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Oxygen delivery and the restoration of the muscle energetic balance following exercise: Implications for delayed muscle recovery in patients with COPD

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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 (O_2) 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 (^{31}P-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 O_2 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 O_2 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 dimished 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

69 the adequacy of post-exercise O_2 supply to the skeletal muscle in patients with COPD are
70 relatively scarce and conflicting. For instance, the pulmonary O_2 consumption (VO_2) time
71 constant at the offset of a cycling exercise has been correlated with microvascular reoxygenation
72 kinetics measured by near-infrared spectroscopy in the quadriceps of patients with COPD
73 (NIRS)(56), suggestive of O_2 supply-limited muscle oxidative metabolism. This interpretation
74 has, however, been challenged by the recent evidence that pulmonary and muscle VO_2 may be
75 dissociated during recovery from exercise (34), making inferences specific to muscle oxidative
76 metabolism from pulmonary VO_2 post-exercise rather tenuous. More recently, slower
77 microvascular deoxygenation recovery kinetics, measured by NIRS, have been reported
78 following neuromuscular electrical stimulation in patients with COPD compared to sedentary
79 controls (2). This finding was interpreted as evidence that impeded recovery of muscle functional
80 capacity is caused by metabolic abnormalities. However, as the NIRS-derived deoxyhemoglobin
81 signal reflects the balance between O_2 utilization and supply, in the absence of any direct
82 measurements of limb blood flow or oxidative metabolism, an inadequate hemodynamic
83 response should not be ruled out and could account for the slower kinetics in patients with
84 COPD.

85 Therefore, it is still unclear whether the interaction between post-exercise muscle
86 metabolism and peripheral hemodynamics is altered in patients with COPD compared to healthy
87 sedentary controls. Accordingly, this study sought to examine the dynamics of peripheral O_2
88 transport and metabolism following the cessation of plantar flexion exercise. We hypothesized
89 that, if contractile dysfunction is the predominant mechanism delaying muscle recovery in
90 patients with COPD, 1) post-exercise limb and capillary hemodynamics would not be different
91 between controls and patients, 2) as a result, both the convective and diffusive components of O_2

92 transport would adequately match the metabolic demand both in controls and patients, and 3)
93 post-exercise PCr and pH kinetics would be similar between controls and patients with COPD.
94

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 $\cdot$ π (vessel diameter/2)^2 $\cdot$ 60, where blood flow is in milliliters per min. Arterial O_2 saturation (SaO_2) 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 O_2 content (CaO_2) was calculated, utilizing baseline SaO_2 and hemoglobin (Hb), as the sum of bound O_2 ($1.34 \cdot Hb \cdot SaO_2$) and dissolved O_2 ($0.003 \cdot PO_2$), based upon a normal

Hb association curve (64). O_2 delivery was then calculated as the product of CaO_2 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 (HbO_2), deoxygenated (HHb) and total (Hbtot) Hb levels as well as a "tissue" oxygenation index (TOI, i.e. HbO_2/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 PO_2 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 O_2-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 O_2 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:

$$Y(t) = Y_{end} + Y_{amp}\,(1-e^{-(t-TD/\tau)}) \qquad (1)$$

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 (Vi_{PCr}) was calculated from the derivative of equation (1) at time zero:

$$Vi_{PCr} = k \cdot \Delta[PCr] \qquad (2)$$

in which Δ [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 (Vi_{PCr}) during the recovery period and [ADP] obtained at the end of exercise as previously described (68):

$$V_{max} = Vi_{PCr}\,(1 + (K_m/[ADP]_{end}^{2.2})) \qquad (3)$$

in which K_m (the [ADP] at half the highest oxidation rate) is 30 μM 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^+_{efflux} can be calculated from the rates of proton production from the CK reaction (H^+_{CK}, in mM/min) and mitochondrial ATP production (H^+_{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:

$$V_{eff} = \beta_{total} \cdot dpH/dt + \gamma \cdot ViPCr + m \cdot ATP_{ox}$$

To improve precision, we use a modified version of this calculation (29) in which the total proton disappearance (i.e., $\int Edt$) 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:

$$r^2 = 1 - (SS_{reg}/SS_{tot}) \qquad (4)$$

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 O_2 delivery and O_2 diffusional conductance:

The convective O_2 delivery dynamics during the recovery period in controls and patients with COPD is displayed in **Figure 2**. The initial post-exercise convective O_2 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 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 is documented in **Figure 3**. The slope of each line from the origin, which reflects O_2 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

296 Both the post-exercise TOI time constant (controls: 48 ± 74 s; COPD: 53 ± 56 s; $P >$
297 0.05) and mean response time (controls: 63 ± 78 s; COPD: 62 ± 59 s; $P > 0.05$) were not
298 significantly different between groups. Similarly, the deoxy-Hb recovery time constant (controls:
299 39 ± 22 s; COPD: 57 ± 46 s; $P > 0.05$) and mean response time (controls: 75 ± 63 s; COPD: 76 ±
300 78 s; $P > 0.05$) were not significantly different between groups.

