High-intensity exercise impairs extradiaphragmatic respiratory muscle perfusion in patients with COPD

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News and Noteworthy

We simultaneously assessed the blood flow index (BFI) in three respiratory muscles during hyperpnoea and high-intensity constant-load cycling sustained at comparable levels of work of breathing and respiratory neural drive in patients with COPD. We demonstrated that high-intensity exercise interferes with respiratory muscle perfusion as intercostal, scalene and abdominal BFI increased during hyperpnoea but not during cycling. Insufficient adjustment in respiratory muscle perfusion during exercise was associated with greater dyspnoea sensations in patients with COPD.

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Abstract

The study investigated whether high-intensity exercise interferes with inspiratory and expiratory muscle perfusion in patients with COPD. We compared respiratory local muscle perfusion between constant-load cycling (sustained at 80% WRpeak) and voluntary normocapnic hyperpnoea reproducing similar work of breathing (WoB) in 18 patients (FEV_1:58±24% predicted). Local muscle blood flow index (BFI), using indocyanine green dye and fractional oxygen saturation (%$StiO_2$) were simultaneously assessed by near-infrared spectroscopy (NIRS) over the intercostal, scalene, rectus abdominis and vastus lateralis muscles. Cardiac output (impedance cardiography), WoB (oesophageal/gastric balloon catheter), and diaphragmatic and extradiaphragmatic respiratory muscle electromyographic activity (EMG) were also assessed throughout cycling and hyperpnoea. Minute ventilation, breathing pattern, WoB and respiratory muscle EMG were comparable between cycling and hyperpnoea. During cycling, cardiac output and vastus lateralis BFI were significantly greater compared to hyperpnoea [by +4.2(2.6-5.9) L/min and +4.9(2.2-7.8) nmol/s], respectively, ($p<0.01$). Muscle BFI and %$StiO_2$ were respectively lower during cycling compared to hyperpnoea in scalene [by -3.8(-6.4- -1.2) nmol/s and -6.6(-8.2- -5.1)%], intercostal [by -1.4(-2.4- -0.4) nmol/s and -6.0(-8.6- -3.3)%] and abdominal muscles [by -1.9(-2.9- -0.8) nmol/s and -6.3(-9.1- -3.4)%] ($p<0.001$). The difference in respiratory (scalene and intercostal) muscle BFI between cycling and hyperpnoea was associated with greater dyspnoea (Borg CR10) scores ($r= -0.54$ and $r= -0.49$, respectively, $p<0.05$). These results suggest that in patients with COPD 1) locomotor muscle work during high-intensity exercise interferes with extradiaphragmatic respiratory muscle perfusion and that 2) insufficient adjustment in extradiaphragmatic respiratory muscle perfusion during high-intensity exercise may partly explain the increased sensations of dyspnoea.

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Keywords: perfusion, exercise, NIRS, COPD, respiratory muscles

Introduction

The ability to measure respiratory muscle blood flow allows the investigation of a number of physiological and pathophysiological factors that limit exercise tolerance in healthy individuals and in patients with chronic cardiorespiratory diseases.

However, traditional techniques for assessing respiratory muscle blood flow are highly invasive, exposing the individuals to unnecessary health risks (14). Near-Infrared Spectroscopy in conjunction with infusions in the circulation of the light-absorbing tracer indocyanine green dye, (NIRS-ICG technique) has been increasingly applied over the past decade to provide a less invasive and reliable method for assessing absolute and relative values (blood flow index) of local respiratory (and locomotor) muscle perfusion at rest and during exercise in healthy participants and in patients with Chronic Obstructive Pulmonary Disease (COPD) (14, 32, 33, 36, 54).

In this context, the theory of blood flow redistribution from the locomotor to respiratory muscles during high-intensity exercise (22, 23) is based on evidence in healthy and trained subjects showing a decrease in locomotor muscle blood flow when respiratory muscle work is artificially increased (and cardiac output is maximal), or an increase in locomotor muscle blood flow when respiratory muscle work is decreased (37, 38). Based on these findings, it has been postulated that owing to the high work of breathing sustained by patients with COPD during exercise, blood flow may increase in favor of the respiratory muscles, thereby compromising locomotor muscle blood flow (85).

