Muscle Oxidative Capacity Is Reduced in Both Upper and Lower Limbs in COPD

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ABSTRACT

Introduction  Skeletal muscle atrophy, weakness, mitochondrial loss and dysfunction are characteristics of chronic obstructive pulmonary disease (COPD). It remains unclear whether muscle dysfunction is localized to the lower limbs, because findings are inconsistent in the few studies where upper and lower limb muscle performance properties were compared within an individual. This study determined whether muscle oxidative capacity is low in both upper and lower limbs of COPD patients compared with controls. Methods  Oxidative capacity of the forearm and medial gastrocnemius were measured using near-infrared spectroscopy to determine the muscle O\textsubscript{2} consumption recovery rate constant \((k, \text{min}\textsuperscript{-1})\) in 20 COPD (GOLD 2/3/4, \(n=7/7/6\)) and 20 smokers with normal spirometry (CON). Muscle \(k\) is linearly proportional to oxidative capacity. Steps/day and vector magnitude units (VMU)/min were assessed using triaxial accelerometry. Differences between group and limb were assessed by 2-way ANOVA. Results  There was a significant main effect of group (\(F=11.2, \eta_p^2=0.13, P=0.001\)): \(k\) was lower in both upper and lower limb muscles in COPD (1.01±0.17, 1.05±0.24 min\textsuperscript{-1}) compared with CON (1.29±0.49, 1.54±0.60 min\textsuperscript{-1}). There was no effect on \(k\) of limb (\(F=1.8, \eta_p^2=0.02, P=0.18\)) or group x limb interaction (\(P=0.35\)). VMU/min was significantly lower in COPD (-38%; \(P=0.042\)). Steps/day did not differ between COPD (4738±3194) and CON (6372±2107; \(P=0.286\)), although the difference exceeded a clinically important threshold (>600-1100 steps/day). Conclusion  Compared with CON, muscle oxidative capacity was lower in COPD in both upper (-20%) and lower (-30%) limbs. These data suggest that mitochondrial loss in COPD is not isolated to locomotor muscles.

KEY WORDS: Mitochondria; Near-infrared spectroscopy; Exercise intolerance; Oxygen consumption; Inactivity; Dyspnea
INTRODUCTION

Skeletal muscle dysfunction is one of the most important extra-pulmonary manifestations impairing quality-of-life of patients with chronic obstructive pulmonary disease (COPD). Loss of muscle mass, strength and power, loss of type I (oxidative) muscle fibers, capillary rarefaction, mitochondrial loss and mitochondrial dysfunction have each been identified and appear to progress with COPD severity(1-6).

Investigations of muscle dysfunction in COPD typically focus on locomotor muscles, especially of the quadriceps, because of their key role in ambulation, autonomy(7) and quality of life. However, prevalence and progression of disease-associated adaptations in upper limbs or trunk muscles are less well characterized. Muscle dysfunction isolated to the lower limbs within an individual implies causal mechanisms proximal to reduced locomotion, while dysfunction of both upper and lower limbs suggests a wider range of systemic mechanisms contributing to muscular impairments, e.g. chronic inflammation, oxidative stress, hypoxemia, nutrition, cigarette smoke or other circulating variables(2, 4, 8-10).

Few studies, largely limited to functional performance measures (strength, endurance), have compared upper and lower limb muscles within individual COPD patients; and their findings are variable. Bernard et al.(11) found greater loss of strength in the lower than the upper limb of COPD patients compared with age-matched non-smoking controls. Weakness and low isometric strength was found in both upper and lower limbs of COPD patients compared with age-matched healthy controls(12-14), but biceps brachii endurance was better preserved than in the quadriceps in a large study of COPD patients(13). These findings contrast with smaller cohorts studied by
Miranda et al.(7) and Vilaró et al.(15), who showed that endurance and fatigability(7), and isometric strength(15), were more impaired in upper than lower limbs of COPD patients. Currently, there are no muscle biopsy studies comparing properties of arm and leg muscles within the same individuals. Therefore, the systemic or local cellular adaptations, that underlie strength or endurance deficits in COPD patients, are not known.