301 *Metabolic offset kinetics:*

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

306

307

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 O_2 delivery and O_2 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 O_2 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 blow 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 O_2 delivery to O_2 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 O_2 extraction, rather than O_2 delivery, to cope with the changes in skeletal muscle O_2 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

399 the capacity to transport O_2 in the periphery is actually well preserved in patients with COPD,
400 especially when employing a small muscle mass exercise paradigm that does not heavily tax
401 central hemodynamics or the pulmonary system (59). This conclusion should, however, be put in
402 perspective with the results from prior studies employing high-intensity cycling exercise, which
403 have consistently documented that O_2 transport to limb locomotor muscles was compromised in
404 patients with COPD due to impaired central and peripheral hemodynamics (3, 6, 7, 42, 43, 54),
405 likely caused, indirectly, by abnormal respiratory-mechanics and gas exchange (5, 13, 66, 72).

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

407 Confirming the results from previous work by our group (37), but with a larger sample
408 size, this study has documented that the PCr recovery time constant and the peak rate of
409 mitochondrial phosphorylation of the plantar flexor muscles were not significantly different
410 between sedentary controls and patients with COPD (**Table 3**). Given the strong dependence of
411 this measurement on the level of muscle activity, these results confirm that, despite a significant
412 group differences in overall physical activity, measured by accelerometry (due to the extreme
413 inactivity of COPD patients), both groups, actually, exhibited characteristics of a sedentary
414 lifestyle and, thus, the same level of muscle disuse. Such metabolic findings add to the growing
415 evidence suggesting that skeletal muscle mitochondrial capacity, assessed *in vivo*, is actually
416 preserved with COPD when the level of physical activity is not significantly different between
417 patients and controls (37, 65), and that, therefore, much of the decline in mitochondrial capacity
418 reported in these patients can likely be attributed to muscle disuse. For instance, 8 weeks of
419 supervised endurance and strength training in patients with COPD restored mitochondrial
420 phosphorylation capacity in the quadriceps to that of the controls (51). Furthermore, Shields et
421 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

445 expression, but not MCT1, has been documented in the vastus lateralis of patients with COPD
446 (16). Considering the severe physical impairment of the patients recruited in the study of Green
447 *et al.* (SaO_2 ~ 90%, VO_2peak ~ 8.6 $ml.min^{-1}.kg^{-1}$) and the role of physical activity in modulating
448 MCT4 content (26), here again, it is possible that extreme inactivity in these patients may help to
449 explain the discrepancy between studies. Future studies examining both H^+ efflux rate and
450 skeletal muscle MCT content at different stages of the disease across a wide range of exercise
451 intensities, while also controlling for the level of physical activity, will help clarify these
452 divergent results.

453 *Experimental considerations and limitations*

454 A known limitation of the NIRS technique is that the signal originates from both Hb and Mb, and
455 the relative contribution of Mb to the overall NIRS signal has been a matter of contention (47).
456 Although this may influence our estimation of microvascular PO_2 due to different O_2 affinity
457 between Hb and Mb, it is unlikely that this issue actually confounded our interpretation of the
458 data. Indeed, most experimental models have demonstrated that the contribution of Mb to the
459 NIRS signal is actually quite minimal, such that the NIRS signal qualitatively reflects blood O_2
460 saturation (30, 44-46, 63, 74). In addition, it has previously been documented that the Mb content
461 in the skeletal muscle of patients with COPD was not significantly different from controls (59) .
462 Therefore, the contribution of Mb to the NIRS signal was likely not different between groups in
463 the present study. Along the same lines, adipose tissue thickness, which has the potential to
464 complicate NIRS interpretation, was not significantly different between groups and therefore
465 also did not likely confound our interpretation of the data.

466 This study did not assess the recovery of functional capacity following exercise and
467 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+}-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

490 suggest that the persistent muscle force deficit following exercise previously documented in
491 patients with COPD is not likely a consequence of lower intracellular O_2 availability or
492 metabolic abnormalities, but, instead, may be linked to impaired Ca^{2+} handling within the
493 contractile apparatus, at least, in the majority of non-hypoxemic COPD patients, as in this study.

494

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501

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504 No conflicts of interest, financial or otherwise, are declared by the author(s).

505

506 **Table 1. Subject characteristics**

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

Potassium ($mM.L^{-1}$)	4.0	±	0.3	3.8	±	0.3

Data expressed as mean ± SD. Body mass index, BMI; FVC, forced vital capacity; FEV_1, 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.

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

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.

517

518 Table 3. Mitochondrial function and proton handling assessed via post-exercise metabolic

519 kinetics in controls and patients with COPD.

	Controls	COPD
Mitochondrial function		
PCr recovery time constant (s)	46 ± 20	49 ± 21
IC 95 (s)	16 ± 12	15 ± 9
Peak mitochondrial phosphorylation rate ($mM.min^{-1}$)	23 ± 10	24 ± 10
Proton (H^+) handling		
Initial H^+ efflux ($mM.min^{-1}$)	4.1 ± 3.0	4.0 ± 3.8

520 Values expressed as mean ± SD. IC 95, 95% confidence interval.

521

522

523 Figure Legends

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

529 Figure 2 – The recovery kinetics of convective O_2 delivery following dynamic plantar flexion
530 exercise in controls and patients with COPD. The figure insert illustrates the immediate post-
531 exercise convective O_2 delivery and the corresponding oxidative ATP demand in both groups.
532 Neither convective O_2 delivery nor oxidative ATP demand were significantly different between
533 controls and patients with COPD ($P > 0.05$) indicative of a similar matching of O_2 supply and
534 demand in both groups. Values are presented as mean ± SEM.

535 Figure 3. The relationship between microvascular partial pressure of O_2 (PO_2) and initial post-
536 exercise PCr resynthesis rate, an index of O_2 utilization, during the recovery from plantar flexion
537 exercise in controls and patients with COPD. The slope of the lines from the origin reflects O_2
538 diffusional conductance according to Fick's law. Values are presented as mean ± SEM.

