Heterogeneity of blood flow and metabolism during exercise in patients with Chronic Obstructive Pulmonary Disease

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Abstract

The study investigated whether the capacity to regulate muscle blood flow (Q) relative to metabolic demand (VO_2) is impaired in COPD. Using six NIRS optodes over the upper, middle and lower vastus lateralis in 6 patients, (FEV_1:46±12%predicted) we recorded from each: a) Q by indocyanine green dye injection, b) VO_2/Q ratios based on fractional tissue O_2 saturation and c) VO_2 as their product, during constant-load exercise (at 20%, 50% and 80% of peak capacity) in normoxia and hyperoxia (F_IO_2:1.0). At 50 and 80%, relative dispersion (RD) for Q, but not for VO_2, was greater in normoxia (0.67±0.07 and 0.79±0.08, respectively) compared to hyperoxia (0.57±0.12 and 0.72±0.07, respectively). In both conditions, RD for VO_2 and Q significantly increased throughout exercise; however, RD of VO_2/Q ratio was minimal (normoxia: 0.12 to 0.08 vs hyperoxia: 0.13 to 0.09). Muscle Q and VO_2 appear closely matched in COPD patients, indicating a minimal impact of heterogeneity on muscle oxygen availability at submaximal levels of exercise.

Keywords: Chronic obstructive pulmonary disease, muscle perfusion, muscle metabolism, exercise, NIRS

1. Introduction

Limb muscle dysfunction is a major systemic consequence of chronic diseases such as chronic obstructive pulmonary disease (COPD) (Maltais et al., 2014) chronic heart failure (Okita et al., 2013) diabetes (Bauer et al., 2007), and peripheral vascular disease (Creager and Libby, 2011) because of its ~~important clinical~~ ~~implications~~ adverse impact on daily physical activity, exercise capacity, quality of life and even survival (Graham et al., 2011, Hulsmann et al., 2004, Patel et al., 2014, Powers et al., 2016).

A major consequence of locomotor muscle dysfunction is the limitation in oxygen availability caused by reduced arterial oxygen saturation and/or reduced muscle perfusion (Q) (Casey and Joyer, 2011). Reduction in either of these during exercise may cause mismatch between local muscle metabolic demand (VO_2) and blood flow (Q) resulting in VO_2/Q heterogeneity in different regions within the exercising muscles (Brass et al., 2004, Heinonen et al., 2015, Koga et al., 2014). Ineffective matching of regional muscle Q to VO_2 (i.e., large regional muscle VO_2/Q heterogeneity) may cause overall impairment of muscle oxygen availability (Casey and Joyer, 2011, Piiper and Haab, 1991, Walley, 1996) thus limiting exercise capacity.

In patients with COPD the well documented lower limb muscle morphological and structural alterations, namely muscle fiber type alteration and atrophy, reduced capillarization, poor oxidative capacity and mitochondrial dysfunction (Maltais et al., 2014) could potentially lead to regional muscle VO_2/Q heterogeneity during exercise of progressively increasing loads (Brass et al., 2004, Mizuno et al., 2003). However, in patients with COPD, studies have demonstrated ~~proposed the existence of a metabolic muscle reserve, manifested by~~ an adequate increase in limb muscle oxygen delivery to support a proportional increase in muscle VO_2 when the locomotor muscles are freed from the constraints of the cardiopulmonary system during

single-leg extension or cycling exercise breathing pure oxygen (37, 39, 53, 54). This evidence suggests that the peripheral muscles of patients with COPD may preserve the capacity to regulate lower leg muscle Q relative to VO_2 across various exercise intensities (Maltais et al., 1998, 2001, Richardson et al., 1999, 2004), thereby suggesting minimal ~~regional~~ peripheral muscle VO_2/Q heterogeneity.

Elegant studies in healthy subjects have used imaging methods such as positron emission tomography (PET) and magnetic resonance spectroscopy (MRS) to evaluate heterogeneity of lower limb muscle perfusion and metabolism at rest and various exercise intensities during low intensity one-leg static or cycling exercise (Heinonen et al., 2010, 2011, 2012, Kalliokoski et al., 2000, 2001, 2003, 2005, Laaksonen et al., 2003, Mizuno et al., 2003). These studies revealed that while at rest and exercise there was some regional muscle variation in VO_2, perfusion remained closely matched to metabolic demand, thereby suggesting very little functional heterogeneity of muscle perfusion and metabolism. Recently our group developed a new technique using near-infrared spectroscopy (NIRS) to assess regionally ~~and simultaneously~~ the degree of heterogeneity of muscle VO_2 to Q and their ratio, VO_2/Q, during cycling exercise of progressive intensity in healthy subjects (Vogiatzis et al., 2015). The findings of this study (Vogiatzis et al., 2015) confirmed previous suggestions (Heinonen et al., 2010, 2011, 2012, Kalliokoski et al., 2000, 2001, 2003, 2005, Laaksonen et al., 2003, Mizuno et al., 2003) by demonstrating that in healthy individuals there is tight local matching between Q and VO_2 and a likely minimal impact of heterogeneity on muscle oxygen availability (Vogiatzis et al., 2015). Accordingly, the aim of the present study was to employ this technique in patients with COPD in order to investigate whether the capacity to regulate regional muscle Q relative to VO_2 is disturbed a) at rest b) during progressive exercise and c) as a function of arterial oxygenation in patients with COPD.

We reasoned that if with increasing exercise loading in normoxia local muscle VO_2/Q heterogeneity increased, this would suggest that the peripheral muscles of patients with COPD do not possess the capacity to adequately regulate oxygen supply relative to metabolic demand. However, if in normoxia local muscle VO_2/Q heterogeneity was unaffected by progressively increasing exercise intensity or systemic oxygen delivery, this would suggest tight matching between local muscle Q and VO_2 during exercise in COPD. We tested our hypotheses by performing two identical protocols of progressive exercise loading (i.e., 20%, 50% and 80% of peak watts) in both normoxia and hyperoxia (F_IO_2: 1.0). We used hyperoxia as ~~in the latter condition~~ alleviation of ventilatory constraints along with an increase in local muscle oxygen delivery by oxygen administration (Maltais et al., 2001, Richardson et al., 1999) would be expected to mitigate regional muscle VO_2/Q heterogeneity compared to normoxia.