We have previously demonstrated that in patients with COPD, intercostal muscle blood flow progressively increased during voluntary hyperpnoea over a wide range of exercise ventilations up to maximal (85). However, during graded cycling, intercostal muscle blood flow fell progressively from rest to the early stages of exercise, whilst cardiac output was rising. When cardiac output plateaued during high-intensity exercise (between 75%-100% of peak work), a greater fall in intercostal muscle perfusion occurred contrasting sharply with the respiratory muscle perfusion responses during voluntary hyperpnoea (85). Furthermore, when COPD patients breathed 21% oxygen in helium (i.e., Heliox) or 100% oxygen to reduce respiratory muscle load, there was no redistribution of blood flow between locomotor and respiratory muscles in either direction at or near peak exercise, thereby challenging the theory of blood flow redistribution between the locomotor and respiratory muscles (55, 83, 90). The aforementioned studies in COPD were, however, focused on the assessment of intercostal muscle blood flow acknowledging potential limitations such as partitioning of blood flow

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between the intercostal muscles and the diaphragm and/or movement-related artefacts (76, 83). In addition, assessment of intercostal muscle blood flow alone may not necessarily reflect the global respiratory muscle perfusion requirements during exercise in this population (76, 83).

Accordingly, the objective of this exploratory study was to investigate whether high-intensity exercise interferes with respiratory muscle perfusion in patients with COPD. Due to the inability to assess diaphragm perfusion, we focused on assessing the blood flow index and oxygenation of other muscles of respiration namely scalene, intercostal and abdominal muscles during cycling and during voluntary normocapnic hyperpnoea sustained at comparable levels of minute ventilation and breathing pattern aiming to reproduce a comparable work of breathing (WoB). We also assessed key variables such as central hemodynamic responses, diaphragmatic and extradiaphragmatic respiratory muscle activation, and locomotor muscle perfusion during both experimental conditions. We hypothesized that if at the same WoB the intercostal, scalene and abdominal muscle blood flow index were lower during cycling compared to hyperpnoea, this would suggest that high-intensity exercise interferes with respiratory muscle perfusion in patients with COPD.

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Methods

Study group

Eighteen clinically stable patients with COPD (FEV_1: 58 ± 24% predicted) according to the Global Initiative for Chronic Obstructive Lung Diseases (GOLD) participated in the study. Exclusion criteria included no participation in exercise-training programs in the year before, no long-term oxygen use and not presenting cardiovascular conditions limiting exercise tolerance, severe orthopaedic conditions, psychiatric or cognitive disorders and/or progressive neurological or neuromuscular disorders.

Study design

The Ethical Committee Research of KU Leuven/UZ Leuven, Belgium approved the study (protocol ID: S58513). Prior to patient enrolment into the study, associated risks and potential benefits of participation were explained, and patients provided their written informed consent. The study conformed to the standards set by the Declaration of Helsinki and has been registered to a database (ClinicalTrials.gov, Identifier: NCT03240640). The study is part of a broader Randomized Clinical Trial (RCT) aiming to investigate the effects of Inspiratory

Muscle Training, by Tapered Flow Resistive Loading on the shortness of breath and on postural control (Clinical Trial Identifier: NCT03240640). Data in Table 1 (baseline characteristics) and Tables 2-6 in 16 out of 18 patients obtained during rest and hyperpnoea have appeared in a recent publication of our group (73), whilst data recorded during cycling have not appeared anywhere in that, or in any other, report.

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Preliminary assessments

All patients underwent the following preliminary assessments prior to visit 1: anthropometrics, pulmonary function (61, 89) and functional capacity (six-minute walking distance test and physical activity assessments). The six-minute walking distance test was performed according to the ATS guidelines (8). Physical activity in terms of steps per day was assessed by a validated for patients with COPD activity monitor (68, 71) using standardized methodology (21).

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Experimental design

Experiments were conducted in 3 visits (Figure 1). During visit 1, patients performed assessment of respiratory muscle strength (46) and a symptom-limited cardiopulmonary exercise test on an electromagnetically braked cycle ergometer to determine peak work rate (WRpeak) (70).

