we used here) to achieve the desaturation target. This, of course, requires knowledge in the PN range prior to the oxidative capacity test, which is a modification to the protocol of Ryan et al. (2012) where the PN range measurement is performed last. This has the concern that a period of ischemia (even briefly) may affect mitochondrial function and therefore influence the assumptions inherent in the measurement of oxidative capacity from $m\dot{V}O_2$ recovery kinetics e.g. an ischemic preconditioning effect (Crisafulli et al., 2011). Our findings, however, that muscle k in older controls (~1.75 min^{-1}) and COPD (~1.45 min^{-1}) were consistent with that predicted from biopsy studies (Meyer et al., 2013) suggest that brief ischemia does not harm the validity of the test.

4.4 Clinical implications

Low muscle oxidative capacity is associated with exercise intolerance in COPD and therefore may contribute to reduce physical activity and quality of life in these patients (Maltais et al., 2014; Meyer et al., 2013). We studied the *gastrocnenius* muscle as a primary locomotor muscle for walking, and which is also extensively activated during standing and in sway. Rehabilitation (cycling and walking) is known to ameliorate oxidative capacity deficits in quadriceps (Maltais et al., 1996) and is associated with a reduction of dyspnea and leg fatigue symptoms during exercise in COPD. Therefore, reliable measurements of skeletal muscle structure and function, independent of disease-

related impairments in pulmonary function or muscle blood flow, are of crucial importance to monitor the peripheral consequences of COPD. This reliable, non-invasive, short-duration, relatively inexpensive and well-tolerated assessment of oxidative capacity in COPD muscle may also enable a better targeting to therapeutic strategies to improve physical activity, exercise tolerance and quality of life in these patients.

4.5 Additional considerations

Our approach focused on biological variability, in that test-retest precision of *k* was assessed in smokers with and without COPD with the same day and session, and the NIRS probe was not removed from the muscle between repeated tests. Southern et al. (2013) assessed the test-retest variability between consecutive days in young healthy participants, an approach that included the combined effects of both methodological and biological variability. This may partly explain why variability for *k* assessment in our study (ICC range 0.88-0.93; CV = 9.9 %) was higher than Southern et al. (2013) (ICC range 0.26-0.59; CV = 10.6 %). In addition, Southern et al. (2013) assessed reliability in 15 participants whereas our study investigated 56. This difference also contributes to the greater ICC found in our study. Nevertheless, our data show that the approach is reliable in COPD and older controls where muscle quality is reduced compared with young subjects.

Furthermore, Southern et al. (2013) found greater day to day test-retest variability of the NIRS oxidative capacity test when using self-metered exercise (specifically, exercise against elastic resistance bands) compared with monitored exercise using a custom-built plantar flexion ergometer. We were specifically interested in assessing a pragmatic approach to the NIRS oxidative capacity test using self-metered exercise in order to assess the applicability of the assessment in the clinical setting without the requirement for additional specialized equipment. We used a manually-applied resistance to plantar flexion administered by the same researcher in all participants. The intensity of the 10-15 contractions was assessed indirectly by feel and also monitored in the TSI trace in real time. This meant that the operator could instruct the participant to alter the intensity or duration of contractions during the test itself to achieve an 'optimal' deoxygenation signal (and, by implication, $m\dot{V}O_2$ response). We believe that this approach might have advantages over delivery of a standardized metered exercise dose in patients with chronic disease where there is wide variability in O_2 delivery and O_2 utilization responsiveness. Our high ICC values for *k* support the

suggestion, and that our pragmatic approach provides a reliable assessment of muscle oxidative capacity suitable for clinical research or routine assessment in COPD patients.

4.6 Limitations

There are few limitations to report for this study. The CON group were slightly, but significantly younger than the COPD patients (Table 1). It is possible that the younger age in CON may contribute to the mean effect size of k in COPD (-0.3 min^{-1}). Nevertheless, the average difference in age was small (5 years on an average age of 65 years), and is not the only variable that affects muscle oxidative capacity. For example, physical activity, occupation, drug therapies and comorbidities are expected to play a significant role in the etiology of loss of muscle oxidative capacity in COPD (Maltais et al., 2014; Wagner, 2006).

Two COPD patients used nasal cannula O_2 delivery during the NIRS assessment. However, these participants' data lay well within the range of the group as a whole (Δk was -0.05 and -0.13 min^{-1}). We could identify no reason additional to treat these data differently from the group. In addition, while baseline arterial O_2 saturation was measured by pulse oximetry, we found no influence of SpO_2 on the reproducibility of k. The brief single leg plantar-flexion contractions were insufficient to alter SpO_2 and we found no differences in muscle HbO_2 among the 13 participants with poor reproducibility of k and the rest of the group. We therefore believe that arterial oxygenation did not influence our results. However, this should be monitored in future studies to ensure that the assumption of constant SpO_2 is met.

