Oxygen delivery-utilization mismatch in contracting locomotor muscle in COPD:

peripheral factors

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Abstract
Central cardiorespiratory and gas exchange limitations imposed by chronic obstructive pulmonary disease (COPD) impair ambulatory skeletal muscle oxygenation during whole-body exercise. This investigation tested the hypothesis that peripheral factors *per se* contribute to impaired contracting lower limb muscle oxygenation in COPD patients. Submaximal neuromuscular electrical stimulation (NMES; 30, 40 and 50 mA at 50 Hz) of the *quadriceps femoris* was employed to evaluate contracting skeletal muscle oxygenation whilst minimizing the influence of COPD-related central cardiorespiratory constraints. Fractional O\(_2\) extraction was estimated by near-infrared spectroscopy (deoxy-hemoglobin/myoglobin concentration; deoxy-[Hb/Mb]) and torque output was measured by isokinetic dynamometry in 15 non-hypoxemic patients with moderate-to-severe COPD (Sp\(O_2\)=94±2%; FEV\(_1\)=46.4±10.1%; GOLD II and III) and 10 age- and gender-matched sedentary controls. COPD patients had lower leg muscle mass than controls (LMM=8.0±0.7 kg vs. 8.9±1.0 kg, respectively; p<0.05) and produced relatively lower absolute and LMM-normalized torque across the range of NMES intensities (p<0.05 for all). Despite producing less torque, COPD patients had similar deoxy-[Hb/Mb] amplitudes at 30 and 40 mA (p>0.05 for both) and higher deoxy-[Hb/Mb] amplitude at 50 mA (p<0.05). Further analysis indicated that COPD patients required greater fractional O\(_2\) extraction to produce torque (i.e., ↑Δdeoxy-[Hb/Mb]/torque) relative to controls (p<0.05 for 40 and 50 mA) and as a function of NMES intensity (p<0.05 for all). The present data obtained during submaximal NMES of small muscle mass indicate that peripheral abnormalities contribute mechanistically to impaired contracting skeletal muscle oxygenation in non-hypoxemic, moderate-to-severe COPD patients.
Introduction

Reduced physical capacity is a hallmark of patients suffering from chronic obstructive pulmonary disease (COPD; refs. 20, 37). A complex interplay of central and peripheral mechanisms has been ascribed to contribute to the early onset of fatigue in these patients (1, 14, 38). Notwithstanding inherent cardiorespiratory abnormalities, mounting evidence suggests a role for skeletal muscle dysfunction in limiting exercise in COPD (20).

Mechanical efficiency, defined as the chemical conversion of energy to external mechanical work, has been reported previously to be reduced in COPD (2, 43). Recent evaluations of skeletal muscle energetics using $^{31}$P-magnetic resonance spectroscopy ($^{31}$P-MRS) revealed that, despite the presence of preserved mitochondrial function, patients with COPD exhibit greater ATP cost of muscle contraction when compared to age- and physical activity-matched healthy counterparts (29, 30). This implies that a given absolute work rate would require greater energy supply to match the metabolic demand of skeletal muscles of COPD patients. Furthermore, it is important to note that COPD is associated with peripheral vascular dysfunction, a condition that likely impairs contracting muscle blood flow and thus $O_2$ delivery (6, 12, 17, 27). However, it remains unclear whether peripheral skeletal muscle dysfunction *per se* plays a mechanistic role in the mismatch between $O_2$ delivery and utilization ($\dot{Q}O_2$ and $\dot{V}O_2$, respectively) during transitions in metabolic demand in COPD. As dictated by Fick’s law of diffusion, a $\dot{Q}O_2/\dot{V}O_2$ imbalance lowers the driving force for blood-myocyte $O_2$ transfer (microvascular partial pressure of $O_2$; $PO_2$mv) and contributes significantly to impaired oxidative metabolism and contractile performance (25, 47, 53). Resolution of this issue is therefore essential to understanding muscle metabolic control and (poor) exercise capacity in COPD and facilitate the development of novel and effective therapeutic strategies.