During visit 2 (>48 hours after *visit 1*) patients underwent a constant-load cycling test at 80% WRpeak to the limit of tolerance (i.e., exercise duration 366 ±109 sec) aiming to record the ventilatory responses (i.e., mean tidal volume, breathing frequency and minute ventilation) that patients were requested to reproduce during the hyperpnoea trial on visit 3 (*see* below). The limit of exercise tolerance was defined as the time point at which patients signalled the inability to continue exercising or could not maintain the required pedalling rate (50 – 60 revolution/min) despite being encouraged by the investigators to carry on cycling. Before and after the constant-load cycling test, assessment of isometric quadriceps strength and quadriceps muscle contractile fatigue (Magstim Co Ltd, Whitland, UK) were performed (16).

During visit 3 (>48 hours after *visit 2*) patients initially performed a voluntary normocapnic hyperpnoea trial reproducing the ventilatory responses (i.e., mean tidal volume, breathing frequency and minute ventilation) recorded for each patient during the last 3 minutes of the constant-load exercise test performed during visit 2. Patients were seated on a chair, with bent knees (at an angle approximately 90°), and the back of the trunk was straight without been supported by the back of the chair, whilst both arms were extended forward with the palms

touching the knees.Hyperpnoea was sustained to the point patients could not maintain ventilatory responses to levels comparable to those during exercise. During the hyperpnoea test investigators provided continuous verbal guidance aiming to ensure a maximum variation in minute ventilation less than 5% throughout the test. This was facilitated by visual feedback on breathing parameters that was provided real-time on a screen monitor (73). Normocapnia was maintained by having subjects inspire from a Douglas bag containing 5% CO_2, 21% O_2 and 74% N_2, connected to a two-way non-rebreathing valve (model 2700, Hans Rudolph) by a piece of tubing (85, 86). Following a sufficient resting period [average 27 min (range: 25-31 min)], patients performed a constant-load cycling test at 80% WRpeak to the limit of tolerance. During hyperpnoea and constant-load exercise, recordings of pulmonary gas exchange and ventilatory variables were performed breath-by-breath (Vmax 229; Sensor Medics, San Diego, CA). Arterial oxygen saturation was measured continuously by a pulse oximeter and blood pressure was assessed every minute by an automated cuff monitor integrated to the cycle ergometer. Breathlessness and leg discomfort were measured by the modified Borg scale (11). During cycling, patients performed inspiratory capacity (IC) manoeuvres every two minutes to identify the degree of dynamic lung hyperinflation assuming constant total lung capacity (TLC) (64).

Subject preparation

Subjects were prepared first with a combined EMG diaphragm-electrode catheter with oesophageal and gastric balloons that were inserted nasally after topical anesthesia for the assessment of activation of the diaphragm (EMG), as well as oesophageal (Pes) and gastric (Pga) pressure measurements. Seven out of the eighteen (n=7/18) patients refused to undergo measurements of diaphragm EMG, Pes and Pga with the oesophageal catheter system. Thus, data on diaphragmatic activation, respiratory pressures and work of breathing represents 11 out of 18 patients. There were no significant differences in physiological responses during cycling and hyperpnoea between patients with diaphragm EMG and respiratory pressures measurements (n=11, male: n=7 and female: n=4) compared to those without these measurements (n=7, male: n=4 and female: n=3). Subjects were prepared with a venous catheter (Insyte Autoguard BC Winged, 22GA, 0.9 x 25mm) for the measurement of the respiratory and locomotor muscle blood flow index. Using a sterile technique, the catheter was introduced percutaneously into the right or left antecubital forearm vein, oriented in the proximal direction. The catheter was used to inject a bolus of ICG, while it was kept patent

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throughout the experiment by periodic flushing with saline. One patient did not have respiratory muscle perfusion measures due to contraindications regarding ICG injections.