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

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Bibliography

1. **Allen DG, Lamb GD, and Westerblad H.** Skeletal muscle fatigue: cellular mechanisms. *Physiological reviews* 88: 287-332, 2008.
2. **Azevedo Dde P, Medeiros WM, de Freitas FF, Ferreira Amorim C, Gimenes AC, Neder JA, and Chiavegato LD.** High oxygen extraction and slow recovery of muscle deoxygenation kinetics after neuromuscular electrical stimulation in COPD patients. *Eur J Appl Physiol* 116: 1899-1910, 2016.
3. **Borghi-Silva A, Oliveira CC, Carrascosa C, Maia J, Berton DC, Queiroga F, Jr., Ferreira EM, Almeida DR, Nery LE, and Neder JA.** Respiratory muscle unloading improves leg muscle oxygenation during exercise in patients with COPD. *Thorax* 63: 910-915, 2008.
4. **Broxterman RM, Ade CJ, Wilcox SL, Schlup SJ, Craig JC, and Barstow TJ.** Influence of duty cycle on the power-duration relationship: observations and potential mechanisms. *Respiratory physiology & neurobiology* 192: 102-111, 2014.
5. **Bruce RM, Turner A, and White MJ.** Ventilatory responses to muscle metaboreflex activation in chronic obstructive pulmonary disease. *J Physiol*, 2016.
6. **Chiappa GR, Borghi-Silva A, Ferreira LF, Carrascosa C, Oliveira CC, Maia J, Gimenes AC, Queiroga F, Jr., Berton D, Ferreira EM, Nery LE, and Neder JA.** Kinetics of muscle deoxygenation are accelerated at the onset of heavy-intensity exercise in patients with COPD: relationship to central cardiovascular dynamics. *J Appl Physiol* 104: 1341-1350, 2008.
7. **Chiappa GR, Queiroga F, Jr., Meda E, Ferreira LF, Diefenthaeler F, Nunes M, Vaz MA, Machado MC, Nery LE, and Neder JA.** Heliox improves oxygen delivery and utilization during dynamic exercise in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 179: 1004-1010, 2009.
8. **Clarenbach CF, Thurnheer R, and Kohler M.** Vascular dysfunction in chronic obstructive pulmonary disease: current evidence and perspectives. *Expert Rev Respir Med* 6: 37-43, 2012.
9. **Debigare R, Cote CH, Hould FS, LeBlanc P, and Maltais F.** In vitro and in vivo contractile properties of the vastus lateralis muscle in males with COPD. *The European respiratory journal* 21: 273-278, 2003.
10. **Eickhoff P, Valipour A, Kiss D, Schreder M, Cekici L, Geyer K, Kohansal R, and Burghuber OC.** Determinants of systemic vascular function in patients with stable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 178: 1211-1218, 2008.
11. **Eliason G, Abdel-Halim SM, Piehl-Aulin K, and Kadi F.** Alterations in the muscle-to-capillary interface in patients with different degrees of chronic obstructive pulmonary disease. *Respir Res* 11: 97, 2010.
12. **Ferreira LF, Townsend DK, Lutjemeier BJ, and Barstow TJ.** Muscle Capillary Blood Flow Kinetics Estimated from Pulmonary O2 Uptake and near-Infrared Spectroscopy. *J Appl Physiol*, 2005.
13. **Gagnon P, Bussieres JS, Ribeiro F, Gagnon SL, Saey D, Gagne N, Provencher S, and Maltais F.** Influences of spinal anesthesia on exercise tolerance in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 186: 606-615, 2012.
14. **Gifford JR, Trinity JD, Layec G, Garten RS, Park SY, Rossman MJ, Larsen S, Dela F, and Richardson RS.** Quadriceps exercise intolerance in patients with chronic obstructive pulmonary disease: the potential role of altered skeletal muscle mitochondrial respiration. *Journal of applied physiology* 119: 882-888, 2015.
15. **Gosker HR, van Mameren H, van Dijk PJ, Engelen MP, van der Vusse GJ, Wouters EF, and Schols AM.** Skeletal muscle fibre-type shifting and metabolic profile in patients with chronic obstructive pulmonary disease. *The European respiratory journal* 19: 617-625, 2002.