It is essential to acknowledge that previous studies utilizing high intensity whole-body exercise (such as supra-gas exchange threshold cycling exercise; ref. 11) have not been designed to examine the potential contribution of peripheral dysfunction to contracting skeletal muscle $\dot{Q}O_2/\dot{V}O_2$ mismatch in COPD. Such experimental protocols employ exercise modalities which recruit a large muscle mass and, consequently, are constrained by central cardiorespiratory limitations imposed by COPD (e.g., dynamic hyperinflation, expiratory flow limitation and augmented respiratory muscle recruitment). Consistent with this notion, interventions aimed at ameliorating these central limitations in COPD (via heliox, pharmacological bronchodilators and
respiratory muscle unloading; refs. 8, 10, 11, see also refs. 32, 44) are capable of improving skeletal muscle $\dot{Q}O_2/\dot{V}O_2$ balance during high intensity cycling exercise. These studies provide a mechanistic link between central, but not peripheral, limitations characteristic of COPD and contracting skeletal muscle $\dot{Q}O_2/\dot{V}O_2$ mismatch. However, due to the multifactorial nature of exercise intolerance in COPD, a different experimental approach is required to explore the potential contribution of peripheral factors in determining skeletal muscle $\dot{Q}O_2/\dot{V}O_2$ mismatch during submaximal contractions \textit{in vivo}.

Electrically-evoked contractions allow the investigation of skeletal muscle function whilst minimizing the influence of COPD-related central cardiorespiratory constraints and potential motivational bias (36, 51). Therefore, the purpose of the current investigation was to examine the dynamic (mis)matching between skeletal muscle $\dot{Q}O_2$ and $\dot{V}O_2$ (non-invasive frequency-modulated near-infrared spectroscopy; NIRS) across a range of submaximal contractions elicited via neuromuscular electrical stimulation (NMES) in non-hypoxemic, stable moderate-to-severe COPD patients. It was hypothesized that, compared to age- and gender-matched sedentary controls, COPD patients would manifest marked $\dot{Q}O_2/\dot{V}O_2$ mismatch and, therefore, greater changes in skeletal muscle deoxygenation (i.e., NIRS-derived deoxy-hemoglobin/myoglobin concentration; deoxy-[Hb/Mb], an index of fractional O$_2$ extraction) during submaximal electrically-evoked contractions. Empirical validation of this hypothesis would provide novel evidence that peripheral abnormalities \textit{per se} contribute to contracting muscle oxygenation deficits in COPD patients.
Methods

Fifteen males (aged >60 yr, body mass index <30 kg/m$^2$) with COPD according to the GOLD criteria (GOLD; ref. 20) were referred from the outpatient clinic of the São Paulo Hospital (Federal University of São Paulo, Brazil) for study participation. Patients were non-entitled to long-term or exertional O$_2$ supplementation and presented with moderate-to-severe airway obstruction; i.e., forced expiratory volume in one second (FEV$_1$)/forced vital capacity (FVC) <0.7 and post-bronchodilator FEV$_1$ between 30 and 80% predicted (GOLD spirometric stages II and III). No patient had been previously enrolled in a pulmonary rehabilitation program. All patients were clinically stable as indicated by no change in medical therapy (including oral corticosteroid use) or exacerbation of symptoms within the preceding 12 weeks. Current COPD treatment included short- and long-acting bronchodilators combined or not with inhaled corticosteroids. Main exclusion criteria were: locomotor or neurological deficits; malignancy; cardiac failure; insulin-dependent diabetes mellitus; distal arteriopathy; α1-antiprotease deficiency; recent surgery; endocrine, hepatic or renal disorder; anticoagulant or oral corticosteroid use. Ten age-matched, never-smoking males with no cardiovascular, respiratory or metabolic diseases served as controls. Healthy non-smoking participants had not undertaken any regular physical activity in the preceding year. The Research Ethics Committee of Federal University of São Paulo approved the study and informed consent was obtained from all participants.

Regular physical activity

The Baeccke’s Questionnaire was administered to obtain an index of regular physical activity (3). The questionnaire includes 16 questions involving three domains: 1) occupational physical activity (8 questions); 2) physical exercise in leisure time (4 questions); 3) leisure and locomotion-related physical activities (4 questions). The total score is the arithmetic mean of the domain scores.