Finally, we did not assess oxidative capacity from muscle biopsy in this study in relation to NIRS measures in these COPD patients. By their nature, NIRS assessment and muscle biopsy sample different muscle regions, complicating direct comparison. Assessment of quadriceps oxidative capacity by NIRS and biopsy was previously established in healthy young subjects ($r > 0.6$; Ryan et al., 2014). Validation of accuracy of the NIRS assessment of muscle oxidative capacity in COPD therefore awaits further investigation.

5. CONCLUSION

We found that a non-invasive NIRS-based assessment of oxidative capacity of *gastrocnemius* muscle was well tolerated and reliable in middle-aged to elderly smokers with or without COPD. Our data

were consistent with direct assessment of muscle citrate synthase activity or oxidative capacity from biopsy studies (Meyer et al., 2013) in that *gastrocnemius k* (a direct correlate of muscle oxidative capacity) was 25% lower in COPD than in smoker controls without pulmonary obstruction. We found high test-retest reliability of the NIRS oxidative capacity test in both COPD (CV = 9.9%; ICC = 0.88) and CON groups (CV = 9.9%; ICC = 0.93). Our attempts to identify objective markers of NIRS test quality were less successful: nevertheless, performance of repeated assessments in the same visit can identify outlying results, and these were associated with small $m\dot{V}O_2$ and deoxygenation responses during dynamic contractions, and participants with a large skinfold or high skin melanin. Together these data suggest that poor-quality assessments occur when the exercise stimulus is insufficient for mitochondrial activation and/or the NIRS signal contains little light reflected from active muscle. Low post-contraction TSI was unrelated to NIRS test reliability, suggesting that O_2 supply is sufficient for NIRS test validity at least down to TSI of ~30% of the individuals physiologic range. Therefore it is recommended to err towards a more intense exercise rather than the maintenance of a high muscle oxygenation to optimize NIRS assessment of muscle oxidative capacity. Our findings support the reliability of non-invasive muscle oxidative capacity assessment by NIRS in COPD, which may be helpful to track the efficacy of interventions in COPD such as pulmonary rehabilitation that are designed to redress skeletal muscle dysfunction.

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CONFLICTS OF INTEREST

No conflicts to declare.

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REFERENCES

1. Agustí, A.G., Noguera, A., Sauleda, J., Sala, E., Pons, J., Busquets, X., 2003. Systemic effects of chronic obstructive pulmonary disease. Eur. Respir. J. 21(2), 347-360.
2. Allaire, J., Maltais, F., Doyon, J.F., Noël, M., LeBlanc, P., Carrier, G., Simard, C., Jobin, J., 2004 Peripheral muscle endurance and the oxidative profile of the quadriceps in patients with COPD. Thorax. 59(8), 673-678.
3. Bland, J.M., Altman, D.G. 1999. Measuring agreement in method comparison studies. Statistical Methods in Medical Research. 8, 135-160.
4. Casaburi, R., 2001. Skeletal muscle dysfunction in chronic obstructive pulmonary disease. Med. Sci. Sports. Exerc. 33(7Suppl), S662-S670.
5. Coronell, C., Orozco-Levi, M., Méndez, R., Ramírez-Sarmiento, A., Gáldiz, J.B., Gea, J., 2004. Relevance of assessing quadriceps endurance in patients with COPD. Eur. Respir. J. 24(1), 129-136.
6. Couillard, A., Prefaut, C., 2005. From muscle disuse to myopathy in COPD: potential contribution of oxidative stress. Eur. Respir. J. 26(4), 703-719.
7. Crisafulli, A., Tangianu, F., Tocco, F., Concu, A., Mameli, O., Mulliri, G., Caria, M.A., 2011. Ischemic preconditioning of the muscle improves maximal exercise performance but not maximal oxygen uptake in humans. J. Appl. Physiol. 111(2), 530-536.
8. Decramer, M., Rennard, S., Troosters, T., Mapel, D.W., Giardino, N., Mannino, D., Wouters, E., Sethi, S., Cooper, C.B., 2008. COPD as a lung disease with systemic consequences--clinical impact, mechanisms, and potential for early intervention. COPD. 5(4), 235-256.
9. Engelen, M.P., Schols, A.M., Does, J.D., Wouters, E.F., 2000. Skeletal muscle weakness is associated with wasting of extremity fat-free mass but not with airflow obstruction in patients with chronic obstructive pulmonary disease. Am. J. Clin. Nutr. 71(3), 733-738.
10. Erickson, M.L., Ryan, T.E., Young, H.J., McCully, K.K., 2013 Near-infrared assessments of skeletal muscle oxidative capacity in persons with spinal cord injury. Eur. J. Appl. Physiol. 113(9), 2275-2283.
11. Faul, F., Erdfelder, E., Lang, A.G., Buchner, A., 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods. 39(2), 175-191.
12. Ferrari, M., Mottola, L., Quaresima, V., 2004. Principles, techniques, and limitations of near infrared spectroscopy. Can. J. Appl. Physiol. 29(4), 463-487.