To address this, we determined upper (medial forearm) and lower (gastrocnemius) limb muscle oxidative capacity in moderate to very-severe COPD patients compared with age-matched smokers with normal spirometry (CON). We used near-infrared spectroscopy (NIRS(1)) to determine non-invasively the recovery rate constant ($k$) of muscle oxygen consumption (m$\dot{V}$O$_2$) isolated from influences of circulatory or pulmonary function. m$\dot{V}$O$_2$ recovery $k$ is directly proportional to muscle oxidative capacity measured in single muscle fibers ($r^2=0.77$). NIRS-based oxidative capacity assessment has been validated against magnetic resonance spectroscopy and biopsy in skeletal muscle(18, 19). Based on previous reports of upper and lower limb muscle endurance in COPD patients(13), we hypothesized that, compared with CON, COPD patients would have a greater deficit in muscle oxidative capacity in the gastrocnemius than in the forearm.

MATERIALS AND METHODS

**Participants**

A group of current or former smokers with at least 10 pack-years smoking history were enrolled: twenty moderate to very-severe COPD patients (GOLD stage 2-4) and twenty age- and sex-matched participants with normal spirometry (CON) (Table 1). Current or former smokers
without COPD were selected as the appropriate comparator group for COPD patients with a smoking history. This attempts to isolate the effects of COPD on muscle oxidative capacity and control for the potential influence of smoking history. It is recognized that the control group in this study do not necessarily represent a “normal” or “healthy” condition. All participants were 45 to 90 years old. Controls had pre-bronchodilator FEV$_1$/FVC $\geq 0.70$ and FEV$_1$ $\geq 80\%$predicted. COPD patients (post-bronchodilator FEV$_1$/FVC $<0.70$ and FEV$_1$ $<80\%$predicted) were in a stable state, with no exacerbation within previous 4-weeks. Exclusion criteria were: presence of significant disease other than COPD (a significant disease was defined as a disease which may: (i) put the patient at risk by participation; (ii) influence the results of the study, such as ischemic heart disease, chronic musculoskeletal or renal disease; or (iii) limit the patient’s ability comply with the protocol); active participation in pulmonary rehabilitation or participation in the past 18 months; pregnancy in women. Participants were informed about study procedures and risks and gave written informed consent. The study was approved by the Human Subjects Committee of The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center (20403-01).

**Measurements**

Each participant visited the laboratory once, during which NIRS muscle and spirometry tests were performed. Participants completed symptom (COPD Assessment Test, CAT; modified Medical Research Council Dyspnea scale, mMRC) and health-related quality of life (St. George’s Respiratory Questionnaire, SGRQ; 36-Items Short Form Health Survey, SF-36) questionnaires. The SGRQ and SF-36 physical activity-related domains were used to evaluate daily physical activity (PA). In addition, PA was objectively assessed in all COPD patients and 6 CON using 7-day triaxial accelerometry (Dynamport MoveMonitor, McRoberts BV, NL).
**NIRS muscle oxidative capacity test**

The non-dominant forearm and medial *gastrocnemius* muscles were assessed using continuous-wave, spatially-resolved spectroscopy NIRS (PortaMon, Artinis BV, NL). NIRS measures relative concentration of deoxygenated (HHb+Mb) and oxygenated (HbO$_2$+MbO$_2$) hemoglobin and myoglobin within the muscle up to ~1.5 cm under the probe. From these measurements, relative changes in total hemoglobin and myoglobin (THb = HHb+Mb+HbO$_2$+MbO$_2$) were calculated. Tissue saturation index (TSI, an index of tissue oxygen saturation) was calculated from these measurements.