Leg muscle mass

Dominant leg muscle mass was measured (from the gluteal furrow to the minimum circumference above the ankle) using dual energy X-ray absorptiometry (DEXA; Lunar DPX IQ,
Lunar Radiation Corp.; Madison, WI, USA). Leg muscle mass (LMM; expressed in kg) was then obtained as the limb mass minus the sum of fat and bone mass.

**Spirometry**

Spirometric tests were performed using the CPFTM system (Medical Graphics Corporation; St. Paul, MN, USA) with airflow being measured by a calibrated pneumotachograph. Patients completed at least three acceptable maximal forced expiratory maneuvers before and after 400 µg of inhaled salbutamol; in the present communication, only post-bronchodilator data are reported. FVC and FEV1 were recorded and expressed as percent of the predicted value.

**Knee extensor muscle torque evaluation**

Isometric knee extension torque development of the dominant leg was assessed using an isokinetic dynamometer (Con-Trex™, CH 8046; Zurich, Switzerland) both in response to maximal voluntary and electrically-evoked contractions. Positioning and stabilization of each subject were standardized. The mechanical axis of rotation of the lever arm was aligned to the axis of rotation of the knee. The resistance pad at the end of the lever arm was strapped to the distal part of tibia; the exact position varied according to the subject’s leg length. Correction for the effect of gravity on neuromuscular performance was made by incorporating limb mass to the calculation of torque production. This was done prior to experimental procedures by fixing the dominant leg to the lever arm positioned at 100° for 5 s while muscles remained relaxed and the limb rested on support. Subsequently, subjects performed two maximal isometric tests separated by a 5 min resting period. During the voluntary isometric tests, subjects were asked to sustain the lever arm at 100° for 5 s while the force applied was recorded instantaneously (peak torque expressed in Nm). For the electrically-evoked contractions, subjects were specifically told to avoid any concomitant voluntary muscle activation.

**Electrical stimulation protocol**

Knee extensor muscles (quadriceps femoris) of the dominant leg were electrically stimulated (Dualpex 961™, Quark; Brazil) with the subjects seated comfortably on the isokinetic dynamometer and with the NIRS electrodes placed on the thigh (see below). Four NMES
Electrodes were placed on the: (1) middle third and (2) proximal portion of the vastus lateralis belly; (3) middle third of the vastus medialis; and (4) proximal portion of the rectus femoris. Electrode placement varied slightly among subjects to evoke maximum visible contractions. A 400 μs-wide, pulsed, biphasic and symmetric rectangular current at 50 Hz was used. Stimulations were applied three times at each progressively higher intensity (order: 3x30, 3x40, and 3x50 mA) with an “on-off” cycle of 20:120 s. Each set was separated by a 5 min resting period. A priori analysis revealed no significant differences among the variables of interest among the 3 consecutive contractions at a given NMES intensity (p>0.05). The present communication thus presents the responses observed during the first contraction of each NMES intensity.

Near-infrared spectroscopy (NIRS)

A frequency-domain multi-distance NIRS system (OxiplexTS, ISS; Champaign, IL, USA) was used to evaluate skeletal muscle oxygenation. Principles of operation and algorithms have been described in detail elsewhere (21). This system provides real-time, absolute (µM) values of the chromophores of interest, i.e. oxygenated and deoxygenated hemoglobin/myoglobin (oxy-[Hb/Mb] and deoxy-[Hb/Mb]; respectively), as well as total hemoglobin/myoglobin (total-[Hb/Mb]). As described in detail by our group (11) and others (e.g., 5, 18, 23, 28), deoxy-[Hb/Mb] was selected as the preferential index of muscle deoxygenation during contractions as this signal is relatively insensitive to blood volume changes and interpreted, therefore, as a proxy for muscle fractional O₂ extraction.