2. Materials and Methods

2.1 Study Group

Six clinically stable patients with COPD classified by the Global Initiative for Chronic Obstructive Lung Disease (18) as spirometric stages II (n=2) and III (n=4) were recruited for the study according to the following inclusion criteria: 1) a post-bronchodilator forced expiratory volume in one second (FEV_1) < 80% predicted without significant reversibility (<12% change of the initial FEV_1 value or < 200 ml) and 2) optimal medical therapy according to GOLD (GOLD, 2016). Patients had a history compatible with COPD, and at least 10 pack/years of smoking history. Exclusion criteria included: 1) Orthopedic, neurological, and other pathologic conditions or severe pain syndromes that could interfere with exercise, 2) respiratory diseases other than COPD (i.e. asthma), 3) clinical signs of acute heart failure, known unstable or moderate-severe heart disease (i.e. arrhythmia, ischemic heart disease or cardiomyopathy), 4) patients under administration of vaso-active medications, 5) engagement in any exercise-training program in the last 3 months, and 6) any hospital admission or COPD exacerbation within the previous 4

weeks. Patients were informed of any risks and discomfort associated with the protocol, and written informed consent was obtained prior to the start of the study. The study was approved by University Chest Hospital Ethics Committee and conformed to the guidelines of the Declaration of Helsinki.

2.2 Experimental Design

Experiments were conducted in two visits. In visit 1, patients underwent an incremental exercise test in atmospheric air (normoxia, F_IO_2: 0.21) to the limit of tolerance to establish peak work rate (WR_{peak}). The incremental exercise tests were performed on an electromagnetically braked cycle ergometer (Ergo-line 800; Sensor Medics, Anaheim CA) with ramp load increments of 5 or 10 W/min to the limit of tolerance (the point at which the work rate could not be tolerated due to severe sensations of dyspnea and/or leg discomfort) with the patients maintaining a pedaling frequency of 40-50 rpm. Tests were preceded by a 3-min rest period, followed by 3-min of unloaded pedaling. Pulmonary gas exchange and ventilatory variables were recorded breath-by-breath (Vmax 229; Sensor Medics), heart rate and arterial oxygen saturation (SpO_2) were determined using the R-R interval from a 12-lead on-line electrocardiogram (Marquette Max; Marquette Hellige, Freiburg, Germany) and a pulse oximeter (Nonin 8600; Nonin Medical, North Plymouth, MN), respectively. Intensity of dyspnea and leg discomfort during the tests was assessed using the modified Borg scale (Borg, 1982).

In visit 2, following resting measurements, patients performed, in a balanced order sequence, two graded exercise tests, separated by 120 min of rest, breathing either atmospheric air (F_IO_2: 0.21, normoxia) or pure oxygen (F_IO_2: 1.0, hyperoxia). Exercise in normoxia and hyperoxia was sustained for 5 minutes at each of four work rates, corresponding to unloaded pedaling and then 20%, 50% and 80% of WR_{peak}. Including the resting measurements at each condition (i.e., normoxia and hyperoxia) this protocol, therefore, produced 10 measurement conditions in each of the six patients.

2.3 Preliminary assessment

All patients underwent the following baseline measurements prior to the study: anthropometric indices, pulmonary function parameters, six–minute walking distance test and quadriceps muscle strength and endurance assessment. Body composition was estimated by a bioelectric impedance device (Maltron BF 907, Essex, UK). Fat free mass index was obtained by dividing fat free mass (FFM) in kg by height. Spirometry and single breath transfer factor for carbon monoxide was measured according to the American Thoracic Society (ATS) and European Respiratory Society (ERS) standards (GOLD, 2016). Post bronchodilator static lung volumes were assessed using whole body plethysmography (Table 1). The six-minute walking distance test was performed according to ATS guidelines (ATS, 2002) and was expressed as fraction of reference values (Troosters et al., 1999). Quadriceps muscle strength and endurance was assessed using the maximal isometric voluntary contraction technique of the knee extensors following a standardized protocol and was expressed as fraction of reference values (Allaire et al., 2004, Swallow et al., 2007). Furthermore, subjects were instructed to maintain a tension representing 60% of their maximal voluntary contraction (MVC) until exhaustion. A computer screen served as a feedback mechanism to help subjects maintain the determined submaximal tension. Subjects were strongly encouraged to pursue until tension dropped to 50% of MVC. Peripheral muscle endurance was thus assessed by the time to fatigue, defined as the time at which the isometric contraction reached 50% MVC (Allaire et al., 2004).

2.4 Subject preparation

Subjects were prepared with peripheral venous and arterial catheters. In brief, using local anesthesia (2% lidocaine) and sterile technique, catheters were introduced percutaneously into the left femoral vein (central venous catheter model AK-04301 Arrow International, Durham, NC) and the right radial artery (Angiocath 20 gauge, 1.16 in., model 381134, Becton Dickinson),

both oriented in the proximal direction. The catheters were used a) to collect arterial and femoral venous blood samples, b) to inject indocyanine green dye (ICG) into the venous line and c) to continuously sample arterial blood after each ICG injection for muscle blood flow calculation. The catheters were kept patent throughout the experiment by periodic flushing with heparinized (1 unit/ml) saline.

Within an hour after execution of the exercise protocol, subcutaneous fat thickness in the upper, middle and lower part of vastus lateralis muscle was assessed by Ultrasound imaging (LOGIQ Book XP; GE Healthcare Products, Milwaukee, WI). An 8-MHz linear array was used while capturing the image in B-mode. Ultrasound gel was applied to the center of the template before placing the transducer on the skin. The skin was marked after removal of each of the six optodes to verify the correct placement of the transducer. After a clear image was identified, the image was saved. This procedure was repeated 3 times for each of the six-optode locations and a mean value was calculated. The images were labeled with the participant's number and optode location of measurement.

2.5 Regional O_2 saturation ($StiO_2$) measured by NIRS.

Three pairs of NIRS optodes were placed on the skin over the upper, middle, and lower vastus lateralis (Figure 1) and secured by double-sided adhesive tape to measure muscle Q and oxygenated hemoglobin/myoglobin (Hb/Mb) signals using three identical spectrophotometers (NIRO 200 spectrophotometer, Hamamatsu Photonics, Hamamatsu, Japan), because these devices have only two channels each, thus requiring three separate spectrophotometers. The light emission and collection points in each optode were 40 mm apart, corresponding to a penetration depth of 20 mm. The oxygenation data assessed by NIRS (Spatially Resolved Technique) were the changes in the ratio of oxygenated to total haemoglobin, an absolute index of local oxygen saturation ($StiO_2$). $StiO_2$ takes into account changes in blood volume during exercise whilst it reflects the dynamic balance between oxygen delivery and demand (Boushel et al., 2001, Tew et

al., 2010). $StiO_2$ data were weighted by the measured blood flow beneath the probe of measurement in order to correct also potential influence of skin blood flow changes during the exercise (Grassi and Quaresima 2016, Tew et al., 2010). For the analysis $StiO_2$ data were averaged over 10 s immediately before ICG injection (Vogiatzis et al., 2015).

