Oxygen delivery and the restoration of the muscle energetic balance following exercise:

Implications for delayed muscle recovery in patients with COPD

Authors: Gwenael Layec 1,2,6, Corey R. Hart2,3, Joel D. Trinity1,2,6, Oh-Sung Kwon1,2, Matt J. Rossman2,3, Ryan M. Broxterman 1,2, Yann Le Fur5, Eun-Kee Jeong4, and Russell S. Richardson 1,2,6

1 Department of Medicine, Division of Geriatrics, University of Utah, Salt Lake City, Utah, USA
2 Geriatric Research, Education, and Clinical Center, George E. Whalen VA Medical Center, Salt Lake City, Utah, USA
3 Department of Exercise and Sport Science, University of Utah, Salt Lake City, Utah, USA
4 Department of Radiology and Utah Center for Advanced Imaging Research, University of Utah, Salt Lake City, Utah, USA
5 CRMBM, Aix-Marseille Universite, CNRS 7339, Marseille, France
6 Department of Nutrition and Integrative Physiology, University of Utah, Salt Lake City, Utah, USA

Corresponding author: G. Layec
VA Medical Center, Bldg 2, 500 Foothill Dr., Salt Lake City, Utah 84148, USA.
E-mail: gwenael.layec@utah.edu

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Abstract

Patients with chronic obstructive pulmonary disease (COPD) experience a delayed recovery from skeletal muscle fatigue following exhaustive exercise that likely contributes to their progressive loss of mobility. As this phenomenon is not well understood, this study sought to examine post-exercise peripheral oxygen (O₂) transport and muscle metabolism dynamics in patients with COPD, two important determinants of muscle recovery. Twenty four subjects, 12 non-hypoxemic patients with COPD and 12 healthy subjects with a sedentary lifestyle, performed dynamic plantar flexion exercise at 40% of maximal work rate (WR_max) with phosphorus magnetic resonance spectroscopy (³¹P-MRS), near-infrared spectroscopy (NIRS), and vascular Doppler ultrasound assessments. The mean response time of limb blood flow at the offset of exercise was significantly prolonged in patients with COPD (Controls: 56±27 s; COPD: 120±87 s; *P*<0.05). In contrast, the post-exercise time constant for capillary blood flow was not significantly different between groups (Controls: 49±23 s; COPD: 51±21 s; *P*>0.05). The initial post-exercise convective O₂ delivery (Controls: 0.15±0.06 L.min⁻¹; COPD: 0.15±0.06 L.min⁻¹) and the corresponding oxidative adenosine triphosphate (ATP) demand (Controls: 14±6 mM.min⁻¹; COPD: 14±6 mM.min⁻¹) in the calf were not significantly different between controls and patients with COPD (*P*>0.05). The PCr resynthesis time constant (Controls: 46±20 s; COPD: 49±21 s), peak mitochondrial phosphorylation rate, and initial proton efflux were also not significantly different between groups (*P*>0.05). Therefore, despite perturbed peripheral hemodynamics, intracellular O₂ availability, proton efflux, and aerobic metabolism recovery in the skeletal muscle of non-hypoxemic patients with COPD are preserved following plantar flexion exercise and, thus, are unlikely to contribute to the delayed recovery from exercise in this population.
Introduction

Exercise intolerance is a frequent complaint, and an important predictor of mortality (55), in patients with COPD. This attenuated exercise capacity, perhaps initiated by a COPD-driven downregulation of oxidative capacity in skeletal muscle (49) can be so debilitating that patients progressively become unable to perform activities of daily living. Interestingly, this downward spiral appears to be aggravated by a prolonged recovery of functional capacity following exercise (48). For instance, after performing a knee-extension exercise to the point of exhaustion, both post-exercise maximum voluntary contraction and electrically evoked quadriceps twitch force were significantly depressed in patients with COPD compared to healthy controls, and remained so even following ~1h of rest (41). As daily life is characterized by repetitive activities, this delayed recovery is likely an important contributor to the poor exercise tolerance, the diminished physical activity, and, ultimately, the loss of mobility experienced by patients with COPD. Therefore, elucidating the prevailing physiological determinants of impaired muscle recovery from exercise in this population is an important and clinically relevant endeavor.

Immediately after exercise, the rate of skeletal muscle recovery is influenced by mechanisms intrinsic to contractile function (e.g. Ca\(^{2+}\) handling), the restoration of energetic balance, and \(O_2\) delivery to muscle. With regard to contractile function, the effects of exhaustive exercise on \(Ca^{2+}\) handling have yet to be investigated as, currently, the effects of COPD on the contractile apparatus during exercise are still unclear in humans (9, 37). In contrast, a prolonged recovery of the muscle energy stores (i.e. phosphocreatine [PCr]) and pH after exercise has already been documented in the lower limb of hypoxemic (75) and non-hypoxemic patients with COPD (51, 57, 62, 65), likely as a consequence of the detrimental effects of both emphysema per se (49) and muscle disuse (37, 51) on muscle oxidative capacity. Finally, studies focused upon
the adequacy of post-exercise O\textsubscript{2} supply to the skeletal muscle in patients with COPD are relatively scarce and conflicting. For instance, the pulmonary O\textsubscript{2} consumption (VO\textsubscript{2}) time constant at the offset of a cycling exercise has been correlated with microvascular reoxygenation kinetics measured by near-infrared spectroscopy in the quadriceps of patients with COPD (NIRS)(56), suggestive of O\textsubscript{2} supply-limited muscle oxidative metabolism. This interpretation has, however, been challenged by the recent evidence that pulmonary and muscle VO\textsubscript{2} may be dissociated during recovery from exercise (34), making inferences specific to muscle oxidative metabolism from pulmonary VO\textsubscript{2} post-exercise rather tenuous. More recently, slower microvascular deoxygenation recovery kinetics, measured by NIRS, have been reported following neuromuscular electrical stimulation in patients with COPD compared to sedentary controls (2). This finding was interpreted as evidence that impeded recovery of muscle functional capacity is caused by metabolic abnormalities. However, as the NIRS-derived deoxyhemoglobin signal reflects the balance between O\textsubscript{2} utilization and supply, in the absence of any direct measurements of limb blood flow or oxidative metabolism, an inadequate hemodynamic response should not be ruled out and could account for the slower kinetics in patients with COPD.