In the present study, we used a single channel consisting of eight laser diodes operating at two wavelengths (690 and 830 nm, four at each wavelength) and a photomultiplier tube. The frequency was modulated at 110 MHz. The laser diodes and photomultiplier tube are connected to a lightweight plastic probe by optical fibers consisting of two parallel rows of emitter fibers and one detector fiber bundle comprising source-detector separations of 2.0, 2.5, 3.0, and 3.5 cm for both wavelengths. The probe was positioned longitudinally on the middle third of the vastus lateralis muscle (∼15-20 cm above the patella) immediately below the NMES electrode #1. To minimize motion artifacts and contamination of the signal by ambient light, after careful shaving and drying of the area, the probe was secured with Velcro straps around the thigh. The near-infrared spectrometer was calibrated on each test day after a warm-up period of at least 30 min. The calibration was performed by placing the optical probe on a calibration block (phantom)
with known absorption and reduced scattering coefficients. Correction factors were determined and implemented automatically by the equipment's software for the calculation of the absorption coefficient ($\mu_A$) and reduced scattering coefficient ($\mu'_s$) for each wavelength (26), i.e., no constant value for photon scattering was assumed herein.

Statistical analyses

Mean and standard deviation (SD) were the measures of central tendency and dispersion. Between group differences were assessed by unpaired Student’s $t$ tests. Comparisons across intensity of NMES were performed using mixed-effects analysis of variance (generalized linear mixed model) considering intra- and inter-subject random effects. The probability of a type I error was established at 0.05 for all tests.
Results

Participant characteristics

As shown in Table 1, COPD patients presented with significantly lower LMM than controls despite similar BMI. As expected by the inclusion criteria, all patients had resting SpO\textsubscript{2} >92%. Although physical activity scores were slightly lower in COPD compared to that achieved by controls (p<0.05), these scores characterize both groups as sedentary (3; see discussion below).

Torque production

Patients with COPD produced lower absolute torque across the entire range of NMES intensities when compared to controls (Fig. 1, p<0.05 for all). Similar results were found when adjusting torque for LMM by DEXA (data not shown). Results are thus presented herein as absolute torque (Nm). As expected, torque production increased as a function of NMES intensity in both groups (Fig. 1; p<0.05 for all).

When expressed as a percentage of MVC, torque production also increased with NMES intensity in both COPD (30 mA=10.7±6.4; 40 mA=18.1±9.0; 50 mA=22.4±8.5 %MVC) and control (30 mA=11.7±7.0; 40 mA=20.2±7.3; 50 mA=27.1±8.3 %MVC; p<0.05 for all). However, between-group differences in torque (%MVC) were observed only at 50 mA (p<0.05).

Skeletal muscle oxygenation

Representative deoxy-[Hb/Mb] responses during submaximal NMES stimulation of the quadriceps femoris of COPD patients and control subjects are depicted in Fig. 2. No differences in resting deoxy-[Hb/Mb] were found between COPD (30 mA=11.6±2.2; 40 mA=11.5±2.3; 50 mA=11.6±2.3 µM) and control (30 mA=10.0±1.4; 40 mA=10.1±1.5; 50 mA=10.3±1.4 µM; p>0.05 for all). Similarly, there were no differences in resting deoxy-[Hb/Mb] among NMES intensities within groups (p>0.05 for all).

Despite producing less torque, COPD patients had similar deoxy-[Hb/Mb] amplitudes at 30 and 40 mA (p>0.05 for both) and higher deoxy-[Hb/Mb] amplitude at 50 mA (p<0.05) when compared to control (Fig. 3). As expected, deoxy-[Hb/Mb] amplitudes increased as a function of NMES intensity in both groups (Fig. 3; p<0.05 for all).
Consistent with altered torque and deoxy-[Hb/Mb] responses during NMES in COPD, patients displayed greater changes in deoxy-[Hb/Mb] per unit of torque output (i.e., ↑Δdeoxy-[Hb/Mb]/torque) across the range of NMES intensities when compared to controls (Fig. 4; p<0.05 for 40 and 50 mA). Different from controls, Δdeoxy-[Hb/Mb]/torque in COPD was progressively greater as a function of NMES intensity (Fig. 4; p<0.05 for all).
Discussion

The present investigation demonstrates, for the first time, that non-hypoxemic moderate-to-severe COPD patients display altered torque production and ambulatory muscle deoxygenation across a range of submaximal NMES compared to age- and gender-matched sedentary controls. Specifically, patients produced lower torque values in association with impaired muscle oxygenation (i.e., greater changes in deoxy-[Hb/Mb]) during NMES relative to controls. This resulted in greater muscle fractional $O_2$ extraction per torque output (i.e., $\Delta$deoxy-[Hb/Mb]/torque) in COPD than control. These results support the hypothesis that local peripheral abnormalities contribute mechanistically to impaired contracting skeletal muscle oxygenation in COPD patients.