With the participant supine on an exam bed, the belly of the calf and forearm muscles were identified. Palpation during a series of brief isometric muscle contractions was used to optimize placement of the plastic-wrapped NIRS probe(1) longitudinally over a highly activated region of the medial forearm and medial *gastrocnemius*. The NIRS probe was then secured in position using an elastic bandage. A rapid-inflation pressure-cuff (SC12D, Hokanson, USA) was placed on the upper arm or proximal thigh on the same limb as the NIRS probe and attached to an electronically-controlled rapid cuff-inflator (E20, Hokanson, USA). The participant was asked to relax and minimize upper limb (UL) or lower limb (LL) movement, except when instructed. Participants were familiarized with rapid cuff-inflation and to the brief muscle contractions required in the protocol. Arterial occlusion pressure was determined for both limbs from a tolerated cuff-pressure within the range of 175-275 mmHg (mean: UL = 221±24, and LL = 218±31 mmHg) that resulted in a rise in HHb+Mb, a fall in HbO$_2$+MbO$_2$ and stable THb signal over 15-20 s. Repeated dynamic muscle contractions were made at ~1 Hz by squeezing a foam tennis ball (forearm) or a plantar-flexion/re-laxation against a light resistance (lower leg) (hereafter referred to as muscle contractions).
Initially, participants lay at rest for 2-3 min to establish a stable baseline TSI. After this, ~10-15 s of light muscle contractions were performed to increase m\(\dot{V}O_2\) and the limb subjected to arterial occlusion until a stable minimum TSI was reached or for 5 min (whichever occurred first). Cuff pressure was then released and the subsequent reactive reoxygenation monitored until resting baseline was reestablished (typically ~8 min for UL, and ~3.5 min for LL). This was used to determine an individualized muscle saturation physiological range (Figure 1). Finally, two muscle oxidative capacity assessments were performed for each limb. Each consisted of: 1) ~10-15 s muscle contractions to increase m\(\dot{V}O_2\) and desaturate the muscle to ~50% of the physiological range(1); 2) a series of intermittent arterial occlusions (5 occlusions for 5 s, 10 for 10 s, each separated by 5-20 s recovery) (Figure 1). Each of the assessments lasted ~6 min and were separated by ~2 min of rest. Participants were provided ~15 min between UL and LL testing. UL was always tested first. At the end of the procedure the skinfold at the NIRS site was measured to estimate adipose tissue thickness (ATT, mm; Table 1) (Lange Skinfold Caliper, BetaTechnology Inc., Santa Cruz, CA).

For each intermittent arterial occlusion during oxidative capacity tests, the linear rate of decline in TSI (desaturation in \%s\(^{-1}\)) was determined and a point value for relative m\(\dot{V}O_2\) reported. Because the rate of tissue deoxygenation during arterial occlusion is inversely proportional to m\(\dot{V}O_2\), its value is reported as positive (\%s\(^{-1}\); Figure 2). The primary study outcome was the exponential m\(\dot{V}O_2\) recovery rate constant \(k\) (min\(^{-1}\)), which was estimated using non-linear least-squares regression (Figure 2) (OriginPro v8.6, OriginLab Co., Northampton, USA(1)). This protocol has strong test-retest reliability in smokers with and without COPD (ICC \(\geq 0.88\))(1).
**Pulse oximetry**

Arterial oxygen saturation was estimated during resting phase at the beginning of testing using fingertip pulse oximetry (SpO2; Rad-5 Pulse Oximeter MasimoSET®, Masimo Co., Irvine, CA).

**Spirometry**

Participants inhaled two puffs of metered dose albuterol sulfate ~15 min before spirometric testing. Spirometry was performed in accordance with American Thoracic Society guidelines using a dual-beam Doppler ultrasound-based spirometer (EasyOne Pro, Ndd Medical, Zürich, Switzerland). Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) were measured from the greatest FEV1 and FVC from up to eight maximum expiratory maneuvers, where the greatest two measurements were within 150 mL.

**Physical activity by triaxial accelerometry**

All COPD patients and 6 CON underwent 7 days of PA monitoring using triaxial accelerometry (Dynaport MoveMonitor, McRoberts BV, NL). Each participant was instructed in the correct positioning of the monitor in small of the back, and to adjust the elastic waistband to ensure the device was in contact with the body and comfortable. Participants were asked to wear the PA monitor for as long as possible during each 24-hour period, and to remove it only for bathing and swimming. The PA monitor was worn from the end of the study visit until 7 full days had elapsed. Data were processed using the manufacturer’s protocols.
Daily PA measurements were accepted as valid if they met the criteria that the monitor was worn for at least 8 hours/day during waking hours (20) and for at least 5 days/week (not necessarily consecutive, without distinction between weekdays and weekends). Compliance with these conditions was ~94%. PA is reported as the mean number of steps per day (total accumulated during each 24 hour period), and as the mean count of vector magnitude units per minute (VMU/min) during “daytime” hours between 8 AM and 11 PM. VMU is the vectorial sum of movements measured at 100Hz from acceleration in the three orthogonal planes (sagittal, frontal, transversal) measured over a one-minute time period. The acceleration signal is converted to a digital representation and processed to obtain an “activity count”, i.e. VMU (for additional details see e.g.(21)).

Statistics

Coefficient of variation (CV) and intraclass correlation coefficient (ICC) assessed within-subject test-retest reproducibility. Two-way ANOVA was used to identify differences between group (COPD and CON) and limb (UL and LL). Associations were investigated by regression (Pearson). Significant differences were accepted at \( P \leq 0.05 \). Results are presented as mean ± SD, unless otherwise specified.