2.6 Estimation of the regional SvO_2

To estimate regional oxygen saturation in venous blood (SvO_2) from the regional NIRS oxygen signal ($StiO_2$), we used the linear relationship [SvO_2 = -40.5 + 1.265 x $StiO_2$] between directly measured femoral venous oxygen saturation ($SfvO_2$) and the blood flow - weighted average $StiO_2$ over the six optodes (Figure 2) previously derived in healthy subjects (Vogiatzis et al., 2015). The relationship between directly measured $SfvO_2$ and the perfusion - weighted average $StiO_2$ over the six optodes for the COPD patients at rest and during exercise was also found to be linear in COPD [SvO2 = 3.40 + 0.533 x StiO2] with the values superimposed on those of healthy subjects (Figure 2). The reason we used the healthy subject regression relationship was because of their wider range of venous oxygen saturation and $StiO_2$ values (Figure 2). Unfortunately, the range of both of these variables in each of COPD patients over all conditions was insufficient to reliably determine individual relationships (Figure 2).

Estimation of the regional ratio of VO_2 to Q (VO_2/Q).

For the estimation of the regional VO_2 to Q the following equation was applied (61):

$$VO_2/Q = CaO_2 - CvO2 = 0.000139 \times [Hb] \times (SaO_2 - SvO_2)$$

where, VO_2 is the local (regional) VO_2, Q is the local blood flow rate (each in ml/min), CaO_2 is the inflowing arterial oxygen concentration, CvO_2 is the local out-flowing venous oxygen concentration; [Hb] is hemoglobin concentration, g/dl, SaO_2 is the percentage arterial oxygen saturation, and SvO_2 is the percentage local venous oxygen saturation as estimated according to the formula presented in the previous paragraph.

2.7 Regional muscle blood flow (Q) measured by NIRS.

To measure regional Q under each optode, a 5-mg/ml bolus of ICG (Pulsion ICG, ViCare Medical) was injected into the femoral vein followed by a rapid 10-ml flush of isotonic saline (Boushel et al., 2000). Tissue microcirculatory ICG following the injection was detected transcutaneously by the NIRO 200, measuring light attenuation at 775, 813, 850, and 913 nm, and was analyzed using an algorithm incorporating the modified Beer-Lambert law (Duncan et al., 1995, van der Zee et al., 1992). Since the measured light attenuation in the tissue is influenced by ICG as well as oxy- and deoxyhemoglobin and myoglobin concentration, the contribution of ICG to the light absorption signal was determined using dedicated NIRS software (N200ICG MFC Application) (Vogiatzis et al., 2015).

Blood flow under each of the six optodes was calculated from the rate of tissue ICG accumulation over time according to the Sapirstein principle (Saperstein, 1956).

2.8 Estimation of the Regional VO_2

Regional VO_2 was calculated as the product of regional VO_2/Q and the corresponding value of regional Q (Vogiatzis et al., 2015): $VO_2 = (VO_2 / Q) \times Q$

2.9 Blood-gas analysis and calculations.

Percentage of arterial and venous oxygen saturation (SaO_2, SvO_2), arterial and venous tensions of O_2 (PaO_2, PvO_2) and CO_2 ($PaCO_2$, $PvCO_2$) and pH, haemoglobin concentration and lactate concentration were measured from arterial and femoral venous blood samples by electrodes and CO-oximetry (ABL 625, Radiometer, Copenhagen, Denmark). Arterial and venous oxygen content (CaO_2, CvO_2) were computed as follows: $[C(z)O_2 = (1.39 \times Hb \times SxO_2) + (0.003 \times PxO_2)]$ where the symbol (z) denotes either arterial (a) or femoral venous (v) blood. The blood-gas analyzer was auto-calibrated every 4 h throughout the day, and calibrating gases of known concentrations were run before each set of measurements. Blood-gas measurements were corrected for subject's tympanic temperature taken during withdrawal of each arterial

blood gas sample. The product of regional muscle blood flow and arterial oxygen content was used to calculate regional muscle oxygen delivery. Regional muscle fractional oxygen extraction was calculated by dividing regional arterio-venous oxygen difference by arterial oxygen content multiplied by 100.

2.10 Statistical analysis.

Data are reported as means ± SD or SEM. The Shapiro-Wilk test revealed that all data were normally distributed. As an index of regional heterogeneity, we calculated the coefficient of variation (the ratio of standard deviation to mean), also termed relative dispersion (RD), of all components (i.e., VO_2, Q, VO_2/Q, $StiO_2$) individually over the six optodes for each condition in each COPD patient. Paired sample t-test was used to compare mean values of subcutaneous adipose tissue thickness among different sites of vastus lateralis. Linear regression was used to determine the relationship between $SfvO_2$ and $StiO_2$ as well as the relationship between the RDs of $StiO_2$ and VO_2/Q across all subjects, exercise intensities and conditions. Two-way ANOVA with repeated measures was applied to detect differences across the various workloads between normoxia and hyperoxia for all the aforementioned dispersion indexes and physiological responses. When ANOVA detected statistical significance, pair-wise differences were identified using Tukey's honestly significant difference *post hoc* procedure. Data were analyzed using the SPSS statistical program, version 18 (SPSS Inc., Chicago, IL). The level of significance was set at $p<0.05$.

3. Results

3.1 Subject characteristics

Subject demographic and peak exercise performance characteristics are shown in Tables 1 and 2. Patients exhibited moderate to severe airway obstruction with increased static lung volumes (i.e., residual volume, functional residual capacity and total lung capacity), moderate to severe reduction in carbon monoxide diffusion capacity, and mildly reduced arterial oxygen

tension (Table 1). Patients were characterized by reduced peak exercise capacity with moderate hemoglobin desaturation, and impaired functional capacity (Table 2).

Subcutaneous adipose tissue thickness was greater ($p<0.001$-0.05) in the upper part of vastus lateralis (A1=10.41 ± 1.97 mm and A2= 10.40 ± 1.33 mm) compared to the middle (B1= 5.10 ± 1.64 mm and B2=6.50 ± 2.02 mm) and the lower (C1= 3.93±1.30 mm and C2=5.16 ± 1.60 mm) part of vastus lateralis (Figure 1), whilst there were no significant differences in adipose tissue thickness between the medial and lateral site of the muscle.

3.2 Ventilatory, hemodynamic, blood gases and metabolic responses to exercise

Table 3 summarizes ventilatory, cardiovascular, gas exchange and metabolic responses at rest and during exercise in normoxia and hyperoxia. Mean arterial blood pressure, minute ventilation, arterial, and venous lactate concentration were lower in hyperoxic compared to normoxic exercise ($p<0.05$-0.001), whilst arterial and venous partial tensions of both oxygen and carbon dioxide as well as arterial oxygen content were greater in hyperoxia compared to normoxia ($p<0.05$-0.001). Heart rate, arterio-venous oxygen difference and pH did not significantly differ between the two F_IO_2 conditions during exercise (Table 3).