Comparison with previous research

As mentioned above, the previous finding of relatively faster dynamics and greater amplitude of deoxy-[Hb/Mb] change during heavy-intensity cycling exercise in patients with COPD (11) does not provide direct evidence that peripheral disturbances per se might affect negatively skeletal muscle $\dot{Q}_O_2/\dot{V}_O_2$ matching. In fact, Chiappa et al. (11) demonstrated that impaired contracting muscle oxygenation in COPD is associated with blunted central hemodynamic responses (slower kinetics of cardiac output) in those experimental conditions. Moreover, subsequent studies aiming at ameliorating central cardiorespiratory constraints imposed by COPD (via heliox, pharmacological bronchodilators and respiratory muscle unloading; refs. 8, 10, 11, see also refs. 32, 44) improved peripheral deoxy-[Hb/Mb] during cycling exercise and provided a mechanistic link between central impairments and contracting skeletal muscle $\dot{Q}_O_2/\dot{V}_O_2$ mismatch.

The current investigation employed small muscle mass NMES to assess potential peripheral limitations associated with muscle $\dot{Q}_O_2/\dot{V}_O_2$ mismatch in COPD patients, based on the fact that this experimental paradigm minimizes considerably the influence of central respiratory and cardiovascular restraints on lower limb skeletal muscle function (36, 51). In addition, NMES allowed quantification of muscle torque production for a given stimulation intensity and revealed that patients required relatively greater fractional $O_2$ extraction per unit of torque output (i.e., $\Delta$deoxy-[Hb/Mb]/torque; Fig. 4). These abnormalities were aggravated by the intensity of metabolic activation given the progressively greater fractional $O_2$ extraction per unit
of torque production as a function of NMES intensity in COPD (Fig. 4). These results extend those reported previously during high intensity whole-body exercise (8, 10, 11, 46) by demonstrating the participation of peripheral factors in contracting skeletal muscle $\dot{Q}O_2/\dot{V}O_2$ mismatch in COPD.

The implications of muscle $\dot{Q}O_2/\dot{V}O_2$ imbalance include a reduced $O_2$ extraction reserve that mandates an exaggerated fall in intramyocyte $PO_2$ and perturbation of the intracellular environment (e.g., ↑[ADP], ↓[PCr]; refs. 25, 47, 53). These are anticipated to enhance glycolysis and utilization of finite energy sources and contribute to the early onset of leg fatigue in COPD.

Potential peripheral mechanisms of impaired contracting muscle oxygenation in COPD

The NIRS-derived deoxy-[Hb/Mb] signal provides a non-invasive index of muscle microvascular (de)oxygenation (or fractional $O_2$ extraction) that is reflective of the dynamic $\dot{Q}O_2/\dot{V}O_2$ matching during transitions in metabolic demand (18, 28). Relatively greater deoxy-[Hb/Mb] change during NMES in COPD patients (Figs. 2 and 3) thus suggest an impaired adjustment of muscle $\dot{Q}O_2$ relative to $\dot{V}O_2$ (19). As noted above, this response likely stems from increased ATP cost of contractions (and thus $O_2$ cost; refs. 29, 30, 43) and/or peripheral vascular dysfunction (expected to reduce contracting muscle blood flow and thus $O_2$ delivery; refs. 6, 17, 27) in COPD.