13. Gosker, H.R., van Mameren, H., van Dijk, P.J., Engelen, M.P., van der Vusse, G.J., Wouters, E.F., Schols, A.M., 2002. Skeletal muscle fibre-type shifting and metabolic profile in patients with chronic obstructive pulmonary disease. Eur. Respir. J. 19(4), 617-625.
14. Gosker, H.R., Zeegers, M.P., Wouters, E.F., Schols, A.M., 2007. Muscle fibre type shifting in the vastus lateralis of patients with COPD is associated with disease severity: a systematic review and meta-analysis. Thorax. 62(11), 944-949.
15. Hamaoka, T., McKully, K.K., Quaresima, V., Yamamoto, K., Chance, B., 2007. Near-infrared spectroscopy/imaging for monitoring muscle oxygenation and oxidative metabolism in healthy and diseased humans. J. Biomed. Opt. Doi:12:062105-1-062105-16.
16. Haseler, L.J., Lin, A.P., Richardson, R.S., 2004. Skeletal muscle oxidative metabolism in sedentary humans: 31P-MRS assessment of O2 supply and demand limitations. J. Appl. Physiol. 97(3), 1077-1081.
17. Korzeniewski, B., Rossiter, H.B., 2015 Each-step activation of oxidative phosphorylation is necessary to explain muscle metabolic kinetic responses to exercise and recovery in humans. J. Physiol. 593(24), 5255-5268.
18. Maltais, F., Decramer, M., Casaburi, R., Barreiro, E., Burelle, Y., Debigaré, R., Dekhuijzen, P.N., Franssen, F., Gayan-Ramirez, G., Gea, J., Gosker, H.R., Gosselink, R., Hayot, M., Hussain, S.N., Janssens, W., Polkey, M.I., Roca, J., Saey, D., Schols, A.M., Spruit, M.A., Steiner, M., Taivassalo, T., Troosters, T., Vogiatzis, I., Wagner, P.D., 2014. ATS/ERS Ad Hoc Committee on Limb Muscle Dysfunction in COPD. An official American Thoracic Society/European Respiratory Society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care. Med. 189(9), e15-e62.
19. Maltais, F., LeBlanc, P., Jobin, J., Casaburi, R., 2000. Peripheral muscle dysfunction in chronic obstructive pulmonary disease. Clin. Chest. Med. 21(4), 665-677.
20. Maltais, F., LeBlanc, P., Simard, C., Jobin, J., Bérubé, C., Bruneau, J., Carrier, L., Belleau, R., 1996 Skeletal muscle adaptation to endurance training in patients with chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care. Med. 154(2 Pt 1), 442-447.
21. McKully, K.K., Iotti, S., Kendrick, K., Wang, Z., Posner, J.D., Leigh, J.Jr., Chance, B., 1994 Simultaneous in vivo measurements of HbO2 saturation and PCr kinetics after exercise in normal humans. J. Appl. Physiol. 77(1), 5-10.