Therefore, it is still unclear whether the interaction between post-exercise muscle metabolism and peripheral hemodynamics is altered in patients with COPD compared to healthy sedentary controls. Accordingly, this study sought to examine the dynamics of peripheral O\textsubscript{2} transport and metabolism following the cessation of plantar flexion exercise. We hypothesized that, if contractile dysfunction is the predominant mechanism delaying muscle recovery in patients with COPD, 1) post-exercise limb and capillary hemodynamics would not be different between controls and patients, 2) as a result, both the convective and diffusive components of O\textsubscript{2}
transport would adequately match the metabolic demand both in controls and patients, and 3) post-exercise PCr and pH kinetics would be similar between controls and patients with COPD.
Methods:

Subjects:

After obtaining written informed consent, 12 non-hypoxemic patients with COPD and 12 age-matched sedentary subjects, participated in this study. The patients were recruited based upon spirometric evidence of COPD, while the controls subjects were recruited based upon no evidence of regular physical activity above that required for activities of daily living (assessed by both questionnaire and accelerometry). Exclusion criteria for the study included: overt cardiovascular disease, diabetes, obesity, neuromuscular disease, and known cancer. All subjects performed standard pulmonary function tests during an initial visit to the laboratory. The study was approved by the Human Research Protection Programs of both the University of Utah and the Salt Lake City Veterans Affairs Medical Center.

Exercise protocol:

After familiarization with the equipment, individual WR$_{max}$ was determined by performing incremental dynamic plantar flexion exercise until exhaustion. On two separate occasions designed to be balanced, subjects performed constant-load sub-maximal plantar flexion at ~40% of WR$_{max}$ (frequency of 1 Hz). The exercise protocol was performed on two separate days, once in the whole body MRI system (TimTrio, 2.9T Siemens Medical Systems, Erlangen, Germany) to assess metabolism, and repeated on a separate day in the laboratory to assess limb and capillary blood flow as well as microvascular oxygenation using Doppler ultrasound imaging and NIRS, respectively. Specifically, the exercise protocol entailed 1 min of rest, 4 min of the constant-load sub-maximal plantar flexion, followed by 5 min of recovery. Prior to initiation of the hemodynamics protocol, blood samples were collected to assess blood lipids, fasting glucose, metabolism, and to perform a complete blood cell count. All experimental
trials were performed by the participants in a thermoneutral environment following an overnight
fast, having refrained from any physical activity and smoking for 12 hours.

**Popliteal blood flow:**

Measurements of popliteal artery blood velocity and vessel diameter were performed in
the popliteal fossa of the exercising leg proximal to the branching of the medial inferior
genicular artery with a Logic 7 Doppler ultrasound system (General Electric Medical Systems,
Milwaukee, WI). The ultrasound system was equipped with a linear array transducer operating at
an imaging frequency of 9 MHz. vessel diameter was determined at a perpendicular angle along
the central axis of the scanned area. Blood velocity was measured using the same transducer with
a frequency of 5 MHz. All blood velocity measurements were obtained with the probes
appropriately positioned to maintain an insonation angle of 60° or less. The sample volume was
maximized according to vessel size and centered within the vessel. Arterial diameter was
measured off-line every 12 s using automated edge-detection software (Medical Imaging
Applications, Coralville, IA), and mean velocity (Vmean) (angle corrected, and intensity-
weighted area under the curve) was automatically calculated beat by beat (Logic 7). Using
arterial diameter and Vmean, blood flow in the popliteal artery was calculated as blood flow =
Vmean ∙ π (vessel diameter/2)² ∙ 60, where blood flow is in milliliters per min. Arterial O₂
saturation (SaO₂) was monitored at baseline with a finger probe oximeter (OxiMax N-600x,
Nellcor, Pleasanton, CA). Mean arterial pressure (MAP), heart rate, stroke volume, and cardiac
output were determined with a Finometer (Finapres Medical Systems, Amsterdam, The
Netherlands). Leg vascular conductance was calculated as popliteal artery blood flow divided by
MAP. Arterial O₂ content (CaO₂) was calculated, utilizing baseline SaO₂ and hemoglobin (Hb),
as the sum of bound O₂ (1.34 ∙ Hb ∙ SaO₂) and dissolved O₂ (0.003 ∙ PO₂), based upon a normal
Hb association curve (64). O₂ delivery was then calculated as the product of CaO₂ and popliteal artery blood flow.

**Microvascular oxygenation and capillary blood flow:**

Microvascular oxygenation was assessed using the NIRS technique, which provides continuous, non-invasive measurements of oxygenated (HbO₂), deoxygenated (HHb) and total (Hbtot) Hb levels as well as a “tissue” oxygenation index (TOI, i.e. HbO₂/Hbtot). Due to identical spectral characteristics, Hb and myoglobin (Mb) are not separated using NIRS. However, although still somewhat contentious (14), the signal is usually considered to be derived mainly from Hb (30). Changes in microvascular oxygenation of the calf muscle were continuously monitored at 2 Hz using a near infrared frequency resolved spectroscopy oximeter (Oxiplex TS, ISS Inc., Illinois USA). The probe was positioned at the level of the largest circumference of the calf and secured with velcro straps and biadhesive tape. This NIRS device uses intensity-modulated light and the probe consisted of 8 infrared light sources (4 emitting at 690 nm and 4 emitting at 830 nm) and one detection channel (inter-optode distance = 1.5 to 4.5 cm) including a selected light detector (photomultiplier tube), thus providing a measurement of absorption and the scattering coefficient of the tissues. Measurement of adipose tissue thickness under the NIRS sample site was performed with a Logic 7 Doppler ultrasound system (General Electric Medical Systems, Milwaukee, WI). Microvascular PO₂ was derived from the tissue oxygen index (4), assuming that the near infrared spectroscopy signal mainly originates from Hb (30), and then computed from the O₂-hemoglobin dissociation curve (64).