3.3 Effect of exercise intensity and site of measurement on vastus lateralis muscle VO_2 and Q

Mean values for all COPD patients for regional muscle VO_2, Q, arterio-venous oxygen difference, oxygen delivery, fractional oxygen extraction and $StiO_2$ across all exercise intensities in normoxia and hyperoxia are shown for all six-probe positions in Table 4. Looking at mean values, regional muscle VO_2, Q and oxygen delivery increased from rest up to 50% of WRpeak and decreased at 80% of WRpeak in normoxia. In contrast, in hyperoxia, regional muscle VO_2, Q and oxygen delivery increased throughout the exercise protocol ($p<0.01$-0.001). In hyperoxia compared to normoxia across the various workloads mean values for regional fractional oxygen extraction were lower, whereas regional muscle VO_2, Q, oxygen delivery, arterio-venous oxygen difference and $StiO_2$ were greater ($p<0.01$-0.001). Regional muscle VO_2, Q, oxygen delivery,

fractional oxygen extraction and arterio-venous oxygen difference were lower in the upper compared with the lower part of vastus lateralis; $StiO_2$ was higher in the upper part of vastus lateralis muscle (Table 4). In addition, during normoxia the average local muscle VO_2/Q ratio was 0.12±0.02 whilst during hyperoxia it was 0.10±0.01 with no significant difference between the two conditions.

3.4 Distribution of regional muscle VO_2, Q, VO_2/Q.

Figure 3 summarizes the relative dispersion (RD) of regional muscle Q, VO_2, $StiO_2$, and VO_2/Q during the exercise for every condition for each COPD patient. Figure 3 displays how well VO_2 and Q were correlated across the different exercises intensities in each condition such that RD of VO_2/Q and $StiO_2$ were much lower and independent of exercise intensity or F_IO_2. In addition, the correlations between the RDs of $StiO_2$ and VO_2/Q across all subjects, rest, exercise intensities and environmental conditions were linear with an R^2 value of 0.80 ($p<0.001$).

Figure 4 shows the mean RD values for the six patients for the following indexes: i.e., Q, VO_2, VO_2/Q and $StiO_2$ during rest and across the four exercise intensities in normoxia and hyperoxia. For Q, RD increased with increasing exercise intensity exercise ($p<0.001$) in both normoxia and hyperoxia, whilst RD of Q was greater in normoxia compared to hyperoxia ($p=0.024$). For VO_2, RD increased in both normoxia and hyperoxia with increasing exercise intensity ($p<0.001$), with no difference between the two conditions ($p=0.096$). Mean RDs for both VO_2/Q (mean RD ranged between 0.12 and 0.08 in normoxia vs 0.13 to 0.09 in hyperoxia) and $StiO_2$ (mean RD ranged between 0.11 and 0.07 in normoxia vs 0.10 to 0.07 in hyperoxia) were small and moreover were unaffected by exercise intensity or F_IO_2 ($p>0.05$).

4. Discussion

4.1 Main findings

The present study constitutes the first experimental attempt to simultaneously measure regional locomotor muscle Q and VO_2 and their ratio (VO_2/Q) at rest and during progressive

exercise in patients with COPD. The main finding of the study is that in normoxia despite considerable heterogeneity of Q and VO_2 within the different regions of vastus lateralis muscle, local muscle ratio of VO_2/Q (~0.12) as well as average RD of VO_2/Q (~0.10) was minimal and unaffected by increased exercise intensity, thus representing a small amount of heterogeneity and possibly minimal impact of heterogeneity on muscle oxygen availability. This notion is also supported by the finding that neither local muscle ratio of VO_2/Q (~0.10) nor average RD of VO_2/Q (~0.11) differed when regional muscle oxygen delivery significantly increased during hyperoxia (control condition) compared to normoxia. These findings expand previous suggestions (Maltais et al., 2001, Richardson et al., 1999) that patients with COPD posses the capacity to tightly regulate regional muscle Q relative to VO_2, albeit at submaximal levels of exercise.

4.2 Physiological implications of regional muscle VO_2/Q heterogeneity

The present study constitutes the first experimental attempt of simultaneously measuring regional muscle Q and metabolism and their ratio (VO_2/Q) at rest and during progressive exercise in patients with COPD. The findings of the present study indicate a very small average local muscle ratio of VO_2/Q (~0.11) and average RD of VO_2/Q (~0.10) in quadriceps muscles of patients with COPD, whilst both values are highly comparable with those obtained from studies in healthy subjects (30-32, 44, 61). Indeed, studies by Kalliokoski (30-32) and Mizuno (44) using PET imaging, reported average local muscle ratio of VO_2/Q ranging from 0.04 to 0.08 during one-legged isometric or cycling exercise. Recently, a study from our group that employed NIRS to assess VO_2/Q heterogeneity across six locations over the vastus lateralis, reported an average RD of VO_2/Q of 0.13 that was unaffected by progressive exercise loading or environmental condition (normoxia and hypoxia) (61). Taking into consideration the findings of the present and previous studies in healthy subjects, it is suggested that in patients with COPD local muscle Q remains closely matched to local muscle VO_2. This is indicative of little

~~functional heterogeneity of Q to metabolism at least over the range of submaximal levels of exercise imposed on COPD in the present study.~~

4.2 Potential mechanisms accounting for the low heterogeneity of local muscle VO_2/Q ratio

Tight matching between Q and VO_2 across progressively increasing levels of exercise was manifested despite a considerable increase in heterogeneity of Q and VO_2 (Figures 3 and 4). It has previously been demonstrated that heterogeneity of Q within the exercising muscles may induce a decrease in oxygen delivery of less well perfused tissues leading to a fall in mitochondrial PO_2 (Heionen et al., 2015, Kalliokoski et al., 2000, 2001, Koga et al., 2014, Piiper and Haab, 1991, Piiper, 2000, Walley, 1996). However, recent evidence suggests (Cano et al., 2015) that human muscles may exhibit an automatic compensatory mechanism that works in the direction of restoring muscle VO_2 when oxygen availability is reduced. Indeed, Cano et al. (2015) recently demonstrated that the lower the mitochondrial PO_2, the higher will be the PO_2 difference between the muscle microvasculature and the mitochondria, when other factors are equal. This would facilitate the diffusion process and provide an autonomic compensatory mechanism to restore VO_2 by extracting more oxygen from the arterial oxygenation. This in turn pushes the VO_2/Q ratio back towards normal levels (Cano et al., 2015).

This self-limited mechanism is reinforced by our findings as we have demonstrated that regional muscle arterio-venous oxygen difference and regional muscle fractional oxygen extraction were greater in normoxia compared to hyperoxia (Table 4) most likely to mitigate the lower regional muscle oxygen delivery in normoxia (Table 4) (Maltais et al., 2001) and normalize the VO_2/Q ratio. Adversely, during hyperoxia, as the PaO_2 increases (Table 3), the proposed self-correcting mechanism of the exercising muscles may reduce the capillary-to-mitochondrial PO_2 diffusion gradient, thus normalizing the balance between regional muscle oxygen supply and demand (Cano et al., 2015). This may provide an additional mechanism,

which may explain the similar RDs of VO_2/Q observed between the two environmental conditions accross different exercise intensities in patients with COPD (Figure 4).