As reviewed by Wagner (52), a number of potential mechanisms typically invoked to explain skeletal muscle dysfunction in COPD patients could be dismissed due to long-term physical inactivity. On the other hand, the substantial increase in the proportion of type II (fast twitch, glycolytic) muscle fibers in the lower limb of COPD patients relative to physical activity-matched controls (43) could contribute to reduced mechanical efficiency as reported previously (2, 29, 30, 43). Shifts in muscle fiber types in the lower limb muscles of COPD may be regulated by several distinct signaling pathways, including mitogen-activated protein kinase (MAPK; refs. 16, 31). The mechanisms associated with increased cost of developing tension in COPD might derive from differences in myosin ATPase activity and cost of calcium handling between fast- and slow-twitch fiber types (24, 48). In this context, a proportionally greater activation of type II fibers in COPD would be associated with increased ATP cost of contractions (and thus $O_2$ cost; refs. 29, 30, 43) and thereby muscle fractional $O_2$ extraction (present results; Figs. 2 and 3). This is consistent with reports of greater fractional $O_2$ extraction (i.e., $\downarrow PO_2$mv assessed via
phosphorescence quenching) during submaximal electrically-induced twitch contractions in fast-
compared to slow-twitch muscles of the healthy rat hindlimb (35).

Impaired contracting muscle oxygenation in COPD could also originate from blunted
muscle hyperemia secondary to peripheral vascular dysfunction (6, 17, 27). Putative mechanisms
accounting for COPD-related vascular dysfunction include marked sympathetic activation (12),
altered redox balance (i.e., oxidative stress; ref. 27, 34) and vascular inflammation (17).
Although the exact factors underlying excessive reactive oxygen species (ROS) within skeletal
muscle in COPD are not entirely clear (42), fiber type shifting could also play a considerable role
in this respect given that type II fibers are characterized by greater production and lower
scavenging of ROS compared to type I fibers (40). In addition, the possibility exists that chronic
lung inflammation characteristic of COPD evokes systemic responses that culminate in both
peripheral oxidative stress and vascular inflammation (22). One prominent common
pathophysiological consequence is impaired endothelium-dependent vasodilation consequent to
reduced nitric oxide bioavailability (7, 50). Of note, others have assessed directly skeletal muscle
conduit blood flow of COPD patients and reported either preserved or increased values across a
range of submaximal work rates (33, 43, 45). Nevertheless, it is important to consider that
conduit artery blood flow responses may not necessarily reflect those of the muscle
microvasculature (which constitutes the primary locus of O\textsubscript{2} exchange and the site of NIRS
interrogation) during submaximal contractions (23).

The ability to assess torque generation via NMES independently of volitional activation
herein circumvented potential motivational bias and evidenced lower torque in COPD patients
compared to control (Fig. 1). Although not unequivocal (15, 54), a rightward shift of the force-
frequency relationship reported previously in COPD patients (13) would require a higher
stimulation intensity to produce the same absolute force and account for, at least in part, the
reduced torque output observed presently. This behavior could be related to decreased calcium
sensitivity of submaximal force generation reported previously in both slow- and fast-twitch
isolated fibers from the diaphragm of COPD patients (39, 49). Importantly, the current findings
at the level of the microcirculation of increased muscle fractional O\textsubscript{2} extraction per unit of torque
(Fig. 4) bridge the gap between reports of lower mechanical efficiency observed at the mouth
(↑pulmonary \(\dot{V}O_2\)/work rate; ref. 2) and muscle (↑muscle \(\dot{V}O_2\)/work rate using thermodilution;
ref. 43; ↑ATP cost of contraction using \(^{31}\)P-MRS; refs. 29, 30) during transitions in metabolic demand in COPD.

**Clinical implications**

Similar to what has been postulated previously for the respiratory muscles (1), the current data suggest that \(\dot{Q}O_2/\dot{V}O_2\) mismatch (↑Δdeoxy-[Hb/Mb]; Figs. 2 and 3) in the ambulatory muscles may also contribute to reduced exercise capacity in COPD (although primarily during activities that do not engage large muscle mass and require large adjustments in central cardiorespiratory responses; ref. 44). Thus, patients may not only benefit from interventions that ameliorate primarily central function (8, 10, 11), but also from those improving directly lower limb skeletal muscle function. Of note, dietary nitrate supplementation constitutes a potential candidate given its ability to reduce both skeletal muscle fractional \(O_2\) extraction and ATP cost of contractions in healthy young subjects (4, 5).