The estimated capillary blood flow response following the offset of exercise was calculated from a modified version of the method proposed by Ferreira et al. (12, 18) using the kinetics of muscle O₂ consumption and the HHb data, as previously described (38). Specifically,
the PCr resynthesis rate, measured by $^{31}$P-MRS, which is derived almost exclusively from oxidative phosphorylation (58), was used as an index of muscle O$_2$ consumption. Then, as the HHb response determined by NIRS is considered to reflect muscle capillary O$_2$ extraction (i.e. CaO$_2$-CvO$_2$)(30), and based upon the Fick equation, the temporal characteristics of capillary blood flow were estimated using the PCr resynthesis rate to HHb ratio.

$^{31}$P MRS:

$^{31}$P-MRS was performed using a clinical 2.9T MRI system (Tim-Trio, Siemens Medical Solutions, Erlangen, Germany) operating at 49.9 MHz for $^{31}$P resonance. $^{31}$P MRS data were acquired with a dual tuned $^{31}$P-proton ($^1$H) surface coil with linear polarization (Rapid biomedical GmbH, Rimpar, Germany) positioned under the calf at its maximum diameter. The $^{31}$P single-loop coil diameter was 125 mm surrounding a 110 mm $^1$H coil loop. The centering of the coil around the leg was confirmed by T$_1$ weighted $^1$H localizing images and the coil was repositioned if the coil was not actually centered on the calf, as determined by the thickness of the gastrocnemius muscle. After a three-plane scout proton image, advanced localized volume shimming was performed. Before each experiment, two fully relaxed spectra were acquired at rest with 3 averages per spectrum and a repetition time of 30 s. Then, MRS data acquisition was performed throughout the rest-exercise-recovery protocol using a FID (free-induction-decay) pulse sequence with a 2.56 ms adiabatic-half-passage excitation radiofrequency pulse and the following parameters: repetition time = 2 s; receiver bandwidth = 5 kHz; 1024 data points; and 3 averages per spectrum). Saturation factors were quantified by the comparison between fully relaxed (TR = 30 s) and partially relaxed spectra (TR = 2s).
As previously described (36), relative concentrations of PCr, inorganic phosphate (Pi), and ATP were obtained by a time-domain fitting routine using the Advanced Method for Accurate, Robust and Efficient Spectral (AMARES) fitting algorithm (71) incorporated into the CSIAPO software (40). Intracellular pH was calculated from the chemical shift difference between the Pi and PCr signals. The free cytosolic [ADP] was calculated from [PCr] and pH using the creatine kinase equilibrium constant ($K_{CK} = 1.66 \times 10^9$ M) and assuming that phosphocreatine represents 85% of total creatine content (23). The resting concentrations were calculated from the average peak areas of the three relaxed spectra (TR = 30 s; N = 3) recorded at rest, assuming an 8.2 mM ATP concentration. When Pi splitting was evident, the pH corresponding to each Pi pool was calculated separately as pH$_1$ and pH$_2$ on the basis of the chemical shift of each peak relative to PCr. The overall muscle pH was then calculated as pH = pH$_1$ (area Pi$_1$/total Pi area) + pH$_2$ (area Pi$_2$/total Pi area).

**Leg volume:**

Leg volume was calculated based on lower leg circumference (three sites: distal, middle and proximal), leg length and skinfold measurements (25). This method has recently been confirmed to provide a valid estimate for muscle volume across a spectrum of individuals with normal a muscle mass to severe muscle atrophy (39).

**Assessment of Physical Activity**

Physical activity level (PAL) was assessed using both a subjective PAL recall questionnaire and objective accelerometer data. The physical activity level questionnaire included items regarding the average type, frequency, intensity, and duration of physical activity in any given week. After receiving standardized operating instructions, subjects wore an accelerometer (GT1M; Actigraph, Pensacola, FL, USA) quantifying both the number of steps per
day and intensity of movement for seven continuous days, with adherence automatically
recorded by the device. According to the manufacturer specification, thresholds for sedentary,
light, lifestyle, moderate, vigorous, and very vigorous activity were defined as <99, 100-759,
760-1951, 1952-5724, and >5725 counts/min, respectively.

Data analysis:

The limb and capillary blood flow, vascular conductance, PCr, HHb, and TOI recovery
kinetics were determined by fitting the time-dependent changes during the recovery period to a
mono-exponential curve described by the following equation:

\[ Y(t) = Y_{end} + Y_{amp} (1-e^{-(t-TD/\tau)}) \]  

where \( Y_{end} \) is the level of variable measured at end-of-exercise and \( Y_{res} \) refers to the amplitude of
the blood flow response, PCr resynthesized or the resaturation during the recovery. Unlike the
other variables, there is no time delay (TD) in the resynthesis of PCr and therefore TD was fixed
to 0 for PCr kinetics. Then, the initial rate of PCr resynthesis from \(^{31}\)P-MRS (\( \text{Vi}_{\text{PCr}} \)) was
calculated from the derivative of equation (1) at time zero:

\[ \text{Vi}_{\text{PCr}} = k \cdot \Delta[\text{PCr}] \]  

in which \( \Delta \text{[PCr]} \), represents the amount of PCr resynthesized during the recovery and the rate
constant \( k = 1/\tau \) (27).

The peak rate of oxidative ATP synthesis from \(^{31}\)P-MRS (\( V_{max} \) in mM.min\(^{-1} \)) was
calculated using the initial rate of PCr synthesis (\( \text{Vi}_{\text{PCr}} \)) during the recovery period and \([\text{ADP}]\)
obtained at the end of exercise as previously described (68):

\[ V_{max} = \text{Vi}_{\text{PCr}} (1 + (K_m/[\text{ADP}]_{\text{end}}^{2.2})) \]
in which \(K_m\) (the [ADP] at half the highest oxidation rate) is 30 \(\mu\text{M}\) in skeletal muscle (27).