Respiratory muscle pressures and work of breathing

The oesophageal/gastric balloon catheter was used for the assessment of Pes, Pga, transdiaphragmatic pressure (Pdi=Pga-Pes) and diaphragm EMG activation via five diaphragm electromyography electrode pairs (Yinghui Medical Equipment Technology Co. Ltd., Guangzhou, China). After optimal placement (20, 45, 58), the catheter was secured at the patient´s nose with tape. The diaphragm EMG signals were sampled at 2000 Hz (Micro1401-3, Cambridge Electronic Design Limited, Cambridge, UK), amplified (Biomedical amplifier, Guangzhou, China) and then recorded and processed by a data acquisition software (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK). Diaphragm EMG data were converted into root mean square (RMS) and were expressed as percentages of maximum activation (diaphragm EMG, %max) that was recorded during IC maneuvers (i.e., obtained at rest or during exercise, 20, 73). Respiratory flow signals, Pes and Pga signals were continuously sampled at 100 Hz (Micro1401-3, Cambridge Electronic Design Limited, Cambridge, UK), and then recorded and processed by the same data acquisition software (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK). Maximal Pes and Pdi pressures were measured from FRC during sniff maneuvers, and maximal Pga was measured from TLC during forced expiratory capacity maneuvers. Pes, Pga and Pdi were expressed as percentages of maximal activation and were used as indices of global inspiratory, expiratory and diaphragmatic effort, respectively (45). Pes, Pga, Pdi and WoB over one-minute periods was calculated by integrating volume and pressure generated (e.g., Pes WoB= Pes x tidal volume), then multiplied by breathing frequency (e.g., Pes WoB/min= Pes WoB x bf) and presented in L/cmH_2O/min. Pes, Pga and Pdi Pressure-Time Products (PTP) – commonly considered indices of the energy of breathing (46) – were assessed by multiplying each of the pressures by the time of muscle contraction and breathing frequency and presented in cmH_2O/sec/min.

Respiratory muscle activation

Respiratory muscles activation, for scalene [(sca), left posterior triangle of the neck], sternocleidomastoid [(scm), midpoint along the long axis of the right sternocleidomastoid muscle], parasternal intercostal [(picm), right parasternal space of the 2nd and 3rd rib 3 cm lateral to the sternum], 7^{th} intercostal [(7^{th}icm), midaxillary line, right 7^{th} intercostal space], rectus abdominis [(abd), upper right 1/3 of rectus abdominis below the costal cartilage] and

vastus lateralis [(vl), left vastus lateralis muscle 10-12 cm above the knee] was measured by surface electromyography (EMG) (Desktop Direct Transmission (DTS), NORAXON, Scottsdale, USA) (73), sampled at 2000 Hz (Micro1401-3, Cambridge Electronic Design Limited, Cambridge, UK), and then recorded and processed by a data acquisition software (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK). For EMGsca, EMGscm, EMGpicm, EMG7thicm data were expressed as percentages of maximum activation during IC maneuvers (i.e., obtained at rest or during exercise, 20, 73) and for EMGabd data were expressed as percentages of maximum activation during forced expiratory capacity maneuvers (73). EMGvl data of maximum activation were recorded during a maximal voluntary isometric contraction of the knee extensors (40). All ventilatory and respiratory pressures, WoB, respiratory and locomotor muscle activation signals used for comparisons at rest, during hyperpnoea and cycling were the average of all values recorded over the last 60 seconds at rest and during the last 30 seconds of hyperpnoea and cycling.

Central hemodynamic responses

Cardiac output was measured continuously during hyperpnoea and cycling by an impedance cardiography device (PhysioFlow PF05; Manatec Biomedical, Macheren, France, PhysioFlow). Six electrodes were placed according to the manufacturers´ instructions (53). Data points were excluded when signal quality was less than 90% (53). Cardiac output values were recorded at 1-second intervals and were the average over the last 60 seconds during rest and during the last 30 seconds of hyperpnoea and cycling trials. Systemic oxygen delivery was calculated as the product of cardiac output and arterial oxygen content; the latter was calculated using the following formula: [1.39 x hemoglobin concentration [Hb] x %SpO_2] (12). Arterio-venous oxygen content (a-vO_2) difference was calculated by dividing whole-body oxygen uptake by cardiac output (73). The oxygen extraction ratio was calculated as the ratio of the arteriovenous oxygen content (a-vO_2) difference to arterial oxygen content and expressed in percentage. Systemic vascular conductance was calculated by dividing cardiac output by the mean arterial blood pressure (73).