16. **Green HJ, Burnett ME, D'Arsigny CL, O'Donnell DE, Ouyang J, and Webb KA.** Altered metabolic and transporter characteristics of vastus lateralis in chronic obstructive pulmonary disease. *J Appl Physiol* 105: 879-886, 2008.
17. **Halestrap AP and Price NT.** The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. *The Biochemical journal* 343 Pt 2: 281-299, 1999.
18. **Harper AJ, Ferreira LF, Lutjemeier BJ, Townsend DK, and Barstow TJ.** Matching of blood flow to metabolic rate during recovery from moderate exercise in humans. *Experimental physiology* 93: 1118-1125, 2008.
19. **Hartmann SE, Waltz X, Leigh R, Anderson TJ, and Poulin MJ.** Blood Flow during Handgrip Exercise in COPD: Effect of Vitamin C. *Med Sci Sports Exerc* 48: 200-209, 2016.
20. **Heindl S, Lehnert M, Criee CP, Hasenfuss G, and Andreas S.** Marked sympathetic activation in patients with chronic respiratory failure. *Am J Respir Crit Care Med* 164: 597-601, 2001.
21. **Howlett RA, Stary CM, and Hogan MC.** Recovery of force during postcontractile depression in single Xenopus muscle fibers. *American journal of physiology Regulatory, integrative and comparative physiology* 280: R1469-1475, 2001.
22. **Ives SJ, Harris RA, Witman MA, Fjeldstad AS, Garten RS, McDaniel J, Wray DW, and Richardson RS.** Vascular dysfunction and chronic obstructive pulmonary disease: the role of redox balance. *Hypertension* 63: 459-467, 2014.
23. **Jeneson JA, Westerhoff HV, Brown TR, Van Echteld CJ, and Berger R.** Quasi-linear relationship between Gibbs free energy of ATP hydrolysis and power output in human forearm muscle. *The American journal of physiology* 268: C1474-1484, 1995.
24. **Jobin J, Maltais F, Doyon JF, LeBlanc P, Simard PM, Simard AA, and Simard C.** Chronic obstructive pulmonary disease: capillarity and fiber-type characteristics of skeletal muscle. *J Cardiopulm Rehabil* 18: 432-437, 1998.
25. **Jones PR and Pearson J.** Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. *The Journal of physiology* 204: 63P-66P, 1969.
26. **Juel C.** Training-induced changes in membrane transport proteins of human skeletal muscle. *European journal of applied physiology* 96: 627-635, 2006.
27. **Kemp GJ and Radda GK.** Quantitative interpretation of bioenergetic data from 31P and 1H magnetic resonance spectroscopic studies of skeletal muscle: an analytical review. *Magn Reson Q* 10: 43-63, 1994.
28. **Kemp GJ, Thompson CH, Sanderson AL, and Radda GK.** pH control in rat skeletal muscle during exercise, recovery from exercise, and acute respiratory acidosis. *Magn Reson Med* 31: 103-109, 1994.
29. **Kemp GJ, Thompson CH, Taylor DJ, and Radda GK.** Proton efflux in human skeletal muscle during recovery from exercise. *European journal of applied physiology and occupational physiology* 76: 462-471, 1997.
30. **Koga S, Kano Y, Barstow TJ, Ferreira LF, Ohmae E, Sudo M, and Poole DC.** Kinetics of muscle deoxygenation and microvascular PO(2) during contractions in rat: comparison of optical spectroscopy and phosphorescence-quenching techniques. *J Appl Physiol (1985)* 112: 26-32, 2012.
31. **Koga S, Poole DC, Ferreira LF, Whipp BJ, Kondo N, Saitoh T, Ohmae E, and Barstow TJ.** Spatial heterogeneity of quadriceps muscle deoxygenation kinetics during cycle exercise. *J Appl Physiol* 103: 2049-2056, 2007.
32. **Koga S, Poole DC, Fukuoka Y, Ferreira LF, Kondo N, Ohmae E, and Barstow TJ.** Methodological validation of the dynamic heterogeneity of muscle deoxygenation within the quadriceps during cycle exercise. *American journal of physiology* 301: R534-541, 2011.
33. **Koga S, Rossiter HB, Heinonen I, Musch TI, and Poole DC.** Dynamic heterogeneity of exercising muscle blood flow and O2 utilization. *Medicine and science in sports and exercise* 46: 860-876, 2014.

34. **Krustrup P, Jones AM, Wilkerson DP, Calbet JA, and Bangsbo J.** Muscular and pulmonary O2 uptake kinetics during moderate- and high-intensity sub-maximal knee-extensor exercise in humans. *The Journal of physiology* 587: 1843-1856, 2009.
35. **Kutsuzawa T, Shioya S, Kurita D, Haida M, Ohta Y, and Yamabayashi H.** 31P-NMR study of skeletal muscle metabolism in patients with chronic respiratory impairment. *Am Rev Respir Dis* 146: 1019-1024, 1992.
36. **Layec G, Bringard A, Vilmen C, Micallef JP, Fur YL, Perrey S, Cozzone PJ, and Bendahan D.** Accurate work-rate measurements during in vivo MRS studies of exercising human quadriceps. *Magma (New York, NY* 21: 227-235, 2008.
37. **Layec G, Haseler LJ, Hoff J, and Richardson RS.** Evidence that a higher ATP cost of muscular contraction contributes to the lower mechanical efficiency associated with COPD: preliminary findings. *Am J Physiol Regul Integr Comp Physiol* 300: R1142-1147, 2011.
38. **Layec G, Haseler LJ, Trinity JD, Hart CR, Liu X, Le Fur Y, Jeong EK, and Richardson RS.** Mitochondrial function and increased convective O2 transport: implications for the assessment of mitochondrial respiration in vivo. *J Appl Physiol (1985)* 115: 803-811, 2013.
39. **Layec G, Venturelli M, Jeong EK, and Richardson RS.** The validity of anthropometric leg muscle volume estimation across a wide spectrum: from able-bodied adults to individuals with a spinal cord injury. *J Appl Physiol (1985)* 116: 1142-1147, 2014.
40. **Le Fur Y, Nicoli F, Guye M, Confort-Gouny S, Cozzone PJ, and Kober F.** Grid-free interactive and automated data processing for MR chemical shift imaging data. *Magma (New York, NY* 23: 23-30, 2010.
41. **Mador MJ, Deniz O, Aggarwal A, and Kufel TJ.** Quadriceps fatigability after single muscle exercise in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 168: 102-108, 2003.
42. **Maltais F, Jobin J, Sullivan MJ, Bernard S, Whittom F, Killian KJ, Desmeules M, Belanger M, and LeBlanc P.** Metabolic and hemodynamic responses of lower limb during exercise in patients with COPD. *J Appl Physiol* 84: 1573-1580, 1998.
43. **Maltais F, Simon M, Jobin J, Desmeules M, Sullivan MJ, Belanger M, and Leblanc P.** Effects of oxygen on lower limb blood flow and O2 uptake during exercise in COPD. *Med Sci Sports Exerc* 33: 916-922, 2001.
44. **Mancini D.** Application of near infrared spectroscopy to the evaluation of exercise performance and limitations in patients with heart failure. *Journal of biomedical optics* 2: 22-30, 1997.
45. **Mancini DM, Bolinger L, Li H, Kendrick K, Chance B, and Wilson JR.** Validation of near-infrared spectroscopy in humans. *J Appl Physiol* 77: 2740-2747, 1994.
46. **Mancini DM, Wilson JR, Bolinger L, Li H, Kendrick K, Chance B, and Leigh JS.** In vivo magnetic resonance spectroscopy measurement of deoxymyoglobin during exercise in patients with heart failure. Demonstration of abnormal muscle metabolism despite adequate oxygenation. *Circulation* 90: 500-508, 1994.
47. **Marcinek DJ, Amara CE, Matz K, Conley KE, and Schenkman KA.** Wavelength shift analysis: a simple method to determine the contribution of hemoglobin and myoglobin to in vivo optical spectra. *Applied spectroscopy* 61: 665-669, 2007.
48. **Mattson JP and Martin JC.** Emphysema-induced reductions in locomotory skeletal muscle contractile function. *Experimental physiology* 90: 519-525, 2005.
49. **Mattson JP and Poole DC.** Pulmonary emphysema decreases hamster skeletal muscle oxidative enzyme capacity. *Journal of applied physiology* 85: 210-214, 1998.
50. **McDonough P, Behnke BJ, Padilla DJ, Musch TI, and Poole DC.** Control of microvascular oxygen pressures in rat muscles comprised of different fibre types. *The Journal of physiology* 563: 903-913, 2005.