22. Meyer, A., Zoll, J., Charles, A.L., Charloux, A., de Blay, F., Diemunsch, P., Sibilia, J., Piquard, F., Geny, B., 2013. Skeletal muscle mitochondrial dysfunction during chronic obstructive pulmonary disease: central actor and therapeutic target. Exp. Physiol. 98(6), 1063-1078.
23. Miller, M.R., Crapo, R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Enright, P., van der Grinten, C.P., Gustafsson, P., Jensen, R., Johnson, D.C., MacIntyre, N., McKay, R., Navajas, D., Pedersen, O.F., Pellegrino, R., Viegi, G., Wanger, J., 2005. ATS/ERS Task Force. General considerations for lung function testing. Eur. Respir. J. 26(1), 153-161.
24. Montes de Oca, M., Loeb, E., Torres, S.H., De Sanctis, J., Hernández, N., Tálamo, C., 2008. Peripheral muscle alterations in non-COPD smokers. Chest. 133(1), 13-18.
25. Motobe, M., Murase, N., Osada, T., Homma, T., Ueda, C., Nagasawa, T., Kitahara, A., Ichimura, S., Kurosawa, Y., Katsumura, T., Hoshika, A., Hamaoka, T., 2004. Noninvasive monitoring of deterioration in skeletal muscle function with forearm cast immobilization and the prevention of deterioration. Dyn. Med. 3(1), 2.
26. Natanek, S.A., Gosker, H.R., Slot, I.G., Marsh, G.S., Hopkinson, N.S., Man, W.D., Tal-Singer, R., Moxham, J., Kemp, P.R., Schols, A.M., Polkey, M.I., 2013. Heterogeneity of quadriceps muscle phenotype in chronic obstructive pulmonary disease (Copd); implications for stratified medicine? Muscle. Nerve. 48(4), 488-497.
27. Nici, L., 2000. Mechanisms and measures of exercise intolerance in chronic obstructive pulmonary disease. Clin. Chest. Med. 21(4), 693-704.
28. Okushima, D., Poole, D.C., Rossiter, H.B., Barstow, T.J., Kondo, N., Ohmae, E., Koga, S., 2015. Muscle deoxygenation in the quadriceps during ramp incremental cycling: Deep vs. superficial heterogeneity. J. Appl. Physiol. 119(11), 1313-1319.
29. Picard, M., Godin, R., Sinnreich, M., Baril, J., Bourbeau, J., Perrault, H., Taivassalo, T., Burelle, Y., 2008. The mitochondrial phenotype of peripheral muscle in chronic obstructive pulmonary disease: disuse or dysfunction? Am. J. Respir. Crit. Care. Med. 178(10), 1040-1047.
30. Regan, E.A., Hokanson, J.E., Murphy, J.R., Make, B., Lynch, D.A., Beaty, T.H., Curran-Everett, D., Silverman, E.K., Crapo, J.D., 2010. Genetic epidemiology of COPD (COPDGene) study design. COPD. 7(1), 32-43.
31. Ryan, T.E., Erickson, M.L., Brizendine, J.T., Young, H.J., McCully, K.K., 2012. Noninvasive evaluation of skeletal muscle mitochondrial capacity with near-infrared spectroscopy: correcting for blood volume changes. J. Appl. Physiol. 113(2), 175-183.

621 32. Ryan, T.E., Southern, W.M., Reynolds, M.A., McCully, K.K., 2013. A cross-validation of near-
622 infrared spectroscopy measurements of skeletal muscle oxidative capacity with phosphorus
623 magnetic resonance spectroscopy. J. Appl. Physiol. 115(12), 1757-1766.
624 33. Ryan, T.E., Brophy, P., Lin, C.T., Hickner, R.C., Neufer, P.D., 2014. Assessment of in vivo skeletal
625 muscle mitochondrial respiratory capacity in humans by near-infrared spectroscopy: a
626 comparison with in situ measurements. J. Physiol. 592(15), 3231-3241.
627 34. Southern, W.M., Ryan, T.E., Reynolds, M.A., McCully, K.K., 2014. Reproducibility of near-infrared
628 spectroscopy measurements of oxidative function and postexercise recovery kinetics in the
629 medial gastrocnemius muscle. Appl. Physiol. Nutr. Metab. 39(5), 521-529.
630 35. Vogiatzis, I., Zakynthinos, S., 2012. Factors limiting exercise tolerance in chronic ling diseases.
631 Comprehensive. Physiology. 2(3), 1779-1817.
632 36. Wagner, P.D., 2006 Skeletal muscles in chronic obstructive pulmonary disease: deconditioning,
633 or myopathy? Respirology. 11(6), 681-686.
634 37. Whittom, F., Jobin, J., Simard, P.M., Leblanc, P., Simard, C., Bernard, S., Belleau, R., Maltais, F.,
635 1998. Histochemical and morphological characteristics of the vastus lateralis muscle in patients
636 with chronic obstructive pulmonary disease. Med. Sci. Sport. Exerc. 30(10), 1467-1474.
637 38. Wouters, E.F., 2002. Chronic obstructive pulmonary disease. 5: systemic effects of COPD. Thorax.
638 57(12), 1067-1070.
639 39. Wüst, R.C., van der Laarse, W.J., Rossiter, H.B., 2013. On-off asymmetries in oxygen consumption
640 kinetics of single Xenopus laevis skeletal muscle fibres suggest higher-order control. J. Physiol.
641 591(3), 731-744.
642 40. Wüst, R.C., Grassi, B., Hogan, M.C., Howlett, R.A., Gladden, L.B., Rossiter, H.B., 2011. Kinetic
643 control of oxygen consumption during contractions in self-perfused skeletal muscle. J. Physiol.
644 589, 3995-4009.
645 41. Voet, D., Voet, J.G., 2004. Rates of Enzymatic Reactions, in Voet, D., Voet, J.G., Biochemistry, third
646 ed. John Wiley and Sons, Hoboken, pp. 472-482.
647