RESULTS

Participant characteristics

One COPD patient could not tolerate UL arterial occlusion, and therefore the patient and the corresponding age- and sex-matched control were excluded from further analysis. Results are reported from 19 COPD and 19 CON. As intended, groups were matched by age and body characteristics (Table 1). Four COPD patients required nasal cannula \( O_2 \) during the visit (at 2 L.min\(^{-1}\)).
**Symptoms, physical activity and quality of life**

COPD patients had significantly greater CAT and mMRC scores than CON, confirming greater dyspnea in patients (Table 2). Significantly greater (worse) SGRQ total and subscale scores, and lesser SF-36 physical and general health component aggregate scores, confirmed lower quality of life in COPD than CON (Table 2). COPD reported lower scores than CON in physical functioning and role-physical scales of SF-36 (P<0.001), suggesting reduced capacity for performing daily and physical activities.

Triaxial accelerometry wearing compliance was met on 6 days (≥15 hours/day) on average in both in COPD and CON (Table 2B), except for one GOLD 1 patient who withdrew on day 2. Therefore, results are presented for 6 individuals in each group. The characteristics of sub-group of 6 controls who undertook accelerometry assessment were: 5 males, 1 female; 56±26 years; 1.70±0.60 m; 66.0±32.5 kg; FEV₁ 2.44±1.13 L; FVC 3.20±1.53 L. VMU/min was significantly lower (P=0.024) in COPD than CON. Although steps/day were not significantly different between COPD and CON (P=0.286, Table 2B), the mean difference exceeded the clinically important difference established for COPD (600-1100 steps/day(22)).

**Upper and lower limb muscle oxidative capacity**

Resting muscle TSI and adipose tissue thickness of the upper and lower extremities were not significantly different between COPD and CON (Table 3). There was no significant difference between the first and second k measurements within individuals for either limb; therefore, the average of the two repeated k values are reported. Test-retest reliability was high for upper and
lower limb $k$ values in both groups (COPD UL: CV=3.8%, ICC=0.96; LL: CV=4.6%, ICC=0.96; and CON UL: CV=5.4%, ICC=0.98; LL: CV=4.5%, ICC=0.98).

There was main effect of group on $k$ ($F=11.2$, $\eta_p^2=0.13$, $P=0.001$) but not of limb ($F=1.8$, $\eta_p^2=0.02$, $P=0.18$). There was no group-by-limb interaction ($P=0.35$). Compared with CON, COPD patients had significantly lower $k$ in upper (~20%) and lower (~30%) limbs (Table 3, Figure 3). There was no significant association between measures of physical activity (VMU/min; steps/day) and $k$ in the lower limbs of COPD ($r^2=0.06-0.13$; $P>0.05$) or CON ($r^2=0.17-0.25$; $P>0.05$).

**DISCUSSION**

This is the first study to determine skeletal muscle oxidative capacity in both upper and lower extremities of current or former smokers with or without COPD. Given the large volume of muscle biopsy data showing low muscle oxidative capacity in moderate to severe COPD patients, we hypothesized that lower limb muscle oxidative capacity would be more severely impaired, compared with matched smoker controls, than the upper limb. Contrary to our hypothesis, there was no interaction between group and limb for muscle $k$. We found COPD patients had substantially lower muscle oxidative capacity (20-30%) in both locomotor and forearm skeletal muscles compared with smokers without pulmonary obstruction (Table 3, Figure 3). This deficit in muscle oxidative capacity in COPD was accompanied by a lower VMU/min and clinically important (but not statistically significant) reduction in steps/day in COPD. However, there were only weak associations between $k$ and physical activity (VMU/min or steps/day) in the lower limb ($r^2=0.06-0.25$). Together, the findings of low oxidative capacity in upper limb and low oxidative capacity
that was only weakly associated with activity in the lower limb, in COPD patients and controls well matched for smoking history, age and sex, suggest a systemic deficit in muscle oxidative capacity in COPD patients that is not well explained by a low volume and/or intensity of physical activity alone.

**Upper and lower limb muscle oxidative capacity in COPD**

The severity of the muscle oxidative capacity deficit in the lower limbs of COPD patients (~20-30%) is similar to reports from biopsy studies of the quadriceps (~10-50%) (23). There is only one previous report of muscle biopsy analysis from the upper limb (deltoid) of 10 COPD patients, and this showed a trend (P=0.07) for lower citrate synthase activity compared with controls (24). The ~20-30% lower muscle oxidative capacity in COPD would likely translate into similar reduction in oxidative ATP synthesis in COPD (5, 25-27), but could be impacted further should mitochondrial uncoupling be increased (e.g., to mitigate oxidative stress).