During the recovery period, PCr is regenerated throughout the CK reaction as the consequence of oxidative ATP production in mitochondria. Thus, \(H^+_{\text{eflux}}\) can be calculated from the rates of proton production from the CK reaction (\(H^+_{\text{CK}}\), in mM/min) and mitochondrial ATP production (\(H^+_{\text{Ox}}\), in mM.min\(^{-1}\)) on one side and the rate of pH changes on the other side. At this time, ATP production is exclusively aerobic, and lactate production is considered negligible:

\[
V_{\text{eff}} = \beta_{\text{total}} \cdot \text{d}pH/\text{d}t + \gamma \cdot \text{ViPCr} + m \cdot \text{ATP}_{\text{ox}}
\]

To improve precision, we use a modified version of this calculation (29) in which the total proton disappearance (i.e., \(\int \text{Edt}\)) is estimated cumulatively from the start of recovery, then fitted to an exponential function to obtain the initial recovery rate \(E\).

Model variables were determined with an iterative process by minimizing the sum of squared residuals (RSS) between the fitted function and the observed values. Goodness of fit was assessed by visual inspection of the residual plot and the frequency plot distribution of the residuals, Chi square values, and the coefficient of determination (\(r^2\)), which was calculated as follows:

\[
 r^2 = 1 - \left( \frac{\text{SS}_{\text{reg}}}{\text{SS}_{\text{tot}}} \right) \quad (4)
\]

with SSreg, the sum of squares of the residuals from the fit and SStot, and the sum of squares of the residuals from the mean.

**Statistical Analysis:**

The assessment of differences between COPD and controls was performed with either paired t-tests or nonparametric Wilcoxon tests, where appropriate (Statsoft, version 5.5;
Statistica, Tulsa, Oklahoma). Statistical significance was accepted at $P < 0.05$. Results are presented as mean ± SD in tables and mean ± SEM in the figures for clarity.
Results

Subject Characteristics: Subject characteristics are presented in Table 1. Patients with COPD exhibited reduced pulmonary function relative to the healthy sedentary controls, and blood gas characteristics consistent with COPD. Despite considerable effort in terms of seeking out sedentary controls, there was still a significant difference in physical activity between groups (Table 1). However, based on the number of steps per day and the time spent in the different intensity domains measured by accelerometry, all subjects can still be confidently defined as sedentary to low active (69). This similar sedentary lifestyle between groups was further confirmed by the lack of a significant difference in plantar flexion peak power output and limb muscle volume between groups. Adipose tissue thickness was not significantly different between groups (Controls: 0.88 ± 0.21 cm; COPD: 0.73 ± 0.25 cm, $P > 0.05$). One patient and one control subject were current smokers. 2 patients were studied while using supplemental O$_2$ (resting SaO$_2$: 94.5 ± 2.1 %).

Baseline and Plantar flexion exercise

Intracellular metabolite concentrations and pH, in addition to tissue and microvascular oxygenation indices (total Hb, deoxy-Hb, and TOI) at rest and during the last 30s of exercise in both controls and patients with COPD are summarized in Table 2. Apart from the resting concentrations of Pi and Phosphodiester (PDE), which were lower in COPD ($P < 0.05$), pH, phosphorylated compounds ([PCr], [Pi] and [ADP]) as well as microvascular oxygenation were not significantly different between groups ($P > 0.05$) at baseline or at the end of exercise.

Recovery period
Peripheral hemodynamics: Limb blood flow, vascular conductance, and capillary blood flow dynamics during the recovery period in controls and COPD are displayed in Figure 1. While end-exercise limb blood flow was not significantly different between groups (controls: 741 ± 216 ml.min⁻¹; COPD: 704 ± 253 ml.min⁻¹; \( P > 0.05 \)), the mean response time at the offset of exercise was significantly prolonged in patients with COPD (Controls: ~56 s; COPD: ~120 s) (Figure 1; \( P < 0.05 \)). Similarly, end-exercise vascular conductance was not significantly different between groups (controls: 7.5 ± 1.4 ml.min⁻¹.mmHg⁻¹; COPD: 6.0 ± 2.5 ml.min⁻¹.mmHg⁻¹; \( P > 0.05 \)). However, the mean response time at the offset of exercise was significantly prolonged in patients with COPD (Controls: ~61 s; COPD: ~127 s) (Figure 1; \( P < 0.05 \)). In contrast, the time constant for capillary blood flow at the offset of exercise was not significantly different between groups (Figure 1; \( P > 0.05 \)).

Convective O₂ delivery and O₂ diffusional conductance:

The convective O₂ delivery dynamics during the recovery period in controls and patients with COPD is displayed in Figure 2. The initial post-exercise convective O₂ delivery and the corresponding oxidative ATP demand were not significantly different between controls and patients with COPD (\( P > 0.05 \), Figure 2). The relationship between microvascular PO₂ and initial post-exercise PCr resynthesis rate, an index of O₂ utilization, during the recovery from plantar flexion exercise in controls and patients with COPD is documented in Figure 3. The slope of each line from the origin, which reflects O₂ diffusional conductance, was not significantly different between groups (controls: 0.47 ± 0.25 mM.mmHg⁻¹.min⁻¹; COPD: 0.55 ± 0.26 mM.mmHg⁻¹.min⁻¹; \( P > 0.05 \)).

Microvascular oxygenation offset kinetics
Both the post-exercise TOI time constant (controls: 48 ± 74 s; COPD: 53 ± 56 s; \( P > 0.05 \)) and mean response time (controls: 63 ± 78 s; COPD: 62 ± 59 s; \( P > 0.05 \)) were not significantly different between groups. Similarly, the deoxy-Hb recovery time constant (controls: 39 ± 22 s; COPD: 57 ± 46 s; \( P > 0.05 \)) and mean response time (controls: 75 ± 63 s; COPD: 76 ± 78 s; \( P > 0.05 \)) were not significantly different between groups.