FIGURE CAPTIONS

Figure 1: Tissue saturation index (TSI, %) changes during the NIRS muscle assessment. Panel A. Protocol phases, and the parameter calculated from the analyses of TSI signal changes, are indicated at the bottom of the graph. Grey shading indicates brief dynamic plantar-flexion exercise. AO = arterial occlusion; *max* and *min* = highest and lowest TSI values during the Physiologic Normalization (PN) phase; TSI_{LOW} = lowest saturation value reached during oxidative capacity assessments (for further details see *Methods*). Panel B. Expansion of panel A to illustrate the linear regression to determine the deflection point of muscle TSI (arrow) during the sustained arterial occlusion (AO). Grey shading indicates brief dynamic plantar-flexion exercise.

Figure 2: Representative COPD (A, B) and control (C, D) participants' responses during the muscle oxidative capacity assessment. Panels A and C show the TSI profiles during dynamic exercise, and intermittent arterial occlusion during recovery. Panels B and D show the calculated muscle $\dot{V}O_2$ recovery profiles and kinetic fit (dashed line). The letters (a-e) are given to illustrate how the corresponding $m\dot{V}O_2$ value is derived from respective TSI negative slopes during intermittent occlusions. The grey area (EX) indicates the brief dynamic exercise. τ (sec) is the $m\dot{V}O_2$ time constant determined by non-linear least-squares regression. *k,* is the rate constant, which is linearly related to muscle oxidative capacity ($k = (1/\tau).60$, min^{-1}).

Figure 3: **Muscle oxidative capacity (*k*) test-retest analyses.** Comparison of muscle *k* inferred from the two repetitions of muscle $\dot{V}O_2$ recovery kinetics in COPD (n = 28) and controls, CON (n = 28). Continuous line is the linear regression. Dotted line is the line of identity (x = y).

Figure 4: Bland-Altman plot of the agreement between repeated measurements of muscle $\dot{V}O_2$ recovery rate constant (*k*). Closed symbols are COPD patients (n = 28) and open symbols are controls (CON, n = 28). Horizontal dashed lines indicate the 95% limits of agreement (range -0.58, 0.64 min^{-1}).

678 **TABLES**

679

680 **Table 1:** Participant characteristics

	COPD	CON
Characteristics		
N.	28	28
Age (yrs)	65 (±8)	60 (±7) *
Weight (kg)	76 (±15)	79 (±17)
Height (cm)	171 (±11)	170 (±8)
BMI (kg/m^2)	26 (±5)	27 (±5)
Gender (M/F)	17/11	16/12
Race (AA/NHW)	5/23	15/13
FVC (L)	3.3 (±0.9)	3.5 (±0.8)
FEV_1 (L)	1.8 (±0.7)	2.8 (±0.6)
FEV_1 %pred	63.9 (±23.4)	97.9 (±13.6) *
SpO_2 (%)	97 (±1.6)	98 (±1.1)
GOLD stage N. (1/2/3/4)	7/13/5/3	
Resting Muscle Characteristics		
Saturation (TSI) (%)	66 (±6)	68 (±5)
ATT (mm)	2.3 (±1.9) ¶	2.8 (±1.9) ♮

Data are mean (±SD). CON = controls; BMI = body mass index; M = male; F = female; AA = African American; NHW = Non Hispanic White; FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1st second; SpO_2 = arterial oxygen saturation; TSI = tissue saturation index; ATT = adipose tissue thickness

* $p \leq 0.01$ vs. COPD patients; ¶ = COPD n=21; ♮ = CON n=18

681
682
683

Table 2: Reproducibility and coefficient of variation (CV) of *gastrocnemius* muscle oxidative capacity assessed by near-infrared spectroscopy, in smokers with and without COPD.

	k (min^{-1})		**CV**	**ICC**	**1st *vs*. 2nd rep**
	1st rep	**2nd rep**	%		*P*
COPD	1.45 (±0.38)	1.42 (±0.36)	9.85	0.88	N.S.
CON	1.75 (±0.70) *	1.72 (±0.70)	9.94	0.93	N.S.

Data are mean (±SD). CON = controls; k = proportional to muscle oxidative capacity; CV = coefficient of variation; ICC = intraclass correlation coefficient.