NMES has been used as a rehabilitative tool in patients with COPD with relative success in improving lower limb muscle function via multiple mechanisms (36, 51). Nevertheless, in healthy young subjects, chronic NMES has been found recently to produce no improvement in contracting muscle oxygenation despite increasing maximal isometric force (41). Whether similar adaptations (or lack thereof) in \(\dot{Q}O_2/\dot{V}O_2\) control occur in the lower limb muscle of COPD patients following NMES training remain to be determined.

**Experimental considerations**

The present investigation is based on a modest sample size (n=15) of non-hypoxemic moderate-to-severe COPD patients and thus may not be representative of the entire COPD population. In this context, it is plausible that hypoxemic and/or more severe COPD patients may demonstrate even greater impairments in locomotor intramuscular function.

Although the small but significant difference in physical activity between our patients and controls needs to be acknowledged (Table 1), it is important to note that the low scores observed herein categorize both groups as sedentary (3). Moreover, reduced mechanical efficiency (as evidenced by increased ATP, and thus, \(O_2\) cost of muscle contractions) in COPD patients is unlikely to derive from contrasting fitness levels alone (29, 30, 43). Thus, it is reasonable to consider that impaired skeletal muscle oxygenation in COPD patients during
submaximal NMES is, at least to some extent, related to peripheral (intramuscular) abnormalities.

The technical aspects and inherent limitations of NIRS-based technology have been discussed at length elsewhere (e.g., 5, 10, 11, 18, 23, 28, 46). A strength of the current investigation is the utilization of frequency-modulated NIRS, which has the singular advantage over traditional spatially-resolved NIRS systems as it provides continuous measurement of the absolute, as opposed to the relative change in, deoxy-[Hb/Mb].

The present NMES experimental protocol differs from voluntary dynamic exercise in many aspects, including the evoked skeletal muscle recruitment pattern. Although not following strictly the size principle of voluntary motor unit recruitment, NMES induces a non-specific pattern of activation where both slow- and fast-twitch fibers are recruited at low and high intensities (9). This actually represents one of the main clinical advantages of NMES given that virtually all muscle fiber types have the potential to be recruited regardless of the intensity of the protocol. Moreover, this behavior is particularly relevant to those patients with acute exacerbation of COPD who might, arguably, benefit the most from a rehabilitative strategy that imposes essentially no central respiratory or cardiovascular burden (36, 51).

Although strong evidence supports the significant 1) increase in the proportion of type II muscle fibers in the lower limb of COPD patients relative to physical activity-matched controls (43); 2) peripheral oxidative stress (27, 34); and 3) vascular inflammation (17), blood and muscle samples were not taken herein and thus speculations were made regarding their potential contribution to impaired muscle oxygenation in COPD. Nonetheless, to the best of our knowledge, this is the first study to indicate the participation of peripheral factors *per se* on ambulatory muscle \( \dot{Q}_2/\dot{V}_2 \) mismatch in COPD patients. Future studies designed to elucidate the mechanisms responsible for these derangements will be valuable.

**Perspectives and significance**

Non-hypoxemic patients with moderate-to-severe COPD produced lower torque together with greater muscle deoxygenation during submaximal NMES relative to sedentary controls. As a result, COPD patients required greater fractional \( O_2 \) extraction to produce a given torque when compared to controls, a behavior that was aggravated as a function of NMES intensity. The present data obtained during submaximal, electrically-evoked contractions of small muscle mass
suggest that peripheral abnormalities contribute mechanistically to impaired skeletal muscle oxygenation during metabolic transitions in non-hypoxemic, moderate-to-severe COPD patients.
Table 1. General characteristics of COPD patients and controls