In humans, the medial gastrocnemius has a greater expression of type I fibers than the forearm muscles (28, 29), where muscle fiber characteristics favor acute strength and precision of movement over muscle endurance. Because of this, we anticipated that, overall, the lower limb muscles would have a greater oxidative capacity than the upper limb. However, we did not identify a statistical effect of limb, potentially because the study was underpowered for this comparison. The study was powered on the basis of anticipated differences of the primary outcome ($k$) between COPD and controls, and achieved a power (1-β) of 0.89 to detect this large effect (Cohen’s d=1.07) (G*power 3.1). While there was a trend towards an effect of limb in controls (lower limb $k = 1.54\pm0.60$ min$^{-1}$ vs. upper limb $k = 1.29\pm0.49$ min$^{-1}$), the effect size was small (d=0.46) and the
power for this comparison was low (1-β=0.28), suggesting the potential for type II error (false negative interpretation). We estimate that 150 participants would be needed to determine with a power of 1-β=0.8 whether muscle oxidative capacity in middle-age smoker controls differed between upper and lower limbs.

However, we were surprised that muscle oxidative capacity was very similar in upper and lower limb muscles in COPD, and was lower in both muscles than even the upper limb muscles in controls. This highlights the severity of the profound loss of mitochondrial function in the ambulatory muscles of COPD patients, given that forearm muscles typically contain far more type II muscle fibers and are far more fatigable. It also suggests a systemic mechanism may contribute strongly to whole-body mitochondrial loss in COPD(30).

Support for this concept can be found in the study by Rabinovich et al.(31), who reported that reduced mitochondrial density and function (coupling of oxidative phosphorylation) was strongly related with PaO$_2$ and early lactate release during exercise. Circulating cigarette smoke constituents (e.g. reactive aldehydes), oxidative stress and/or systemic inflammation and cytokine expression may impair mitochondrial dynamics or biogenesis(4, 32), or impair the neuromuscular junction(33), interrupting calcineurin signaling and other calcium-sensitive cellular mediators, each contributing to oxidative capacity loss in muscle. While cigarette smoke constituents have been implicated in directly inhibiting oxidative phosphorylation (e.g. by inhibition of cytochrome c oxidase), studies to investigate this in humans have been inconsistent in their findings(32). In a previous NIRS study of 39 smokers without COPD, we were unable to identify a direct effect of
current smoking on muscle oxidative capacity(34). However, the degree to which the loss of skeletal muscle oxidative capacity is pathological or a consequence of mitochondrial functional phenotype adaptations induced by muscle fiber switch and physical inactivity is still unclear(4, 35).

Several studies have reported a preservation of type I fiber number, strength(24, 36) or endurance(13) in upper limb muscles of COPD patients, suggesting that muscle structural alterations are not homogeneously distributed among different muscle groups in COPD(4, 23). One interpretation of this is that reduced ambulation and consequent muscular deconditioning is a primary driver of the low oxidative muscle phenotype in the legs of COPD patients, while upper limb muscles may be better protected through engagement in activities of daily living. Although our physical activity data predominantly reflect activity of the lower limb muscles, overall our data do not support this proposal. Rather they suggest a systemic loss of the oxidative muscle phenotype in COPD(4, 23, 30, 35, 37).

Other structural changes, particularly loss of muscle mass, which is a strong predictor of mortality in COPD(4), has been implicated in reduced endurance (directly related to oxidative capacity) and strength in COPD patients. Franssen et al.(13) compared quadriceps and biceps brachii muscle strength and endurance in COPD patients with and without muscle mass depletion and found a significant reduction in strength only in the lower limbs of those who had lost fat free mass (FFM). However, the authors reported that this difference disappeared after correcting for FFM(13), suggesting that the loss of FFM contributes to muscle weakness in COPD but other factors should be considered to explain endurance impairments in COPD. Although we did not
assess muscle mass or FFM in our study, we sought stable COPD patients without recent exacerbations or uncontrolled weight loss in the previous 6 months, and BMI was not different from controls on average (24±4 kg/m²). Although BMI is a crude index, 6 COPD patients in our study had BMI <21 kg/m² (range 18.2 to 20.3 kg/m²; GOLD 2/3/4 n=2/3/1), suggested as the lower limit of normal for chronic disease patients(4). Nevertheless, the mean $k$ in this sub-group (UL = 0.91±0.12 min⁻¹; LL = 1.05±0.24 min⁻¹) was not significantly different from the COPD group as a whole, suggesting that low oxidative capacity is not a characteristic solely of underweight or undermuscled subjects. Our findings appear relevant to a general COPD population rather than those with overt weight loss or muscle weakness.