* p≤ 0.05 vs. COPD patients

690 **Table 3:** Near-infrared spectroscopy tissue saturation index (TSI) variables of the *gastrocnemius*
691 during post-contraction intermittent arterial occlusion. Mean and range of the saturation nadir
692 ($TSI_{LOW,}$ %) and relative peak muscle oxygen consumption ($m\dot{V}O_2$, $\%.s^{-1}$) during repeated tests in
693 smokers with and without COPD.
694

	1st repetition		2nd repetition		CV	
	TSI_{Low}	$m\dot{V}O_2$	TSI_{Low}	$m\dot{V}O_2$	TSI_{Low}	$m\dot{V}O_2$
	%	% s^{-1}	%	% s^{-1}	%	%
COPD	47.5 (30.4 − 65.9)	1.38 (0.66 − 3.18)	46.7 (26.2 − 65.2)	1.51 (0.37 − 3.74)	3.92	16.11
CON	47.4 (6.5 − 67.8)	1.71 (0.20 − 10.60)	42.2 (12.6 − 69.8)	1.49 (0.20 − 6.55)	5.25	17.65

Data are mean (range). CON = controls; TSI_{LOW} = lowest value reached by tissue saturation index during
695 the arterial occlusions series; $m\dot{V}O_2$ = muscle oxygen consumption
696
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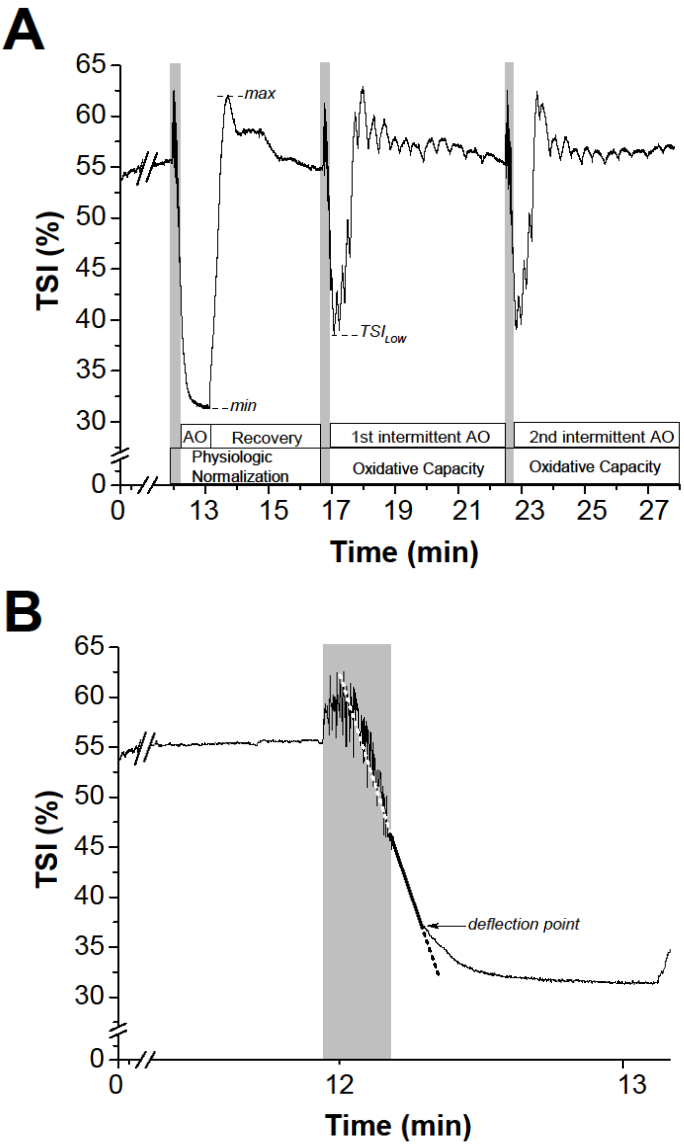
A
TSI (%)
Time (min)
max
min
TSI_Low
AO
Recovery
Physiologic Normalization
1st intermittent AO
Oxidative Capacity
2nd intermittent AO
Oxidative Capacity
B
TSI (%)
Time (min)
deflection point