<table>
<thead>
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<th>COPD (n=15)</th>
<th>Control (n=10)</th>
<th>p</th>
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<tr>
<td>Age (yrs)</td>
<td>65.2 ± 6.1</td>
<td>65.2 ± 4.1</td>
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<td>Height (cm)</td>
<td>166.7 ± 5.2</td>
<td>170.1 ± 7.7</td>
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<tr>
<td>Body mass (kg)</td>
<td>72.3 ± 10.1</td>
<td>74.8 ±12.5</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>26.0 ± 2.9</td>
<td>26.1 ± 4.4</td>
<td>0.93</td>
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<tr>
<td>Leg mass (kg)</td>
<td>10.8 ± 1.1</td>
<td>11.9 ± 2.0</td>
<td>0.15</td>
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<tr>
<td>Leg muscle mass (kg)</td>
<td>8.0 ± 0.7</td>
<td>8.9 ± 1.0</td>
<td>0.02</td>
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<tr>
<td>Leg muscle mass/ leg mass (%)</td>
<td>74.0 ± 3.5</td>
<td>75.7 ± 7.3</td>
<td>0.51</td>
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<td>Leg fat mass (kg)</td>
<td>2.8 ± 0.6</td>
<td>2.9 ± 1.3</td>
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<td>Leg bone mass (kg)</td>
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<td>FEV₁/FVC</td>
<td>43.2 ± 8.8</td>
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<td>-</td>
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<td>FEV₁ (L)</td>
<td>1.3 ± 0.3</td>
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<td>-</td>
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<tr>
<td>FEV₁ (%)</td>
<td>46.4 ± 10.1</td>
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<td>FVC (%)</td>
<td>79.7 ± 10.5</td>
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<td>SpO₂ (%)</td>
<td>94 ± 2</td>
<td>96 ± 1</td>
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<td>Peak torque (Nm)</td>
<td>121.5 ± 28.4</td>
<td>152.0 ± 21.2</td>
<td>0.01</td>
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<tr>
<td>Physical Activity Score</td>
<td>7.0 ± 1.0</td>
<td>7.9 ± 1.0</td>
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Values are mean ± SD. FEV₁, post-bronchodilator forced expiratory volume in 1 second; FVC, forced vital capacity; SpO₂, oxygen saturation by pulse oximetry.
Figure legends

Fig. 1 Skeletal muscle (*quadriceps femoris*) torque production in COPD patients and control subjects during submaximal NMES. \( p<0.05: * \) intra-group difference vs. the previous value; \(^\dagger\) inter-group differences at a given NMES intensity.

Fig. 2 Changes in skeletal muscle (*quadriceps femoris*) deoxy-hemoglobin/myoglobin concentration (\( \Delta \text{deoxy-[Hb/Mb]} \)) in a representative COPD patient (*upper panel*) and control subject (*lower panel*) during submaximal NMES.

Fig. 3 Mean changes in skeletal muscle (*quadriceps femoris*) deoxy-hemoglobin/myoglobin concentration (\( \Delta \text{deoxy-[Hb/Mb]} \)) in COPD patients and control subjects during submaximal NMES. \( p<0.05: * \) intra-group difference vs. the previous value; \(^\dagger\) inter-group differences at a given NMES intensity.

Fig. 4 Mean changes in skeletal muscle (*quadriceps femoris*) deoxy-hemoglobin/myoglobin concentration per unit of torque output (\( \Delta \text{deoxy-[Hb/Mb]}/\text{torque} \)) in COPD patients and control subjects during submaximal NMES. Note that COPD patients demonstrated higher \( \Delta \text{deoxy-[Hb/Mb]}/\text{torque} \) values (i.e., increased fractional O\(_2\) extraction to produce torque) relative to control subjects and as a function of NMES intensity. \( p<0.05: * \) intra-group difference vs. the previous value; \(^\dagger\) inter-group differences at a given NMES intensity.
References


Fig. 1
Fig. 3

Delta Deoxy-[Hb/Mb] (μM)

<table>
<thead>
<tr>
<th>NMES intensity (mA)</th>
<th>COPD</th>
<th>Control</th>
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<tr>
<td>30</td>
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<tr>
<td>50</td>
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</tbody>
</table>

* indicates significant difference at the 0.05 level.
† indicates a trend towards significance.
Fig. 4

Delta Deoxy-[Hb/Mb]/Torque (µM/Nm)

COPD

Control

NMES intensity (mA)