The levels of physical activity we found in our COPD patients were similar to those reported in literature (38-40), while our smoker-controls were less active than reports for similar middle-aged individuals (e.g. ~9,000 steps/day; c.f. Table 2). The groups also differed in pulmonary function (by design), dyspnea and self-reported physical functioning and quality-of-life (Table 2) and one objective measure of activity (VMU/min), but were otherwise not different in smoking history, age, or sex. While we also found no significant difference in steps/day between COPD and CON (P=0.286), the mean difference (1634 steps/day; an effect size of d=0.60) exceeded established clinical importance (600-1100 steps/day) that corresponds to a reduced risk of hospitalization(22) and morbidity. This suggests that physical activity is lower in COPD than controls in our study by a clinically relevant magnitude, and it is likely that the study is underpowered to detect a statistically significant difference between COPD and controls in the secondary outcome of steps/day (1-β=0.44). Nevertheless, we found no association between objectively measured physical activity and $k$, suggesting that factors in addition to – or, perhaps, other than – physical
activity are responsible for low oxidative capacity in muscles of COPD patients. Further study of a larger cohort is warranted to investigate this suggestion.

**Study limitations**

We used non-invasive assessment of muscle oxidative capacity to allow two muscle sites to be investigated. This assessment is reliable and has been validated against gold standard methods (biopsy and magnetic resonance spectroscopy). Nevertheless, this assessment is indirect and does not provide a detailed picture of intramyocyte signaling processes that contribute to mitochondrial loss. We did not assess muscle mass, FFM, muscle endurance or strength in this study, to provide insight into how muscle oxidative capacity loss associates with muscle quantity or performance. This study was also relatively small, enrolling no more than seven subjects per each GOLD 2 to 4 category. Therefore, the study was not sufficiently powered to identify an effect of disease severity on upper and lower limb muscle oxidative capacity. The two NIRS-investigated sites are representative of skeletal muscles involved in daily activities (i.e. grasping or carrying for the forearm and locomotion for the gastrocnemius); the selection of the muscle sites is limited by the NIRS-based technique, which requires the ability to implement repeated arterial occlusions(41). Therefore, a wider systemic assessment of respiratory abdominal or vertebral muscles is not possible with this method. We used accelerometry to provide an objective evaluation of the daily physical activity, which predominantly represents activity of the lower limbs, therefore upper limb activity remains unmeasured in our study.
Clinical implications and future perspectives

Low oxidative capacity in the locomotor muscle is associated with exercise intolerance and therefore may contribute to inactivity, poor quality-of-life and represents a strong therapeutic target(4, 23). It is known that locomotor muscles respond well to endurance exercise training by increasing oxidative capacity, even in COPD patients(4, 13). However, if muscle mitochondrial loss is systemic in COPD - and perhaps even not limited to the muscle tissues - then exercise training would be expected to ameliorate this deficit only in the muscles engaged by training, but not in a wider range of locations or tissues. Ameliorating systemic mitochondrial dysfunction may need additional, possibly pharmaceutical, therapies as an adjunct to exercise training in COPD patients. Systemic mitochondrial loss could contribute to immune system or liver dysfunction and cardiovascular disease, which are each prevalent in COPD patients. As such, the NIRS we used for this study may represent a relatively simple and non-invasive method to investigate the severity of systemic mitochondrial dysfunction and response to therapy. Future studies with a larger cohort are needed to identify whether severity of the pulmonary and/or physical activity impairments are associated with oxidative capacity loss of the upper and lower limbs in COPD. Comprehensive evaluation of exercise capacity and isolated muscle structure and function are needed to identify the clinical correlates and physiological mediators underlying these observations.