Muscle blood flow index by NIRS

To measure respiratory and vastus lateralis muscle blood flow index (BFI), we used the NIRS-ICG derived BFI method (32, 36, 54). Specifically, four sets of NIRS probes from two

commercial Near-Infrared Spectroscopy (NIRS, Continuous Wave, Spatially Resolved Technique, NIRO-200 and a NIRO-200NX; HAMAMATSU Photonics KK) devices were used in combination with the light-absorbing indocyanine green dye (ICG). The four NIRS probes were placed at scalene (right posterior triangle of the neck), 7th intercostal (midaxillary line, left 7th intercostal space) and rectus abdominis (upper left 1/3 of rectus abdominis below the costal cartilage) muscles (73). The fourth NIRS probe was placed over the left vastus lateralis muscle 10-12 cm above the knee (next to EMG electrode) (86). NIRS-ICG derived BFI was calculated by dividing the ICG peak concentration of the muscle by the rise time from 10 to 90% of peak according to established methods and expressed in nanomoles/sec (nmol/sec) (32, 36, 54, 73). In addition, BFI data were adjusted for resting values and expressed as fold change from rest during cycling and hyperpnoea (54). ICG injections for calculating BFI were performed at rest and during the last 30 seconds of hyperpnoea and cycling trials. ICG concentration curves data were exported by NIRS in document file format (i.e., filename extension ´txt´) and stored on disk for off-line analysis. ICG concentration curves in ´txt´ format were analyzed by using the Chart5 version 5.4.2 (ADInstruments) program. Low-pass filtering with a cutoff frequency of 0.5 Hz and smoothing window width (by using the triangular Bartlett window function) of nine points produced the smoothed curve that was used for BFI calculation (36, 54, 73).

Muscle oxygenation by NIRS

For respiratory and vastus lateralis muscle oxygenation, the same NIRS devices were used. Concentration changes in deoxy (Hb+Mb) were used as an index of muscles oxygen extraction and total (Hb+Mb) as an index of blood volume reflecting changes in microvascular conductance (vasodilation or vasoconstriction responses) for respiratory and vastus lateralis muscles (31). In addition, absolute values of NIRS derived fractional tissue O_2 saturation index (%$StiO_2$; i.e., the ratio of [oxy(Hb+Mb)] to [total(Hb+Mb)] expressed as a fraction ([oxy(Hb+Mb)]/[total(Hb+Mb)]*100) that reflect the tissue capacity to match oxygen supply relative to its metabolic demand (31, 52, 84) were also recorded. A path length of 18.6 cm was set up for all respiratory and vastus lateralis muscles. Separation distance between the NIRS light transmitter and receiver probes was 40 mm, thus allowing a maximum NIRS penetration depth of 20 mm. NIRS oxygenation data were sampled at 5 or 6 Hz and averaged during the last 60 seconds at rest and during the last 30 seconds for hyperpnoea and cycling. Adipose tissue thickness (fat + skin layer) were performed by a Harpenden 10-skinfold caliper on the scalene, 7th intercostal space, rectus abdominis and the vastus lateralis muscle (80). The mean

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values (±SD) of the 18 subjects of the adipose tissue for scalene, intercostal, abdominal and vastus lateralis muscles were 3.4 ± 1.6 mm, 8.6 ± 3.8 mm, 11.5 ± 5.0 mm and 7.5 ± 4.4 mm, respectively.

Assessment of quadriceps muscle strength and fatigue

Patients were sitting in a recumbent chair (hips extended at 120° and knees flexed at 90°) with arms crossed in front of the chest (16) for the assessment (right leg) of unpotentiated quadriceps twitch contractions (at 30, 50, 70, 80, 90, 95 and 100% of the maximum stimulator output), maximal voluntary contractions (five isometric MVC for 3 sec) and potentiated quadriceps twitch contractions (five contractions with a twitch at 100% of the power output of the stimulator) before, 10 and 35 min after the constant-load cycle exercise (5). The strain-gauge signal was transformed by an analogue force transducer (546QD; CDS Europe, Milan, Italy), amplified (Biopac mp150; Biopac Systems, Goleta, CA, USA) and then processed with a specific data acquisition and analysis program (AcqKnowledge Software, Biopac Systems, Goleta, CA, USA). The highest values recorded during MVC and during potentiated quadriceps twitch contractions was included in the analysis and expressed in predicted values (3, 74). A fall in potentiated quadriceps twitch contractions of ≥15%, 10 min after exercise was considered as a sign of significant contractile fatigue (16, 74).