51. **McKeough ZJ, Alison JA, Bye PT, Trenell MI, Sachinwalla T, Thompson CH, and Kemp GJ.** Exercise capacity and quadriceps muscle metabolism following training in subjects with COPD. *Respir Med* 100: 1817-1825, 2006.
52. **Montes de Oca M, Torres SH, De Sanctis J, Mata A, Hernandez N, and Talamo C.** Skeletal muscle inflammation and nitric oxide in patients with COPD. *The European respiratory journal* 26: 390-397, 2005.
53. **Moro L, Pedone C, Scarlata S, Malafarina V, Fimognari F, and Antonelli-Incalzi R.** Endothelial dysfunction in chronic obstructive pulmonary disease. *Angiology* 59: 357-364, 2008.
54. **Oelberg DA, Kacmarek RM, Pappagianopoulos PP, Ginns LC, and Systrom DM.** Ventilatory and cardiovascular responses to inspired He-O2 during exercise in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 158: 1876-1882, 1998.
55. **Oga T, Nishimura K, Tsukino M, Sato S, and Hajiro T.** Analysis of the factors related to mortality in chronic obstructive pulmonary disease: role of exercise capacity and health status. *Am J Respir Crit Care Med* 167: 544-549, 2003.
56. **Okamoto T, Kanazawa H, Hirata K, and Yoshikawa J.** Evaluation of oxygen uptake kinetics and oxygen kinetics of peripheral skeletal muscle during recovery from exercise in patients with chronic obstructive pulmonary disease. *Clin Physiol Funct Imaging* 23: 257-262, 2003.
57. **Payen JF, Wuyam B, Levy P, Reutenauer H, Stieglitz P, Paramelle B, and Le Bas JF.** Muscular metabolism during oxygen supplementation in patients with chronic hypoxemia. *Am Rev Respir Dis* 147: 592-598, 1993.
58. **Quistorff B, Johansen L, and Sahlin K.** Absence of phosphocreatine resynthesis in human calf muscle during ischaemic recovery. *The Biochemical journal* 291 (Pt 3): 681-686, 1993.
59. **Richardson RS, Leek BT, Gavin TP, Haseler LJ, Mudaliar SR, Henry R, Mathieu-Costello O, and Wagner PD.** Reduced mechanical efficiency in chronic obstructive pulmonary disease but normal peak VO2 with small muscle mass exercise. *Am J Respir Crit Care Med* 169: 89-96, 2004.
60. **Roseguini BT, Alves CN, Chiappa GR, Stein R, Knorst MM, and Ribeiro JP.** Attenuation of muscle metaboreflex in chronic obstructive pulmonary disease. *Med Sci Sports Exerc* 40: 9-14, 2008.
61. **Rossman MJ, Garten RS, Groot HJ, Reese V, Zhao J, Amann M, and Richardson RS.** Ascorbate infusion increases skeletal muscle fatigue resistance in patients with chronic obstructive pulmonary disease. *Am J Physiol Regul Integr Comp Physiol* 305: R1163-1170, 2013.
62. **Sala E, Roca J, Marrades RM, Alonso J, Gonzalez De Suso JM, Moreno A, Barbera JA, Nadal J, de Jover L, Rodriguez-Roisin R, and Wagner PD.** Effects of endurance training on skeletal muscle bioenergetics in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 159: 1726-1734, 1999.
63. **Seiyama A, Hazeki O, and Tamura M.** Noninvasive quantitative analysis of blood oxygenation in rat skeletal muscle. *J Biochem (Tokyo)* 103: 419-424, 1988.
64. **Severinghaus JW.** Simple, accurate equations for human blood O2 dissociation computations. *Journal of applied physiology: respiratory, environmental and exercise physiology* 46: 599-602, 1979.
65. **Shields GS, Coissi GS, Jimenez-Royo P, Gambarota G, Dimber R, Hopkinson NS, Matthews PM, Brown AP, and Polkey MI.** Bioenergetics and intermuscular fat in chronic obstructive pulmonary disease-associated quadriceps weakness. *Muscle & nerve* 51: 214-221, 2015.
66. **Stark-Leyva KN, Beck KC, and Johnson BD.** Influence of expiratory loading and hyperinflation on cardiac output during exercise. *Journal of applied physiology* 96: 1920-1927, 2004.
67. **Thompson CH, Davies RJ, Kemp GJ, Taylor DJ, Radda GK, and Rajagopalan B.** Skeletal muscle metabolism during exercise and recovery in patients with respiratory failure. *Thorax* 48: 486-490, 1993.
68. **Trenell MI, Sue CM, Kemp GJ, Sachinwalla T, and Thompson CH.** Aerobic exercise and muscle metabolism in patients with mitochondrial myopathy. *Muscle Nerve* 33: 524-531, 2006.