Interestingly, the reported heterogeneity of local muscle VO_2/Q (Figure 4) in the present study appears to be much less than that observed between alveolar ventilation and perfusion (V_A/Q) in the lungs of patients with COPD (Wagner et al., 1977). Indeed, RD of VO_2/Q ratio reported in the present study appears to be only about $^1/_{10}$ of that of V_A/Q in the lung of patients with COPD (Wagner et al., 1977) indicating that muscle heterogeneity of VO_2/Q has less of an impact on O_2 transport than that observed in lung V_A/Q heterogeneity (Cano et al., 2015).

4.3 NIRS-based $StiO_2$ for assessing muscle VO_2/Q heterogeneity

The study of matching local muscle Q to VO_2 in humans during exercise has been challenging and several techniques have been employed such as PET and MRI (Kalliokoski et al., 2006). Although the abovementioned techniques are non-invasive, both are sensitive to motion artifact and require well-established laboratories and experienced personnel for data analysis and interpretation. NIRS is an optical, noninvasive technique that can be easily used in the human muscles either during static or dynamic exercise whilst offers real-time and rapidly responsive absolute index of local oxygen saturation ($StiO_2$).

Along these lines, we have demonstrated that RDs of $StiO_2$ and VO_2/Q were almost identical (Figures 3 and 4) whilst the correlation between the RDs of $StiO_2$ and VO_2/Q across all subjects, exercise intensities and environmental conditions was linear, positive and highly significant with an R^2 value of 0.80. Note, however, that the RD of $StiO_2$ does not depend on the equation relating femoral vein O_2 saturation to $StiO_2$. Whilst NIRS reflects 70% of venular blood and 30% arterial blood (Boushel et al., 2001), however, regional $StiO_2$ must reflect regional venous/intracellular oxygenation since all regions see the same arterial value. Furthermore, the RD of $StiO_2$ itself is a direct measure of heterogeneity in local muscle oxygenation and can thus

be employed as a non-invasive surrogate to VO_2/Q heterogeneity given that the RD of $StiO_2$ closely mirrors the RD of VO_2/Q in patients with chronic diseases (Figures 3 and 4).

4.4 Heterogeneity of regional muscle Q and VO_2

In the present study, we found that heterogeneity of regional muscle Q significantly increased with exercise intensity (Figures 3 and 4) in both environmental conditions. This is different to what we and others have so far reported in healthy individuals across progressive exercise loading where regional muscle Q and VO_2 heterogeneity was unaffected by exercise intensity or environmental condition (Heinonen et al., 2010, 2011, 2012, Kalliokoski et al., 2000, 2001, 2003, 2005, Laaksonen et al., 2003, Mizuno et al., 2003, Vogiatzis et al., 2015). A possible explanation for this discrepancy is that intrinsic abnormalities previously reported in the muscles of COPD patients (Maltais et al., 2014) may impair homogeneous distribution of blood flow within different muscle regions. Indeed, the total number of capillaries and number of capillaries per muscle fiber has been reported as reduced in patients with COPD (Eliason et al., 2010, Gosker et al., 2007a, Maltais et al., 2000). Recently Eliason et al. (2010) demonstrated that muscle-to-capillary interface - a sensitive marker for changes in capillary network of limb muscles - is disturbed in patients with moderate and severe COPD compared to age-matched healthy individuals. Such a structural disturbance would be expected to impair homogeneous perfusion within the exercising muscles during exercise of progressive intensity (Eliason et al., 2010). In addition, it is known that type I fibres demonstrate lower regional muscle Q heterogeneity during exercise (Laughlin et al., 2012), because this fibre type exhibits up-regulation of vasodilation mechanisms and reduced alpha-adrenergic-mediated vasoconstriction compared to type II fibres (Behnke et al., 2011, McAllister, 2003). Thus, the well-documented alternations in muscle fiber composition in COPD (e.g. decrease in proportion of type I fibres and increase in proportion of type II fibres) (Eliason et al., 2010, Gosker et al., 2007a, Maltais et

al., 2000) may further explain inhomogeneous distribution of blood flow within the vastus lateralis muscle during exercise of increasing intensity.

We have also demonstrated that heterogeneity of local muscle VO_2 increased with exercise intensity (Figure 4) under both environmental conditions. A possible explanation is that in patients with COPD the decreased energy dependence on oxidation possibly disrupts the homogeneous distribution of VO_2 as exercise intensity increases (Coyle et al., 1992, Hunter et al., 2001). It is recognized that different muscle fiber types have different metabolic potential and O_2 demands during exercise (Coyle et al., 1992, Hunter et al., 2001). In COPD, the decrease in oxidative type I fiber distribution shifts the metabolic dependence of muscle contraction towards glycolytic metabolism and thus energy production from oxidative ATP formation is much less (Picard et al., 2008, Richardson et al., 1999, Sala et al., 1999, van den Borst et al., 2013). In addition, metabolic alternations in muscle fibres, namely reduced oxidative enzyme activity and mitochondrial dysfunction, may further compromise the oxidative metabolic capacity and therefore may increase the heterogeneity of regional muscle VO_2 in the exercising muscles (Grosker et al., 2007a,b, Maltais et al, 2000, Picard et al., 2008). Another potential explanation for greater heterogeneity of local muscle VO_2 in COPD compared to healthy individuals may be different muscle recruitment patterns during exercise. Specifically, in healthy individuals, it is expected that oxidative type I fibres are activated from the beginning of exercise, whilst type II are expected to be recruited at higher work rates (Jones et al., 2004). However, this may not be the case for the COPD population, where due to alternations in muscle fibre composition (Grosker et al., 2007a,b, Maltais et al, 2000, van den Borst et al., 2013), it is expected that type II fibres be recruited earlier during the exercise task compared to healthy individuals.

The present findings also demonstrate that heterogeneity of Q and VO_2 at moderate and intense exercise loads (50 and 80 % peak) was lower in hyperoxia compared to normoxia

(Figure 5). Two potential mechanisms most likely accounted for this finding. Firstly, by inspiring pure oxygen (i.e., F_IO_2: 1.0), the greater increase in arterial oxygen content and in oxygen delivery observed (Maltais et al., 2001, Richardson et al., 1999) (Table 4), possibly enhanced evenness of oxygen supply to the working muscles, thus resulting in less heterogeneity within the different muscle regions. In addition, it is well documented that hyperoxia causes peripheral vasoconstriction (Welch et al., 1997), which would be expected to preferentially constrict the most well-oxygenated regions, and thereby even out flow differences, especially when metabolic demand is increased during exercise.