Statistical analysis

Data are expressed as mean ± SD at rest, cycling and hyperpnoea or as mean difference with 95% confidence interval (lower and upper limit) for comparisons between the three conditions (i.e., at rest, cycling and hyperpnoea). The normality of all the data was examined by the Shapiro-Wilk test. Ventilatory and breathing pattern parameters, respiratory muscle pressures and WoB, respiratory and locomotor muscle activation, central hemodynamic and respiratory and locomotor blood flow and oxygenation variables recorded at rest, during hyperpnoea, and cycling were compared using repeated-measures ANOVA or by the Friedman test when normal distribution was violated. When ANOVA (or Friedman test) detected significant differences between rest, hyperpnoea, and cycling, pairwise comparisons with Bonferroni correction (for ANOVA) or using Dunn´s Multiple Comparison Test (for Friedman test) were performed as pos-hoc analysis. Changes from rest in respiratory and vastus lateralis muscle BFI and oxygenation variables between cycling and hyperpnoea tests were compared by paired t-tests when normally distributed, or by Wilcoxon signed-rank tests if normal distribution assumptions were not met. Changes in respiratory and vastus lateralis muscle BFI and oxygenation variables

between cycling and hyperpnoea tests among patients with different stages of diseases severity were compared by unpaired t-tests when normally distributed, or by Welch's Test if normal distribution assumptions were not met. Pearson´s correlation coefficient (r) was used to establish associations between BFI (expressed as the difference between cycling and hyperpnoea) and dyspnoea (expressed as the difference between cycling and hyperpnoea) for intercostal, scalene, rectus abdominis, and vastus lateralis muscles. The minimum sample size was calculated based on 80% power and a two-sided 0.05 significance level. An expected effect size [Cohens d] of 0.497 was calculated based on data from a previous study in patients with COPD (FEV_1: 51±18%predicted) (85), which demonstrated a significant decrease in intercostal muscle $\%StiO_2$ during cycling compared to voluntary normocapnic hyperpnoea. Specifically, that study (85) revealed a mean difference in intercostal muscle $\%StiO_2$ of -2.00% with a corresponding pooled SD of 4.02% between cycling (at 75% of peak work rate, ~60 watts) and hyperpnoea sustained at levels of minute ventilation similar to those recorded during cycling (~45 litres/min). The critical sample size was calculated to be 9 patients using repeated-measures ANOVA as the primary statistical analysis method. Taking into consideration the challenges imposed on patients by the invasive procedures, we decided to recruit 18 patients for obtaining a full dataset for the minimum required number of patients. Data were analyzed using the GraphPad Prism statistical software. The level of significance was set at $p<0.05$.

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Results

Subject characteristics.

Subject characteristics are shown in Table 1. Five patients were Global Initiative for COPD (GOLD) stage I, seven patients were GOLD stage II, five patients were GOLD stage III and one patient was GOLD stage IV. Patients demonstrated decreased exercise and functional capacity and inspiratory muscle strength and mildly reduced physical activity levels (77) indicated by the physical activity measures (Table 1).

Breathing pattern, symptoms and locomotor muscle fatigue

Tidal volume, breathing frequency, and duty cycle did not differ between hyperpnoea and cycling sustained at comparable levels of minute ventilation ($p>0.1$ for all comparisons, Table 2). Peak inspiratory flows did not differ between the two conditions ($p=0.97$). During cycling patients demonstrated a significant reduction from rest in inspiratory capacity ($p<0.0001$, Table

2). Specifically, the decrease from rest in inspiratory capacity during cycling did not significantly differ among patients with different stages of disease severity (GOLD I*:* -0.394 ± -0.407, GOLD II: -0.441 ± -0.480, GOLD III-IV: -0.493 ± -0.395 L, p>0.1). Dyspnoea at end of cycling was significantly higher compared to hyperpnoea (p=0.0008, Table 2). Leg discomfort at end of cycling was 7.0 ± 2.8 on the 10-Borg scale. The primary reason for stopping cycling was dyspnoea (n=7), leg discomfort alone (n=4) and the combination of both leg discomfort and dyspnoea (n=7). Compared to resting values of potentiated quadriceps twitch contraction force (11.5 ± 4.3 kg), this was significantly decreased on average by 22 ± 21% and 23 ± 17%, 10 and 35 min after the end of the constant-load exercise test (visit 2), respectively (*10 min*: 9.2 ± 4.5 kg and *40 min*: 9.1 ± 4.2 kg, both p<0.001).