**Metabolic offset kinetics:**

Changes in pH and [PCr] dynamics during the recovery period in controls and patients with COPD are displayed in Figure 4. **Table 3** documents mitochondrial function and proton handling assessed via post-exercise metabolic kinetics in both groups. None of these variables were different between controls and patients with COPD.
Discussion

Patients with COPD experience a delayed recovery from skeletal muscle fatigue in the first minutes following exhaustive exercise that likely contributes to their progressive loss of mobility. As this delayed post-exercise recovery is not well understood, this study sought to examine the interaction between post-exercise muscle metabolism and peripheral hemodynamics following plantar flexion exercise in patients with COPD and healthy sedentary controls. The main findings of this study were that 1) while end-exercise limb blood flow was not significantly different between groups, post-exercise vascular conductance and limb blood flow kinetics, but not capillary dynamics, were slower in patients with COPD compared to controls, 2) despite these altered hemodynamics, convective O₂ delivery and O₂ diffusional conductance appeared to appropriately match muscle metabolic demand both in controls and patients with COPD, and 3) the metabolic recovery and mitochondrial capacity in patients with COPD was not significantly different to the controls. Therefore, in the face of perturbed peripheral hemodynamics, both intracellular O₂ availability and metabolic recovery in the skeletal muscle of non-hypoxemic patients with COPD are actually preserved following exercise and, thus, are unlikely to contribute to the delayed functional recovery from exercise exhibited by this population.

Prolonged post-exercise peripheral hemodynamics in patients with COPD

For a given metabolic demand at the end of the exercise (Figure 2), blood flow and vascular conductance were not significantly different between sedentary controls and patients with COPD. In contrast, the kinetics of post-exercise limb blood flow and vascular conductance were significantly slower in patients with COPD compared to controls (Figure 1). Similarly, two studies (19, 61) reported no difference in exercise-induced blood flow and vascular conductance during steady-state exercise in patients with COPD compared to healthy age-matched controls.
Our results not only extend these findings to the calf muscle, but also reveal that, despite no significant difference in the steady-state blood flow response, post-exercise hemodynamics are actually prolonged in patients with moderate to very severe COPD. This novel finding confirms the importance of studying not only steady-state response, but also post-exercise hemodynamics as it provides a sensitive and unique window to examine the regulation of blood flow.

Interestingly, the prolonged recovery dynamics at the macro-circulatory level did not translate into slower capillary hemodynamics within the working muscle, as the mean response time of capillary blood flow in patients with COPD was not significantly different to that of the controls (Figure 2). While initially somewhat puzzling, this dissociation between macro- and micro-circulatory dynamics likely indicates inefficient blood flow redistribution following exercise. Indeed, neither the PCr resynthesis nor pH kinetics were significantly different between groups (Figure 4) thereby indicating that prolonged limb blood flow recovery did not stem from an elevated ATP demand or metabolic acidosis within the working muscle. Instead, these findings suggest a significant portion of post-exercise blood flow was directed toward other areas of the lower limb, with low metabolic activity, in patients with COPD.

Another possible explanation for the different hemodynamics in the conduit artery and capillaries of the working muscle of patients with COPD might stem from substantial heterogeneity in O₂ delivery to O₂ utilization matching in the exercising muscles (31-33). Specifically, animal studies have revealed that fast-twitch glycolytic fibers rely, to a greater extent, on adjustments in fractional O₂ extraction, rather than O₂ delivery, to cope with the changes in skeletal muscle O₂ demand (33, 50). According to these findings, and given the, well-established, shift in muscle fiber type toward type II glycolytic fibers with COPD (15), it is possible that the region investigated by the NIRS (medial gastrocnemius muscle), might have
relied more on alterations in $O_2$ extraction rather than blood flow to match muscle $O_2$ demand compared to other muscles of the lower limb composed of a greater proportion of slow twitch oxidative fibers.

The potentially abnormal regulation of lower limb hemodynamics in patients with COPD is consistent with disease-related vascular dysfunction in this population. For instance, COPD has been associated with augmented sympathetic activity (20), impaired endothelium-dependent and –independent dilation (8, 10, 22, 53), and alterations in the concentration of circulating vasoactive substances such as nitric oxide (52), (22). Individually or in combination, such phenomena have the potential to impair vascular control and peripheral hemodynamics following exercise. Also relevant to the present findings is the previously documented evidence of an attenuated contribution of the muscle metaboreflex to hemodynamic control in the calf muscles of patients with COPD (60). Specifically, the sympathetically-mediated vasoconstriction of the calf vasculature following stimulation of the metabosensitive afferents was attributed to the metabolic products of handgrip exercise (60). Such maladaptations can lead to the inefficient coupling between blood flow and metabolic demand, especially at times when metabolic demand varies rapidly, such as the onset and offset of exercise. Combined with the current results, these findings suggest a possible overperfusion of muscle tissue with low metabolic demand after exercise in the lower limb. Although such alterations in blood flow distribution may be of little consequence during a small muscle exercise, this has the potential to result in greater perfusion-metabolism mismatch and/or blood pressure dysregulation at the onset and offset of whole-body high-intensity exercise, with the potential to compromise exercise capacity and recovery.