4.5 Methodological considerations

In our earlier study (Vogiatzis et al., 2015) we demonstrated that NIRS could be used to assess the distribution of local muscle VO_2 with respect to local muscle Q in healthy individuals. In the present study regional SvO_2 was inferred from the regional NIRS oxygen saturation signal ($StiO_2$) using a linear relationship between mean $StiO_2$ and femoral venous oxygen saturation established by varying both exercise intensities and F_IO_2 (Figure 2). Although NIRS has been advocated for use in physiological and clinical studies there are, however, limitations in extrapolating regional SvO_2 from $StiO_2$. Specifically, a possible contributor to the NIRS signal is the level of intracellular MbO_2 desaturation during exercise. Evidence exists that MbO_2 desaturation proportionally increases with progressive exercise intensity (Belardinelli et al., 1995), whilst a 50–60% of MbO_2 desaturation can occur only at 50% of maximal VO_2 (Richardson et al., 1995). In addition, femoral venous O_2 represents the sum of all blood returning from the exercising leg, whereas the NIRS based $StiO_2$ signal originates in the exercising vastus lateralis muscle only (Chance et al., 1998) and thus it is possible that the venous blood sampled did not accurately represent quadriceps muscle because of contamination by blood originating from non-exercising tissues. Nevertheless, Mancini et al. (1994) demonstrated an almost identical correlation between SvO_2 and $StiO_2$ during forearm exercise,

when sampling blood from a vein that was closely drained the exercising muscle (Mancini et al., 1994).

In the current study the range of SvO_2 and $StiO_2$ was not sufficiently great to give a reliable relationship, although overall it was highly significant (r=0.38, p=0.003) and the data completely overlaid those previously seen in healthy subjects (Vogiatzis et al., 2015) (Figure 2). For this reason, we used the empirical equation previously found in healthy subjects (Vogiatzis et al., 2015) to estimate local venous oxygen saturation from local $StiO_2$.

Another issue that merits consideration is that microvascular, cutaneous circulation and subcutaneous adipose tissues may affect NIRS muscle signals. Indeed, skin blood flow can increase markedly as core temperature increases during high intensity and prolonged exercise; however, both these exercise conditions were not applied in the present study and thus the potential influence of cutaneous vasodilation on NIRS-derived measures of $StiO_2$ should have been minimal if any. Morover, the study by Tew et al. (2010) as well as a recent study by Messere and Roatta (2013) indicated that the NIRS method used in the present study (Spatially Resolved Spectroscopy) effectively rejects interference by alterations in muscle blood volume and skin blood flow, effectively eliminating contamination from skin and subcutaneous tissues during both static or dynamic exercise (Messere and Roatta, 2013, Tew et al., 2010). Moreover, even the fact that our subjects were elderly and untrained, subcutaneous adipose tissue was only ~10 mm in the upper part of vastus lateralis and ranged between 4-6 mm in the middle and lower part of vastus lateralis. These values are lower from the suggested cut off point of 20 mm, which is reported to make NIRS measurements meaningless in terms of investigating skeletal muscle oxygenation profile responses at rest and during exercise (Grassi and Quaresima 2016).

The present study focused on six regions of vastus lateralis muscle and thus the full extent of heterogeneity of VO_2, Q and VO_2/Q may not have been captured. The reason for choosing just 6 regions was limitation in technology availability of multichannel NIRS. This study

required three separate NIR spectrophotometers, each with two measuring optodes. In addition, there is limited space on the thigh to place a large number of optodes, and also there is potential for light interference between optodes when these are placed too closely to each other (Vogiatzis et al., 2015). Thus, given the current state of technical development, placing additional optodes may not have been helpful. However, if an array of optodes and supporting software and hardware could be developed to increase the spatial resolution, without interference, better capture of the extent of heterogeneity would likely be possible.

Potential contribution to heterogeneity of other currently inaccessible regions of quadriceps muscle by NIRS needs to be further considered. Indeed, the study by Kalliokoski et al. (2000) in healthy individuals showed differences in heterogeneity of Q among the quadriceps muscle regions that was characterized by a decrease in the heterogeneity of Q in those exercising muscles in which Q was the highest (vastus intermedius and vastus medialis) as compared to those muscles with the lowest Q (vastus lateralis and rectus femoris) during exercise. More recently, it was shown that superficial and deep muscle NIRS readings are different in healthy individuals at rest and during exercise (Okushima et al., 2015).

4.6 Conclusions

In conclusion, this study provides evidence that patients with moderately severe COPD maintain the capacity to tightly regulate regional muscle blood flow relative to metabolic demand during submaximal intense exercise loads. Our findings ~~reinforces the notion of a skeletal muscle metabolic reserve previously proposed in these patients. It also~~ indicate that regional muscle metabolism/blood flow heterogeneity is unlikely to be a significant contributor to the overall muscle O_2 availability during the range of submaximal exercise intensities that are often employed in the pulmonary rehabilitation setting.

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Table 1. Demographic, anthropometric and baseline pulmonary function data

	Demographic/Anthropometric
Age, yr	67 ± 7
Gender, m/f	4/2
Height, cm	168 ± 8
Weight, kg	71 ± 3
BMI, kg/m^2	25.4 ± 3.1
FFMI, kg/m^2	18.1 ± 0.9
	Pulmonary function
FEV_1, liters	1.10 ± 0.2
FEV_1, % predicted	46 ± 12
FVC, liters	2.8 ± 0.6
FVC, % predicted	77 ± 13
FEV_1 / FVC	39 ± 9
IC, liters	2.0 ± 0.6
IC, % predicted	74 ± 25
RV, liters	4.3 ± 1.4
RV, % predicted	207 ± 51
FRC, liters	5.9 ± 1.1
FRC, % predicted	181 ± 47
TLC, liters	7.9 ± 1.9
TLC, % predicted	130 ± 14
RV/TLC, %	56 ± 12
IC/TLC, %	27 ± 9
TL_{CO}, % predicted	54 ± 11
PaO_2, mmHg	84 ± 8
$PaCO_2$, mmHg	41 ± 6
SaO_2, %	94 ± 2

Values are expressed as means ± SD for 6 subjects. BMI, body mass index; FFMI, fat free mass index; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; IC, inspiratory capacity; RV, residual volume; FRC, functional residual capacity; TLC, total lung capacity; TL_{CO}, diffusing capacity of the lung for carbon monoxide; PaO_2, partial tension of arterial oxygen; $PaCO_2$, partial tension of arterial carbon dioxide; SaO_2, arterial oxygen saturation.