69. **Tudor-Locke C, Craig CL, Brown WJ, Clemes SA, De Cocker K, Giles-Corti B, Hatano Y, Inoue S, Matsudo SM, Mutrie N, Oppert JM, Rowe DA, Schmidt MD, Schofield GM, Spence JC, Teixeira PJ, Tully MA, and Blair SN.** How many steps/day are enough? For adults. *The international journal of behavioral nutrition and physical activity* 8: 79, 2011.

70. **Tupling R, Green H, Grant S, Burnett M, and Ranney D.** Postcontractile force depression in humans is associated with an impairment in SR Ca(2+) pump function. *American journal of physiology Regulatory, integrative and comparative physiology* 278: R87-94, 2000.

71. **Vanhamme L, van den Boogaart A, and Van Huffel S.** Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 129: 35-43, 1997.

72. **Vogiatzis I, Athanasopoulos D, Habazettl H, Aliverti A, Louvaris Z, Cherouveim E, Wagner H, Roussos C, Wagner PD, and Zakynthinos S.** Intercostal muscle blood flow limitation during exercise in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 182: 1105-1113, 2010.

73. **Whittom F, Jobin J, Simard PM, Leblanc P, Simard C, Bernard S, Belleau R, and Maltais F.** Histochemical and morphological characteristics of the vastus lateralis muscle in patients with chronic obstructive pulmonary disease. *Med Sci Sports Exerc* 30: 1467-1474, 1998.

74. **Wilson JR, Mancini DM, McCully K, Ferraro N, Lanoce V, and Chance B.** Noninvasive detection of skeletal muscle underperfusion with near-infrared spectroscopy in patients with heart failure. *Circulation* 80: 1668-1674, 1989.

75. **Wuyam B, Payen JF, Levy P, Bensaidane H, Reutenauer H, Le Bas JF, and Benabid AL.** Metabolism and aerobic capacity of skeletal muscle in chronic respiratory failure related to chronic obstructive pulmonary disease. *The European respiratory journal* 5: 157-162, 1992.

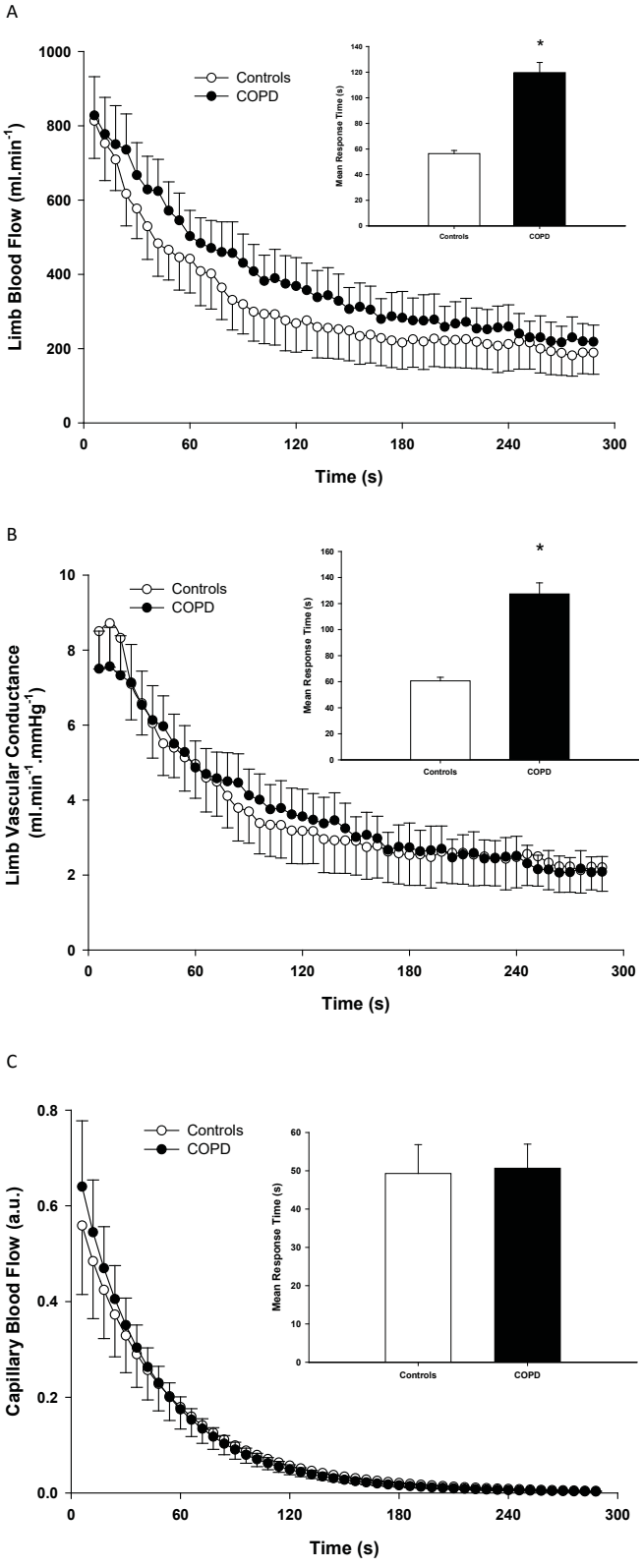
A
Controls
COPD
Limb Blood Flow (ml.min-1)
Time (s)
Mean Response Time (s)
*
Controls
COPD
B
Controls
COPD
Limb Vascular Conductance (ml.min-1.mmHg-1)
Time (s)
Mean Response Time (s)
*
Controls
COPD
C
Controls
COPD
Capillary Blood Flow (a.u.)
Time (s)
Mean Response Time (s)
Controls
COPD