*Matching of peripheral $O_2$ supply and utilization in the leg of patients with COPD*
An important finding of this study was that convective $O_2$ delivery to the plantar flexor muscles appeared to be preserved at the offset of exercise in patients with COPD, and appeared to match muscle metabolic demand (Figure 2). In addition, as illustrated in Figure 3, skeletal muscle $O_2$ diffusional conductance was also not significantly different between sedentary controls and patients with COPD. As a result of this preserved $O_2$ transport system, both the deoxy-Hb recovery time constant and mean response time were not significantly different between groups, implying that muscle $O_2$ extraction in the plantar flexor muscles of patients with COPD was also not significantly different from that of the controls. In agreement with these findings, Richardson et al. (59) documented a similar relationship between peak muscle $O_2$ consumption and $O_2$ delivery, assessed directly across the exercising muscle, in patients with COPD and controls during single leg knee-extensor exercise (59). It should, however, be noted that in the present study, the post-exercise deoxy-Hb response was quite heterogeneous in both groups, which may allude to different determinants of muscle aerobic capacity. However, a discussion of these factors and the existence of different muscle phenotypes in patients with COPD is beyond the scope of the present study. Interestingly, the present functional findings were also supported by morphometric analysis of skeletal muscle fiber capillarization (59). Indeed, according to a series of recent studies, capillary density, capillary to fiber ratio, number of capillaries around a fiber, and capillary to fiber cross-sectional area are similar between healthy sedentary controls and patients with COPD (11, 14, 59, 73). While not unanimous (24), perhaps due to contrasting levels of physical activity between groups in this latter study, the majority of findings suggest that the size of the capillary-fiber interface, an important site of functional resistance to $O_2$ flux, is likely maintained in patients with COPD. Therefore, in combination, these morphometric and functional findings lend strong support to the concept that
the capacity to transport O\textsubscript{2} in the periphery is actually well preserved in patients with COPD, especially when employing a small muscle mass exercise paradigm that does not heavily tax central hemodynamics or the pulmonary system (59). This conclusion should, however, be put in perspective with the results from prior studies employing high-intensity cycling exercise, which have consistently documented that O\textsubscript{2} transport to limb locomotor muscles was compromised in patients with COPD due to impaired central and peripheral hemodynamics (3, 6, 7, 42, 43, 54), likely caused, indirectly, by abnormal respiratory-mechanics and gas exchange (5, 13, 66, 72).

**Metabolic recovery and mitochondrial function in the skeletal muscle of patients with COPD**

Confirming the results from previous work by our group (37), but with a larger sample size, this study has documented that the PCr recovery time constant and the peak rate of mitochondrial phosphorylation of the plantar flexor muscles were not significantly different between sedentary controls and patients with COPD (Table 3). Given the strong dependence of this measurement on the level of muscle activity, these results confirm that, despite a significant group differences in overall physical activity, measured by accelerometry (due to the extreme inactivity of COPD patients), both groups, actually, exhibited characteristics of a sedentary lifestyle and, thus, the same level of muscle disuse. Such metabolic findings add to the growing evidence suggesting that skeletal muscle mitochondrial capacity, assessed \textit{in vivo}, is actually preserved with COPD when the level of physical activity is not significantly different between patients and controls (37, 65), and that, therefore, much of the decline in mitochondrial capacity reported in these patients can likely be attributed to muscle disuse. For instance, 8 weeks of supervised endurance and strength training in patients with COPD restored mitochondrial phosphorylation capacity in the quadriceps to that of the controls (51). Furthermore, Shields et al. (65) recently examined the PCr recovery kinetics in the biceps brachial and quadriceps
femoris muscles in patients with COPD, with the premise that the upper extremities are less affected by the disease-related reduction in physical activity. This approach allowed the effects of disease and deconditioning on skeletal muscle mitochondrial phosphorylation capacity to be parsed out. Interestingly, using such approach, the authors reported a slower PCr recovery halftime in the quadriceps, but not in the biceps brachial, thereby providing additional evidence that muscle disuse may be the predominant factor accounting for the impaired mitochondrial capacity in the skeletal muscle of patients with COPD (65).

It has previously been reported that initial post-exercise H⁺ efflux rate in the calf muscle was similar between hypoxemic patients with COPD and age-matched controls (67). However, given the contrasting difference in end-exercise pH in this prior study (6.93 in controls and 6.68 in COPD), it is unclear whether this result should, in fact, be interpreted as evidence of impaired H⁺ clearance. In the current study, for a given metabolic state and pH (Table 2), initial H⁺ efflux rate at the offset of the exercise was not significantly different between controls and patients with COPD (Table 3), which translated into similar pH recovery kinetics between groups. This is an important finding as excessive accumulation of lactate and exaggerated metabolic acidosis have previously been reported in patients with COPD during both cycling (42) and plantar flexion exercise (35, 57, 75). In light of the present results, this metabolic acidosis appears not to be related to impaired H⁺ transport.

One potential explanation in the present study, is that preserved limb and capillary blood flow in patients with COPD (Figure 1) contributed to preserved H⁺ efflux. During the initial phase of recovery from exercise, H⁺ transport is also regulated by the monocarboxylate transporters 1 and 4 (MCT1 and MCT4) (17), and the sodium-H⁺ antiporter (28). Interestingly, and somewhat in contrast with our functional measurement of H⁺ efflux, a lower MCT4
expression, but not MCT1, has been documented in the vastus lateralis of patients with COPD (16). Considering the severe physical impairment of the patients recruited in the study of Green et al. (SaO2 ~ 90%, VO2peak ~ 8.6 ml.min⁻¹.kg⁻¹) and the role of physical activity in modulating MCT4 content (26), here again, it is possible that extreme inactivity in these patients may help to explain the discrepancy between studies. Future studies examining both H⁺ efflux rate and skeletal muscle MCT content at different stages of the disease across a wide range of exercise intensities, while also controlling for the level of physical activity, will help clarify these divergent results.

*Experimental considerations and limitations*

A known limitation of the NIRS technique is that the signal originates from both Hb and Mb, and the relative contribution of Mb to the overall NIRS signal has been a matter of contention (47). Although this may influence our estimation of microvascular PO₂ due to different O₂ affinity between Hb and Mb, it is unlikely that this issue actually confounded our interpretation of the data. Indeed, most experimental models have demonstrated that the contribution of Mb to the NIRS signal is actually quite minimal, such that the NIRS signal qualitatively reflects blood O₂ saturation (30, 44-46, 63, 74). In addition, it has previously been documented that the Mb content in the skeletal muscle of patients with COPD was not significantly different from controls (59). Therefore, the contribution of Mb to the NIRS signal was likely not different between groups in the present study. Along the same lines, adipose tissue thickness, which has the potential to complicate NIRS interpretation, was not significantly different between groups and therefore also did not likely confound our interpretation of the data.