Table 2. Peak exercise data and functional capacity

WR_{peak}, W	59 ± 16
VO_{2peak}, l /min	1.10 ± 0.4
$VO_{2peak,}$ % predicted	46 ± 7
HR_{peak}, beats/min	121 ± 14
V_{Epeak}, l/min	41 ± 12
V_{Tpeak}, liters	1.21 ± 0.4
f_{peak}, breaths/min	33 ± 3
SpO_2, %	89 ± 2
Borg dyspnoea scores	7 ± 2
Borg leg effort scores	5 ± 3
6-minute walking test, m	419 ± 34
6-minute walking test, % predicted	73 ± 9
Quadriceps muscle force, kg	28 ± 6
Quadriceps muscle force, % predicted	61 ± 12
Quadriceps muscle endurance, sec	35 ± 17
Quadriceps muscle endurance, % predicted	43 ± 15

Values are expressed as means ± SD for 6 subjects. WR_{peak}, peak work rate; VO_{2peak}, peak oxygen uptake; HR_{peak}, peak heart rate; V_{Epeak}, peak minute ventilation; V_{Tpeak}, peak tidal volume; f_{peak}, peak breathing frequency; SpO_2, arterial oxygen saturation assessed by pulse oximetry.

Table 3. Ventilatoty, hemodynamic, blood gases and metabolic responses to exercise

	Normoxia					Hyperoxia				
	Rest	**Unload**	**20%**	**50%**	**80%**	**Rest**	**Unload**	**20%**	**50%**	**80%**
Watts	-	5	14±3	33±5	48±6	-	5	14±3	33±5	48±6
HR, beats/min	72±3	78±3	84±4	95±4	109±6	68±3	74±3	79±4	94±3	105±5
MAP, mmHg	94±8	109±8	112±9	124±11	133±10	94±9	108±0	110±11	116±11†	122±10†
V_E, l/min	13±1	18±2	22±3	29±3	35±4	11±1†	16±2†	20±2†	27±2†	32±2†
V_T, l	0.79±0.07	0.94±0.09	1.06±0.11	1.23±0.13	1.30±0.11	0.69±0.06	0.91±0.09	1.02±0.08	1.18±0.11	1.25±0.13
Bf, breaths/min	17±1	19±2	22±2	24±2	27±3	17±1	18±1	20±2	23±2	27±2
PaO_2, mmHg	85±5	73±3	78±6	75±5	72±3	420±22†	441±25†	450±18†	483±11†	455±15†
PvO_2, mmHg	27±1	23±1	23±1	24±1	25±2	29±1	27±1†	28±2†	28±1†	29±1†
$PaCO_2$, mmHg	39±3	42±2	43±2	43±2	42±2	40±2	43±2	44±2	47±2†	48±3†
$PvCO_2$, mmHg	51±2	55±2	59±2	63±3	64±2	52±2	59±2†	63±3†	69±3†	75±3†
CaO_2, ml/l	192±6	194±7	192±5	190±6	187±8	201±7†	201±5†	199±7†	201±5†	205±6†
$C(a\text{-}v)O_2$, ml/l	110±5	130±7	132±6	131±5	129±5	108±7	123±6	127±7	128±5	129±5
Arterial PH	7.37±0.01	7.36±0.01	7.34±0.01	7.33±0.01	7.38±0.01	7.38±0.01	7.36±0.01	7.35±0.01	7.33±0.01	7.31±0.01
Arterial La, mmol/l	0.82±0.11	1.00±0.06	1.13±0.11	2.18±0.31	3.94±0.54	0.77±0.08	0.87±0.07	0.98±0.10	1.95±0.46	2.77±0.58†
Venous pH	7.34±0.01	7.32±0.01	7.29±0.01	7.25±0.02	7.24±0.01	7.33±0.01	7.30±0.01	7.27±0.01	7.24±0.02	7.20±0.01
Venous La, mmol/l	1.12±0.09	1.25±0.06	1.52±0.15	3.18±0.55	4.50±0.62	1.15±0.08	1.20±0.07	1.25±0.13	2.60±0.66	3.36±0.58†

Values are expressed as means ± SEM for 6 subjects. HR, heart rate; MAP, mean arterial blood pressure, V_E, minute ventilation; V_T, tidal volume; Bf, breathing frequency; PaO_2, partial tension of arterial oxygen, PvO_2, partial tension of venous oxygen, $PaCO_2$, partial tension of arterial carbon dioxide, $PvCO_2$, partial tension of venous carbon dioxide; CaO_2, arterial oxygen content; $C(a\text{-}v)O_2$, arterio-venous oxygen content difference; La, lactate concentration. Crosses denote significant differences from normoxia at identical levels of exercise intensity.

Table 4. Effects of exercise for each site of measurement on vastus lateralis muscle