CONCLUSION

We found that muscle oxidative capacity was low in both upper (forearm) and lower (medial gastrocnemius) limb muscles of moderate to very-severe COPD patients compared with sex and age-matched smokers with normal spirometry. Low oxidative capacity was not limited to the lower limbs or to those with COPD. Low muscle oxidative capacity in COPD may contribute to
the greater respiratory symptoms and reduced muscle endurance and quality of life in COPD patients. These data imply that low oxidative capacity in leg muscles of COPD patients may not solely relate to reduced ambulation and deconditioning, but that systemic factors e.g. inflammation or oxidative stress, are likely strong mediators loss of mitochondrial oxidative phenotype.
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CONFLICTS OF INTEREST

Authors have no conflicts to declare. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The study results do not constitute endorsement by ACSM. A.A. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
References


FIGURE LEGENDS

**Figure 1.** Changes in tissue saturation index (TSI) during the NIRS muscle test. Three protocol phases are shown. The grey shading indicates muscle contractions, always preceded by a resting (i.e. baseline) phase. Data are from the medial *gastrocnemius* muscle of a control individual.

**Figure 2.** Representative COPD and control (CON) responses for the muscle oxidative capacity assessments. Muscle oxygen consumption (mVO₂) recovery following brief contractions is shown, with a mono-exponential fit (dashed line), for the upper (medial forearm muscle, left) and lower (medial *gastrocnemius*, right) limb. COPD, chronic obstructive pulmonary disease; UL, upper limb; LL, lower limb; TSI, tissue saturation index; τ, mVO₂ recovery time constant; k, mVO₂ recovery rate constant (k = (1/τ).60, min⁻¹); CON, control.

**Figure 3.** Median and interquartile ranges of medial forearm (upper limb, UL) and medial *gastrocnemius* (lower limb, LL) skeletal muscle VO₂ recovery rate constant in COPD and control (CON) group. Dotted line indicates the group mean. k, mVO₂ recovery rate constant; UL, upper limb; LL, lower limb; COPD, chronic obstructive pulmonary disease; CON, control; *, main effect of condition (COPD vs CON), $P \leq 0.001$. 
Figure 2

**COPD - UL**

- $\tau = 94 \text{ s}$
- $k = 0.64 \text{ min}^{-1}$

**COPD - LL**

- $\tau = 85 \text{ s}$
- $k = 0.70 \text{ min}^{-1}$

**CON - UL**

- $\tau = 43 \text{ s}$
- $k = 1.41 \text{ min}^{-1}$

**CON - LL**

- $\tau = 45 \text{ s}$
- $k = 1.34 \text{ min}^{-1}$
Figure 3
Table 1. Participant characteristics.

<table>
<thead>
<tr>
<th></th>
<th>COPD</th>
<th>CON</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.</td>
<td>19</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64 (±9)</td>
<td>62 (±7)</td>
<td>0.44</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72 (±17)</td>
<td>78 (±17)</td>
<td>0.23</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171 (±10)</td>
<td>170 (±10)</td>
<td>0.76</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 (±4)</td>
<td>27 (±4)</td>
<td>0.06</td>
</tr>
<tr>
<td>Sex (M / F)</td>
<td>14 / 5</td>
<td>14 / 5</td>
<td>-</td>
</tr>
<tr>
<td>Race (AA / NHW)</td>
<td>5 / 14</td>
<td>11 / 8</td>
<td>-</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>3.1 (±0.9)</td>
<td>3.7 (±0.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>1.6 (±0.8)</td>
<td>2.8 (±0.6)</td>
<td>0.0001</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>47.3 (±15.2)</td>
<td>75.6 (±4.0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>FEV₁ %pred</td>
<td>44.1 (±18.7)</td>
<td>97.1 (±12.1)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Resting SpO₂ (%)</td>
<td>97 (±1.6)*</td>
<td>97 (±1.3)</td>
<td>0.39</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ATS pack.years)</td>
<td>37 (±15)</td>
<td>38 (±19)</td>
<td>0.41</td>
</tr>
<tr>
<td>GOLD stage (2 / 3 / 4)</td>
<td>7 / 6 / 6</td>
<td>0 / 0 / 0</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are mean (± SD). COPD, chronic obstructive pulmonary disease patients. CON, controls. BMI, body mass index. M, male. F, female. AA, African American. NHW, Non-Hispanic White. FVC, forced vital capacity. FEV₁, forced expiratory volume in 1 second. SpO₂, arterial oxygen saturation from finger pulse oximetry. GOLD, Global Initiative for Chronic Obstructive Lung Disease.

* Four COPD patients required nasal cannula O₂ during the visit (at 2 L.min⁻¹)
Table 2. Symptoms and health-related quality of life questionnaire scores (A); Physical activity triaxial accelerometry monitoring (B).