Figure 1

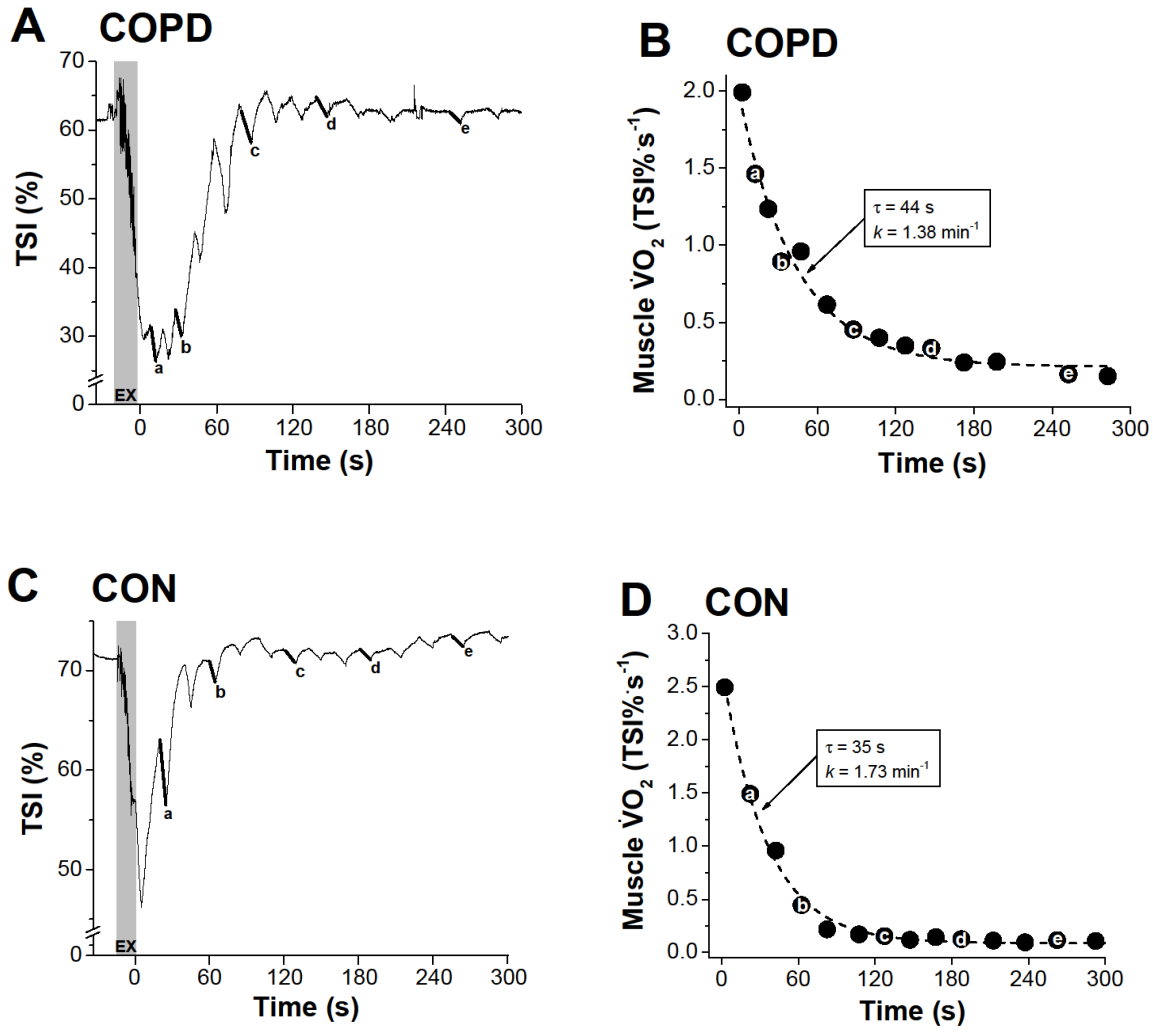
A
COPD
TSI (%)
Time (s)
EX
a
b
c
d
e
B
COPD
Muscle V̇O2 (TSI%·s-1)
τ = 44 s
k = 1.38 min-1
Time (s)
C
CON
TSI (%)
Time (s)
EX
D
CON
Muscle V̇O2 (TSI%·s-1)
τ = 35 s
k = 1.73 min-1
Time (s)