		Normoxia					Hyperoxia				
	Probes	**Rest**	**Unload**	**20%**	**50%**	**80%**	**Rest**	**Unload**	**20%**	**50%**	**80%**
						VO_2 (ml/min/100g)					
Upper	**A1**	0.23	0.52‡	0.71‡	1.01‡	0.59‡	0.25	0.65‡	0.81‡	1.05‡	1.16‡
	A2	0.20	0.47‡	0.52‡	0.52‡	0.26‡	0.32	0.65‡	0.66‡	0.67‡	0.49‡
Middle	**B1**	0.32	0.98	1.84	3.46	2.30	0.31	1.25	2.02	3.20	3.26
	B2	0.26	0.66	1.15	2.03	1.05	0.29	0.95	1.43	2.39	2.49
Lower	**C1**	0.22	1.04	2.33	5.21	4.32	0.32	1.52‡	2.78	5.37	7.07
	C2	0.26	0.93	1.67	3.92	2.27	0.30	1.26‡	2.04	3.49	4.65
	mean	**0.25***	**0.77***	**1.37***	**2.69***	**1.80**	**0.30*†**	**1.05*†**	**1.62*†**	**2.69**	**3.19†**
	SEM	**0.02**	**0.10**	**0.28**	**0.74**	**0.61**	**0.01**	**0.15**	**0.33**	**0.71**	**0.98**
						Q (ml/min/100g)					
Upper	**A1**	1.9	4.5‡	6.2‡	8.7‡	5.2‡	2.0	5.2‡	6.6‡	8.2‡	8.5‡
	A2	1.8	4.1‡	4.7‡	4.6‡	2.3‡	2.6	5.3‡	5.5‡	5.4‡	3.8‡
Middle	**B1**	2.4	7.6	14.1	27.3	18.4	2.2	8.7	14.0	22.4	22.3
	B2	2.1	5.5	9.5	17.2	8.7	2.1	7.0	10.5	17.1	17.3
Lower	**C1**	1.4	7.4	16.1	35.7	30.6	1.9	9.7	17.5	33.4	41.8
	C2	1.8	7.0	12.5	29.9	18.6	2.0	8.6	13.7	23.4	30.0
	mean	**1.9***	**6.0***	**10.5***	**20.6***	**14.0**	**2.1***	**7.4*†**	**11.3*†**	**18.3†**	**20.6†**
	SEM	**0.1**	**0.6**	**1.8**	**5.1**	**4.3**	**0.1**	**0.8**	**1.9**	**4.2**	**5.7**
						Oxygen delivery (ml/min/100g)					
Upper	**A1**	0.38	0.90‡	1.22‡	1.69‡	0.95‡	0.42	1.13‡	1.42‡	1.80‡	1.94‡
	A2	0.34	0.84‡	0.95‡	0.91‡	0.43‡	0.54	1.15‡	1.18‡	1.18‡	0.85‡
Middle	**B1**	0.45	1.50	2.73	5.24	3.39	0.45	1.88	3.06	4.84	4.97
	B2	0.40	1.10	1.87	3.32	1.57	0.43	1.51	2.26	3.71	3.92
Lower	**C1**	0.26	1.42	3.12	6.93	5.79	0.40	2.10	3.80‡	7.32‡	9.45
	C2	0.34	1.38	2.43	5.73	3.36	0.41	1.86	2.99‡	5.12‡	6.82
	mean	**0.36***	**1.19***	**2.05**	**3.97***	**2.58**	**0.44*†**	**1.60*†**	**2.45*†**	**3.99**	**4.66†**
	SEM	**0.03**	**0.12**	**0.35**	**0.97**	**0.81**	**0.02**	**0.17**	**0.42**	**0.93**	**1.29**
						Arterio-venous oxygen difference (ml/100ml)					
Upper	**A1**	11.8	11.5	11.4	11.6	11.9	13.0	12.4	11.8	12.3	12.6
	A2	11.1	10.9	10.8	11.0	11.4	12.4	12.1	11.8	12.4	12.6
Middle	**B1**	12.9	12.1	12.2	12.2	12.1	13.9	13.3	13.0	13.2	13.4
	B2	12.2	11.5	11.5	11.6	11.9	13.9	13.2	12.8	13.2	13.4
Lower	**C1**	15.2	13.7	13.8	13.8	13.6	16.2	15.0	14.7	15.0	15.3
	C2	13.7	12.5	12.5	12.5	12.5	14.7	13.9	13.5	13.8	13.9
	mean	**12.8**	**12.0**	**12.0**	**12.1**	**12.2**	**14.0†**	**13.3†**	**12.9†**	**13.3†**	**13.5†**
	SEM	**0.6**	**0.4**	**0.4**	**0.4**	**0.3**	**0.5**	**0.4**	**0.4**	**0.4**	**0.4**
						Oxygen extraction (%)					
Upper	**A1**	60.8‡	58.3‡	58.4‡	60.2‡	62.7‡	60.6‡	57.7‡	55.6‡	56.9‡	57.5‡
	A2	57.3‡	55.6‡	55.5‡	57.4‡	60.5‡	58.4‡	56.1‡	55.8‡	57.5‡	57.7‡
Middle	**B1**	66.3	61.9	62.6	63.2	63.9	64.8	61.7	60.9	60.9	60.9
	B2	62.9	58.7	59.3	60.3	63.0	64.9	61.3	60.4	61.2	61.1
Lower	**C1**	77.9	69.5	70.9	71.8	71.4	75.6	70.0	69.0	69.6	69.8
	C2	70.8	64.0	64.5	65.2	66.0	68.7	64.8	63.3	63.9	63.3
	mean	**66.0**	**61.3**	**61.9**	**63.0**	**64.6**	**65.5**	**61.9**	**60.8**	**61.7†**	**61.7†**
	SEM	**3.0**	**2.0**	**2.2**	**2.1**	**1.5**	**2.5**	**2.0**	**2.0**	**1.9**	**1.9**
						$StiO_2$					
Upper	**A1**	62.0‡	63.1‡	63.1‡	61.5‡	59.6‡	64.7‡	67.2‡	69.1‡	68.1‡	67.4‡
	A2	64.7‡	65.1‡	65.3‡	63.6‡	61.3‡	66.5‡	68.5‡	68.9‡	67.6‡	67.2‡
Middle	**B1**	57.9	60.4	60.0	59.3	58.8	61.2	63.9	64.6	64.7	64.5
	B2	60.5	62.8	62.5	61.5	59.5	61.2	64.3	65.0	64.5	64.4
Lower	**C1**	48.9	54.8	53.8	52.9	53.1	52.3	57.0	57.9	57.4	57.1
	C2	54.4	58.9	58.7	57.9	57.2	58.0	61.3	62.6	62.2	62.5
	mean	**58.1**	**60.9**	**60.6**	**59.5**	**58.3**	**60.6†**	**63.7†**	**64.7†**	**64.1†**	**63.8†**
	SEM	**2.3**	**1.5**	**1.7**	**1.5**	**1.2**	**2.1**	**1.7**	**1.7**	**1.6**	**1.5**

Values are expressed as means ± SEM for 6 subjects. Regional muscle oxygen consumption (VO_2), blood flow (Q), arterio-venous oxygen difference, oxygen delivery, fractional oxygen extraction and tissue oxygen saturation ($StiO_2$) for all subjects across all exercise intensities in normoxia and hyperoxia are displayed for all 6 probe positions. The number displayed for each probe position represents the mean value of all patients. A1, B1, C1: lateral site; A2, B2, C2: medial site. Asterisks denote significant differences relative to 80% of peak work rate for each condition. Crosses denote significant differences relative to normoxia at identical levels of exercise intensity. Double crosses denote significant differences between upper and lower regions of the vastus lateralis muscle.

Figure captions

Figure 1: Positioning of six optodes over the right vastus lateralis muscle of a patient with COPD.

Figure 2: Linear relationships between measured femoral venous O_2 saturation ($SfvO_2$) and blood flow-weighted average NIRS O_2 saturation ($StiO_2$) over the six optodes for all COPD patients (closed circles) and healthy subjects (open triangles) (61). The linear relationship and its corresponding Pearson correlation coefficient, r, were derived from the data previously obtained in healthy subjects (61).

Figure 3: Heterogeneity by means of relative dispersion (RD) of $StiO_2$ (closed triangles), VO_2/Q ratio (open circles), and VO_2 (closed circles) for all COPD patients under all conditions. Heterogeneity by means of RD of VO_2 tracks that of Q across all conditions and subjects, while RD of $StiO_2$ and VO_2/Q are much lower and unrelated to RD of Q.

Figure 4: Heterogeneity by means of relative dispersion (RD) of Q, VO_2, $StiO_2$ and VO_2/Q as a function of exercise intensity in normoxia (closed circles) and hyperoxia (open circles). The VO_2/Q dispersion appears unaffected by both exercise and hyperoxia, alone and in combination, reflecting tight matching between Q and VO_2 across muscle regions. Values are expressed as means ± SEM for 6 subjects. Asterisks denote significant differences from hyperoxia at identical level of exercise intensities. Crosses denote significant differences from rest for both normoxia and hyperoxia.