<table>
<thead>
<tr>
<th></th>
<th>COPD</th>
<th>CON</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>COPD Assessment test (CAT)</strong></td>
<td>16 [6-29]</td>
<td>5 [0-18]</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td><strong>Modified MRC Dyspnea Scale (mMRC)</strong></td>
<td>2 [0-4]</td>
<td>0 [0-1]</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td><strong>St. George's Respiratory Questionnaire (SGRQ) score:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>36 [11-73]</td>
<td>6 [0-48]</td>
<td><strong>0.0002</strong></td>
</tr>
<tr>
<td>Active</td>
<td>48 [12-100]</td>
<td>12 [0-66]</td>
<td><strong>0.0001</strong></td>
</tr>
<tr>
<td>Symptom</td>
<td>30 [0-56]</td>
<td>7 [0-48]</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>Impacts</td>
<td>31 [0-80]</td>
<td>0 [0-39]</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td><strong>Short Form Health Survey (SF-36) total score:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental Component Summary</td>
<td>46 [35-63]</td>
<td>51 [33-61]</td>
<td>0.405</td>
</tr>
<tr>
<td>Physical Component Summary</td>
<td>39 [22-55]</td>
<td>55 [34-62]</td>
<td><strong>0.0006</strong></td>
</tr>
<tr>
<td>Physical Functioning Scale</td>
<td>38 [19-55]</td>
<td>53 [28-57]</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Role-Physical Scale</td>
<td>37 [18-57]</td>
<td>57 [35-57]</td>
<td><strong>0.0002</strong></td>
</tr>
<tr>
<td>Body Pain Scale</td>
<td>55 [37-62]</td>
<td>62 [33-61]</td>
<td>0.64</td>
</tr>
<tr>
<td>General Health Scale</td>
<td>39 [26-53]</td>
<td>46 [38-63]</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Days of monitoring (n)</strong></td>
<td>6.2 (±0.4)</td>
<td>6.0 (±0.0)</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>Daytime VMU/min (count)</strong></td>
<td>300 (±170)</td>
<td>481 (±113)</td>
<td><strong>0.042</strong></td>
</tr>
<tr>
<td><strong>Steps per day (n)</strong></td>
<td>4738 (±3194)</td>
<td>6372 (±2107)</td>
<td>0.286</td>
</tr>
</tbody>
</table>

**A.** Questionnaire scores are median [range min-max]. **B.** Data are mean (±SD). COPD, chronic obstructive pulmonary disease. CON, controls. VMU = vector magnitude units. GOLD, Global Initiative for Chronic Obstructive Lung Disease. Questionnaire SF-36: The Mental and Physical Component Summary are aggregate scores.
Table 3. Skeletal muscle characteristics.

<table>
<thead>
<tr>
<th></th>
<th>COPD</th>
<th>CON</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upper Limb</strong> (R/L)</td>
<td>3 / 16</td>
<td>4 / 15</td>
<td></td>
</tr>
<tr>
<td>Saturation (TSI) (%)</td>
<td>66 (±6)</td>
<td>67 (±7)</td>
<td>0.22</td>
</tr>
<tr>
<td>ATT (mm)</td>
<td>3.5 (±1.1)</td>
<td>4.1 (±2.2)</td>
<td>0.28</td>
</tr>
<tr>
<td>$k$ (min$^{-1}$)</td>
<td>1.01 (±0.17)</td>
<td>1.29 (±0.49)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Lower Limb</strong> (R/L)</td>
<td>18 / 1</td>
<td>16 / 3</td>
<td></td>
</tr>
<tr>
<td>Saturation (TSI) (%)</td>
<td>67 (±4)</td>
<td>68 (±5)</td>
<td>0.23</td>
</tr>
<tr>
<td>ATT (mm)</td>
<td>4.5 (±2.8)</td>
<td>4.8 (±2.1)</td>
<td>0.77</td>
</tr>
<tr>
<td>$k$ (min$^{-1}$)</td>
<td>1.05 (±0.24)</td>
<td>1.54 (±0.60)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are mean (±SD). COPD, chronic obstructive pulmonary disease patients. CON, controls. R, right. L, left. TSI, tissue saturation index. ATT, adipose tissue thickness. $k$, rate constant of muscle oxygen consumption recovery kinetics. GOLD, Global Initiative for Chronic Obstructive Lung Disease.