Figure 2

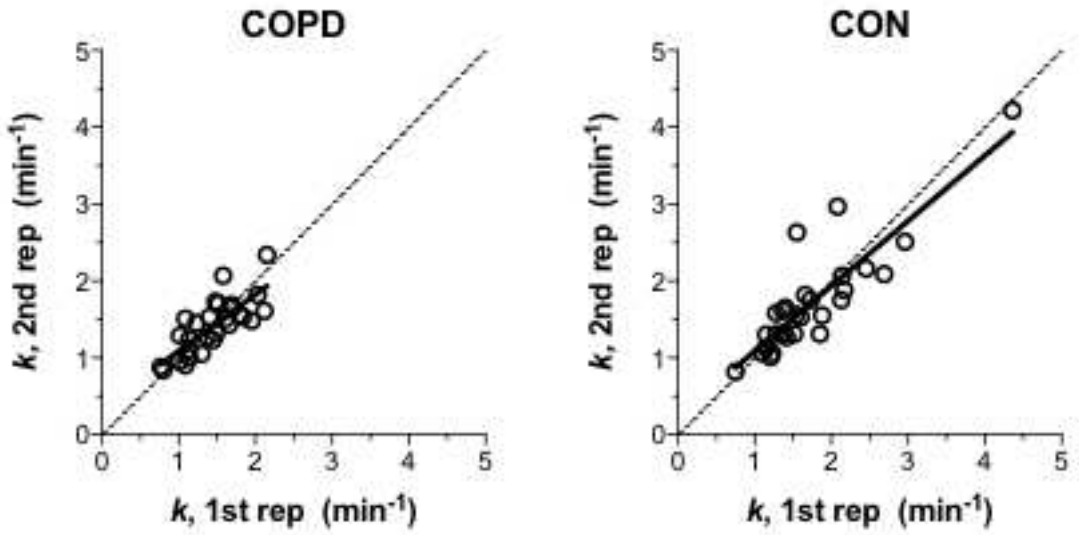

Figure 3

Figure 4

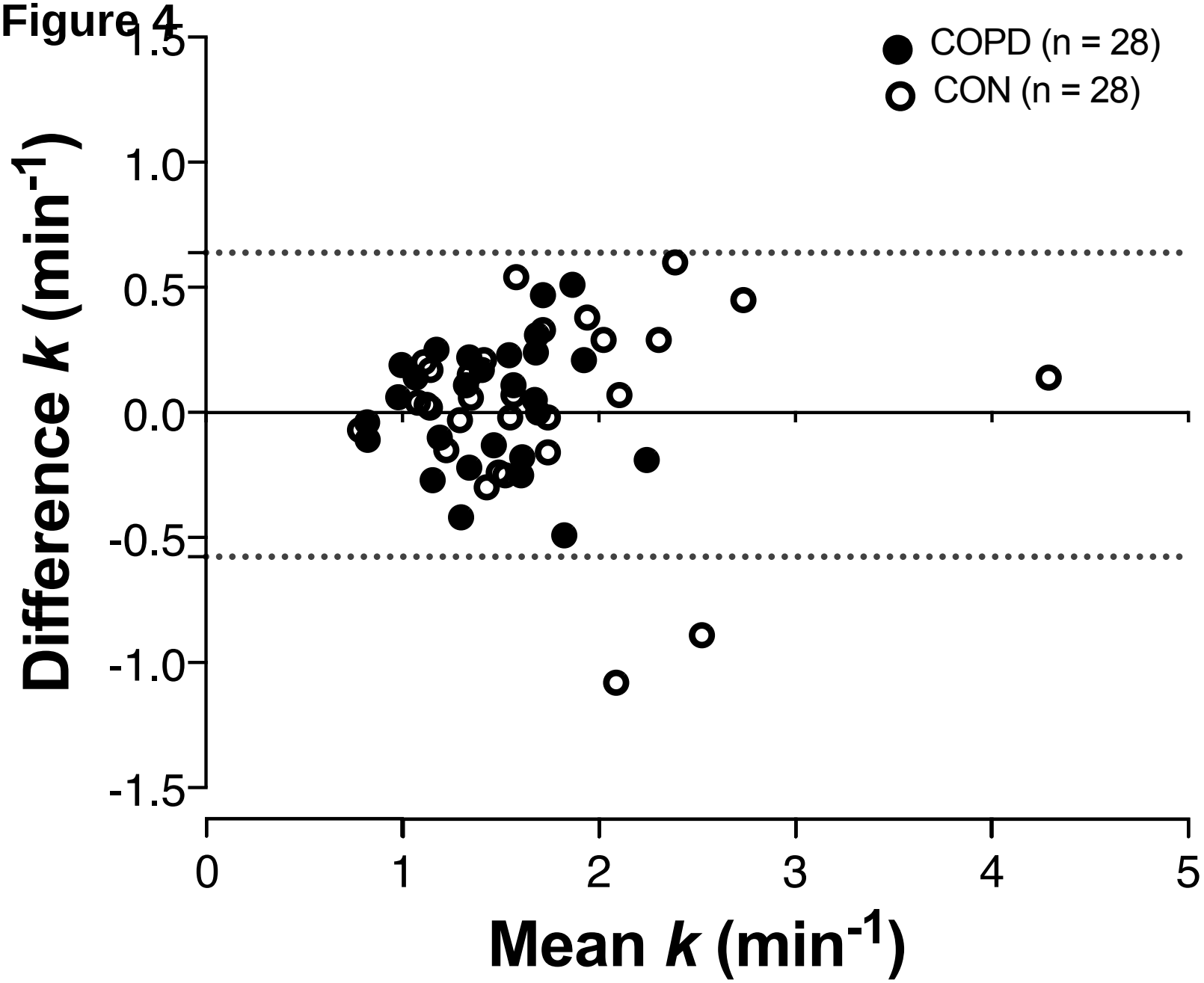

Figure 4