This study did not assess the recovery of functional capacity following exercise and therefore could not directly investigate the link between muscle recovery and exercise tolerance.
However, future studies using an all-out test during small muscle mass exercise to explore the relationships between critical power (and \( W' \)) and the determinants of muscle recovery would provide some valuable information on the factors contributing to exercise intolerance in patients with COPD.

Perspective and implications of the delayed skeletal muscle fatigue recovery following exercise

Overall, the results of this study do not support the hypothesis that the persistent force deficit following exercise experienced by patients with COPD is due to inadequate intracellular \( O_2 \) availability or prolonged metabolic disturbance and acidosis. Possible alternative explanations for the delayed recovery in skeletal muscle fatigue may involve a reduction in \( Ca^{2+} \) sensitivity of the contractile apparatus (21), depressed \( Ca^{2+}-\text{ATPase} \) activity (70), or a reduction in \( Ca^{2+} \) release that stem from an impaired coupling between the dihydropyridine receptor and the ryanodine receptor, which releases \( Ca^{2+} \) from the sarcoplasmic reticulum (1). However, whether impaired \( Ca^{2+} \) handling in the skeletal muscle of patients with COPD actually contributes to the persistent post-contractile depression will require further investigations.

Conclusion

In summary, using an integrative approach, this study revealed altered post-exercise peripheral hemodynamics in the plantar flexor of non-hypoxemic patients with COPD, suggesting inefficient blood flow redistribution within the lower limb. Nevertheless, convective \( O_2 \) delivery and \( O_2 \) diffusional conductance within the exercising muscle appeared to be appropriately matched with metabolic demand and were associated with preserved aerobic metabolism recovery and proton handling in the calf muscle of these patients. Together, these findings
suggest that the persistent muscle force deficit following exercise previously documented in patients with COPD is not likely a consequence of lower intracellular O$_2$ availability or metabolic abnormalities, but, instead, may be linked to impaired Ca$^{2+}$ handling within the contractile apparatus, at least, in the majority of non-hypoxemic COPD patients, as in this study.

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Disclosures:

No conflicts of interest, financial or otherwise, are declared by the author(s).
### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample size</strong></td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>68 ± 6</td>
<td>66 ± 6</td>
</tr>
<tr>
<td><strong>Anthropometric</strong> <strong>characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>173 ± 9</td>
<td>171 ± 9</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>76 ± 13</td>
<td>77 ± 16</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>25 ± 3</td>
<td>26 ± 5</td>
</tr>
<tr>
<td><strong>Limb muscle volume (dL)</strong></td>
<td>21 ± 5</td>
<td>23 ± 4</td>
</tr>
<tr>
<td><strong>Functional</strong> <strong>characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Steps per day</strong></td>
<td>6079 ± 1775</td>
<td>3021 ± 747 *</td>
</tr>
<tr>
<td><strong>Sedentary physical activity (min)</strong></td>
<td>1244 ± 61</td>
<td>1303 ± 43 *</td>
</tr>
<tr>
<td><strong>Light physical activity (min)</strong></td>
<td>104 ± 37</td>
<td>84 ± 43</td>
</tr>
<tr>
<td><strong>Lifestyle physical activity (min)</strong></td>
<td>56 ± 25</td>
<td>31 ± 28 *</td>
</tr>
<tr>
<td><strong>Moderate physical activity (min)</strong></td>
<td>24 ± 15</td>
<td>9 ± 14 *</td>
</tr>
<tr>
<td><strong>Vigorous and very vigorous activity (min)</strong></td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td><strong>Gait Speed (m.s⁻¹)</strong></td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.2 *</td>
</tr>
<tr>
<td><strong>Plantar Flexion maximal work rate (W)</strong></td>
<td>9 ± 3</td>
<td>7 ± 2</td>
</tr>
<tr>
<td><strong>Pulmonary function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FVC (L)</strong></td>
<td>4.8 ± 1.4</td>
<td>3.2 ± 0.7 *</td>
</tr>
<tr>
<td><strong>FVC (%pred)</strong></td>
<td>117 ± 22</td>
<td>80 ± 16 *</td>
</tr>
<tr>
<td><strong>FEV1 (L)</strong></td>
<td>3.4 ± 0.8</td>
<td>1.6 ± 0.5 *</td>
</tr>
<tr>
<td><strong>FEV1 (%pred)</strong></td>
<td>114 ± 18</td>
<td>53 ± 16 *</td>
</tr>
<tr>
<td><strong>FEV1/FVC %</strong></td>
<td>73 ± 8</td>
<td>51 ± 9  *</td>
</tr>
<tr>
<td><strong>Blood Characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Arterial Blood Saturation (%)</strong></td>
<td>94 ± 1</td>
<td>94 ± 2</td>
</tr>
<tr>
<td><strong>Glucose (mg.dl⁻¹)</strong></td>
<td>84 ± 13</td>
<td>91 ± 8</td>
</tr>
<tr>
<td><strong>Cholesterol (mg.dl⁻¹)</strong></td>
<td>202 ± 39</td>
<td>198 ± 28</td>
</tr>
<tr>
<td><strong>Triglycerides (mg.dl⁻¹)</strong></td>
<td>151 ± 81</td>
<td>87 ± 25</td>
</tr>
<tr>
<td><strong>HDL (mg.dl⁻¹)</strong></td>
<td>51 ± 13</td>
<td>71 ± 34</td>
</tr>
<tr>
<td><strong>LDL (mg.dl⁻¹)</strong></td>
<td>130 ± 32</td>
<td>108 ± 25</td>
</tr>
<tr>
<td><strong>WBC (K.ul⁻¹)</strong></td>
<td>5.3 ± 1.4</td>
<td>7.4 ± 3.0</td>
</tr>
<tr>
<td><strong>RBC (M.ul⁻¹)</strong></td>
<td>4.9 ± 0.5</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td><strong>Hemoglobin (g.dl⁻¹)</strong></td>
<td>15 ± 2</td>
<td>14 ± 1</td>
</tr>
<tr>
<td><strong>Hematocrit (%)</strong></td>
<td>45 ± 4</td>
<td>44 ± 3</td>
</tr>
<tr>
<td><strong>Neutrophil (K.ul⁻¹)</strong></td>
<td>2.9 ± 1.1</td>
<td>4.8 ± 3.4</td>
</tr>
<tr>
<td><strong>Lymphocyte (K.ul⁻¹)</strong></td>
<td>1.7 ± 0.5</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td><strong>Monocyte (K.ul⁻¹)</strong></td>
<td>0.5 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td><strong>Bicarbonate (mM.L⁻¹)</strong></td>
<td>25 ± 1</td>
<td>28 ± 3  *</td>
</tr>
<tr>
<td>Potassium (mM.L⁻¹)</td>
<td>4.0 ± 0.3</td>
<td>3.8 ± 0.3</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD. Body mass index, BMI; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s. high density lipoprotein, HDL; low density lipoprotein, LDL; white blood cells, WBC; red blood cells, RBC. *, P < 0.05; significantly different from controls.
Table 2. Metabolic and microvascular oxygenation responses at rest and during steady state submaximal plantar flexion exercise in control and COPD subjects.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorylated compounds and pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCr (mM)</td>
<td>34 ± 5</td>
<td>36 ± 8</td>
</tr>
<tr>
<td>Pi (mM)</td>
<td>1.9 ± 1.0</td>
<td>1.1 ± 0.4 *</td>
</tr>
<tr>
<td>ADP (μM)</td>
<td>8.2 ± 0.3</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>pH</td>
<td>6.97 ± 0.02</td>
<td>6.96 ± 0.04</td>
</tr>
<tr>
<td>PDE (mM)</td>
<td>2.0 ± 1.4</td>
<td>0.6 ± 0.6 *</td>
</tr>
<tr>
<td><strong>Microvascular oxygenation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygenation index (%)</td>
<td>62 ± 4</td>
<td>60 ± 7</td>
</tr>
<tr>
<td>Hb total (µM)</td>
<td>55 ± 20</td>
<td>56 ± 27</td>
</tr>
<tr>
<td>Deoxy-Hb (µM)</td>
<td>21 ± 8</td>
<td>23 ± 13</td>
</tr>
<tr>
<td><strong>End Exercise</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorylated compounds and pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCr (mM)</td>
<td>22 ± 6</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>Pi (mM)</td>
<td>10 ± 4</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>ADP (μM)</td>
<td>43 ± 21</td>
<td>47 ± 47</td>
</tr>
<tr>
<td>pH</td>
<td>6.99 ± 0.06</td>
<td>6.93 ± 0.09</td>
</tr>
<tr>
<td><strong>Microvascular oxygenation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygenation index (%)</td>
<td>60 ± 4</td>
<td>55 ± 9</td>
</tr>
<tr>
<td>Hb total (µM)</td>
<td>58 ± 22</td>
<td>58 ± 29</td>
</tr>
<tr>
<td>Deoxy-Hb (µM)</td>
<td>24 ± 10</td>
<td>28 ± 20</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD. PCr, Phosphocreatine; Pi, Inorganic phosphate; PME, Phosphomonoester; ADP, Adenosine diphosphate; PDE, Phosphodiester. *, $P < 0.05$; significantly different from controls. Hb total and DeoxyHb: total - and deoxy-hemoglobin, respectively.
Table 3. Mitochondrial function and proton handling assessed via post-exercise metabolic kinetics in controls and patients with COPD.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mitochondrial function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCr recovery time constant (s)</td>
<td>46 ± 20</td>
<td>49 ± 21</td>
</tr>
<tr>
<td>IC 95 (s)</td>
<td>16 ± 12</td>
<td>15 ± 9</td>
</tr>
<tr>
<td>Peak mitochondrial phosphorylation rate (mM.min⁻¹)</td>
<td>23 ± 10</td>
<td>24 ± 10</td>
</tr>
<tr>
<td><strong>Proton (H⁺) handling</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial H⁺ efflux (mM.min⁻¹)</td>
<td>4.1 ± 3.0</td>
<td>4.0 ± 3.8</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD. IC 95, 95% confidence interval.
Figure 1 – The recovery kinetics of limb blood flow (panel A), vascular conductance (panel B) and capillary blood flow (panel C) following dynamic plantar flexion exercise in controls and patients with COPD. The figure inserts illustrate the mean response time. Both for limb blood flow and vascular conductance, the mean response time was significantly slower in patients with COPD compared to controls ($P < 0.05$). Values are presented as mean ± SEM.

Figure 2 – The recovery kinetics of convective O$_2$ delivery following dynamic plantar flexion exercise in controls and patients with COPD. The figure insert illustrates the immediate post-exercise convective O$_2$ delivery and the corresponding oxidative ATP demand in both groups. Neither convective O$_2$ delivery nor oxidative ATP demand were significantly different between controls and patients with COPD ($P > 0.05$) indicative of a similar matching of O$_2$ supply and demand in both groups. Values are presented as mean ± SEM.

Figure 3. The relationship between microvascular partial pressure of O$_2$ (PO$_2$) and initial post-exercise PCr resynthesis rate, an index of O$_2$ utilization, during the recovery from plantar flexion exercise in controls and patients with COPD. The slope of the lines from the origin reflects O$_2$ diffusional conductance according to Fick’s law. Values are presented as mean ± SEM.

Figure 4 – The recovery kinetics of phosphocreatine (panel A) and pH (panel B) following dynamic plantar flexion exercise in controls and patients with COPD. Values are presented as mean ± SEM.


Figure 1.
Figure 2.
Figure 3.
Figure 4.