Figure 1.

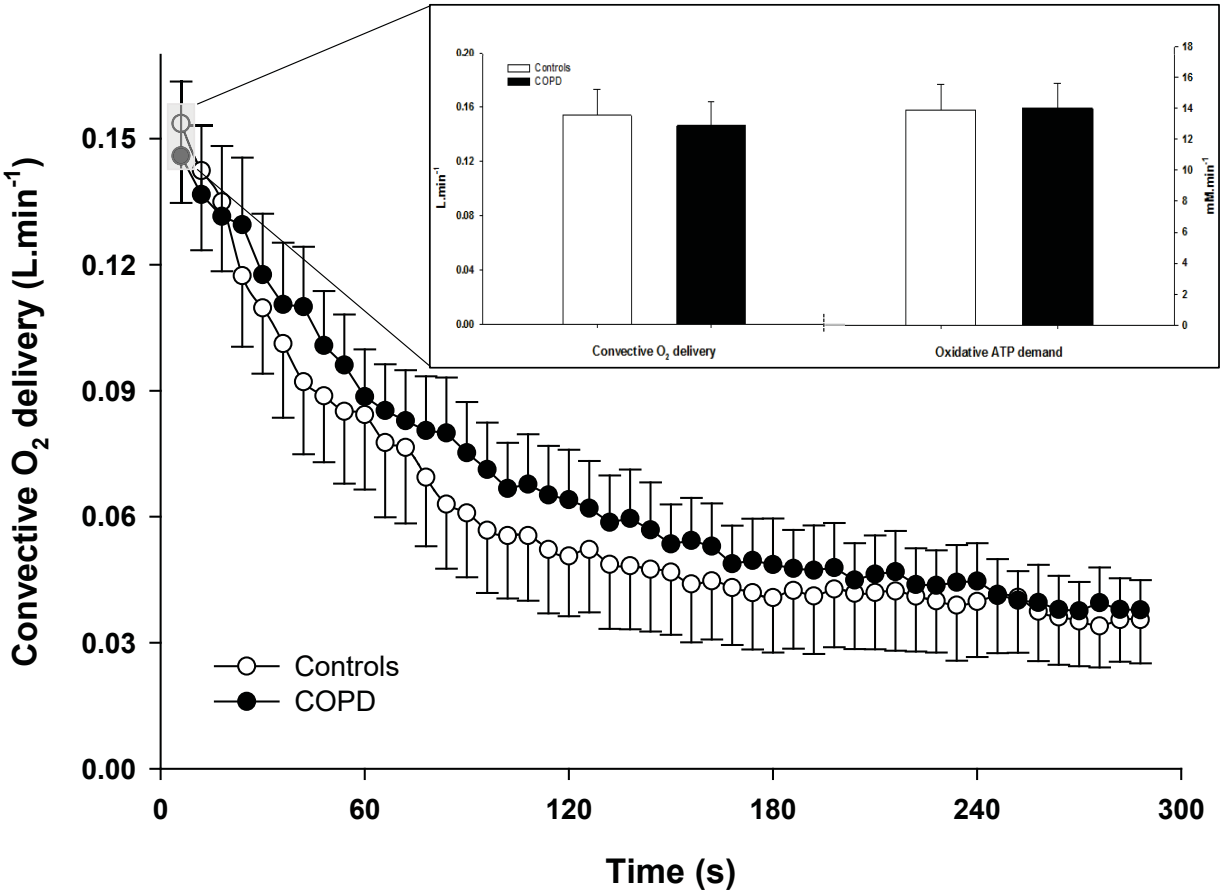
Convective O_2 delivery ($L.min^{-1}$)
0.15
0.12
0.09
0.06
0.03
0.00
0
60
120
180
240
300
Time (s)
Controls
COPD
0.20
0.16
0.12
0.08
0.04
0.00
$L.min^{-1}$
18
16
14
12
10
8
6
4
2
0
$mM.min^{-1}$
Controls
COPD
Convective O_2 delivery
Oxidative ATP demand

Figure 2.

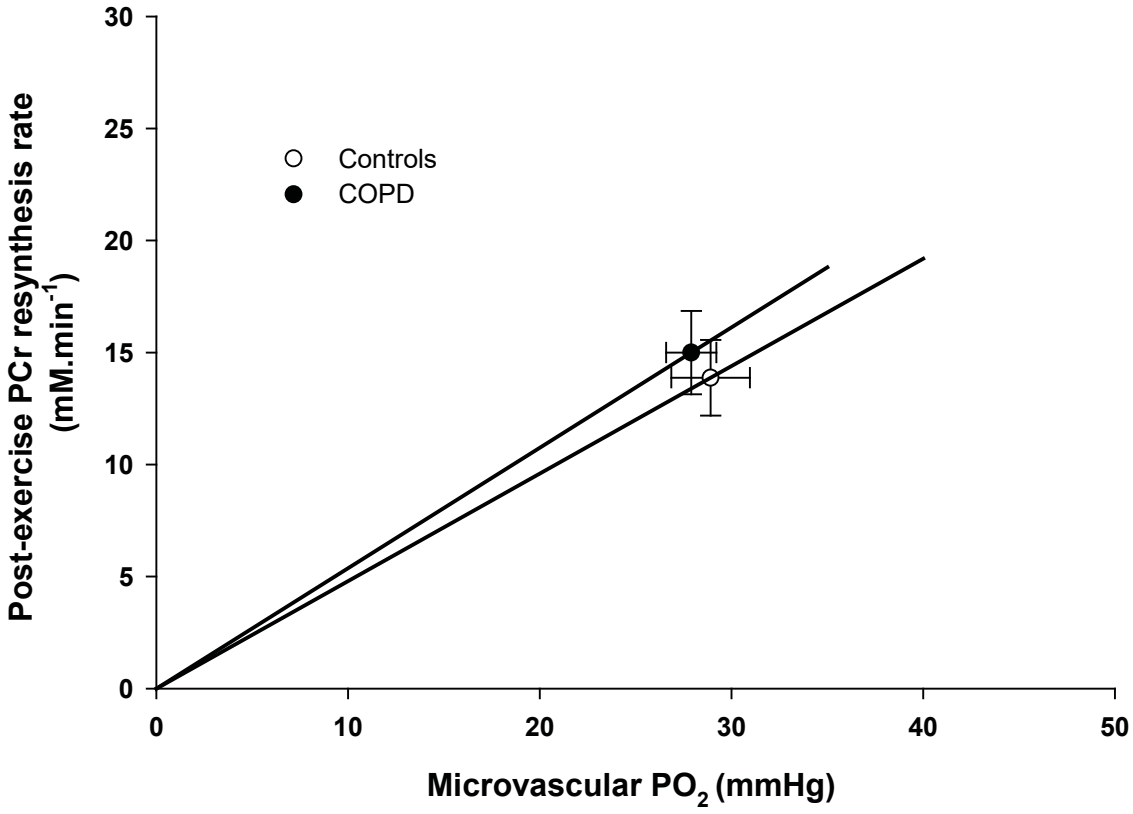
Post-exercise PCr resynthesis rate
(mM.min-1)
Controls
COPD
Microvascular PO2 (mmHg)
0
5
10
15
20
25
30
0
10
20
30
40
50

Figure 3.

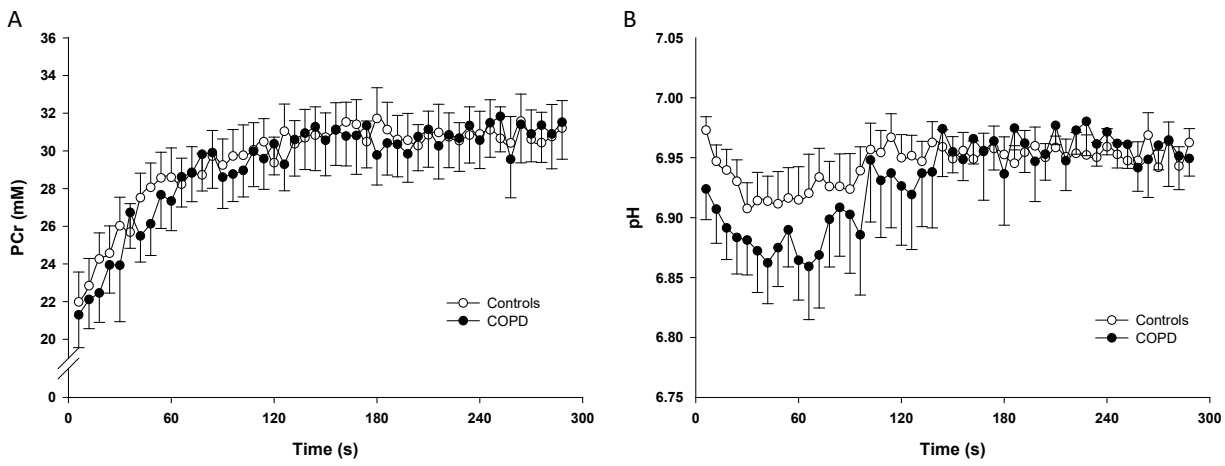

Figure 4.