Respiratory pressures and work of breathing

Pes, Pdi and expiratory Pga significantly increased from rest during both hyperpnoea and cycling trials (p<0.0001 for all comparisons) (Table 2). No significant differences in Pes, Pdi and expiratory Pga were observed between the two conditions (p>0.1 for all comparisons). Inspiratory WoB (both Pes and Pdi) significantly increased from rest during both hyperpnoea and cycling trials (p<0.0001 for all comparisons) but we did not observe any significant differences between the two conditions (p=0.44 and p=0.24, respectively) (Table 2). Expiratory WoB (Pga) significantly increased from rest only during hyperpnoea (p=0.002). However, no significant differences in expiratory WoB were found between hyperpnoea and cycling (p=0.51) (Table 2). The pressure-time products (PTP) of Pes and Pdi significantly increased from rest during both hyperpnoea and cycling (p<0.0001 for all comparisons), whereas no significant differences were found between the two conditions (p=0.35 and p=0.93, respectively) (Table 2). PTP of expiratory gastric pressure significantly increased from rest during both hyperpnoea and cycling (p=0.001 and p=0.003, respectively) and tended to be significantly greater during hyperpnoea compared to cycling (p=0.083) (Table 2).

Activation of respiratory and locomotor muscles

Activation of all respiratory muscles significantly increased from rest during hyperpnoea and cycling (p<0.0001 for all comparisons) (Table 3). Furthermore, only sternocleidomastoid muscle activation was found to be significantly greater during hyperpnoea compared to cycling

(p=0.005). As expected, vastus lateralis muscle activation significantly increased from rest (p=0.0005) and was significantly greater during cycling compared to hyperpnoea (p=0.009).

Central hemodynamic and metabolic responses

Heart rate, stroke volume, cardiac output, and oxygen consumption significantly increased from rest during hyperpnoea and cycling ($p<0.0001$ for all comparisons) and were significantly greater during cycling compared to hyperpnoea (p=0.001-0.0001) (Table 4). Furthermore, arterial oxygen saturation significantly decreased from rest during cycling (p=0.0007) and was significantly lower compared to hyperpnoea (p=0.0018) (Table 4). Systemic oxygen delivery, systemic arteriovenous oxygen content difference and oxygen extraction were significantly greater during cycling compared to hyperpnoea (p=0.0001- p=0.0033, Table 4). Mean arterial blood pressure and systemic vascular conductance were significantly increased from rest during hyperpnoea (p=0.006, p=0.009, respectively) and cycling (p=0.0001, $p<0.0001$, respectively) and were greater during cycling compared to hyperpnoea (p=0.0012, $p<0.0001$, respectively) (Table 4).

Perfusion responses of respiratory and locomotor muscles

During cycling, vastus lateralis muscle BFI significantly increased from rest (p=0.0005) and was greater compared to hyperpnoea (p=0.0005, Figure 2 D and Table 5). However, scalene (p=0.74), 7^{th} intercostal (p=0.072) and abdominal muscle BFI (p=0.093) did not significantly differ from resting levels (Figure 2 A, B and C and Table 5). Moreover, during cycling scalene (p=0.0018), intercostal (p=0.0039) and abdominal (p=0.0045) muscle BFI was significantly lower compared to hyperpnoea (Figure 2 A, B and C and Table 5). Similarly, when BFI values were expressed as fold changes from rest, vastus lateralis muscle BFI during cycling was significantly greater (p=0.001), whilst scalene (p=0.0003), intercostal (p=0.0017) and abdominal (p=0.023) muscle BFI were significantly lower compare to hyperpnoea (Figure 3 A, B and C). In addition, the pattern of change in respiratory muscle BFI (i.e., decrease) and leg muscle BFI (i.e., increase) to cycling versus hyperpnoea was the same across different stages of COPD severity (Table 6).

Oxygenation responses of respiratory and locomotor muscles

During hyperpnoea, total [Hb+Mb] concentration increased from rest in scalene (p=0.0027), 7^{th} intercostal (p=0.079) and abdominal (p=0.028) muscles (Table 5). In addition, during hyperpnoea, total [Hb+Mb] concentration was greater for the scalene (p=0.0061), 7^{th} intercostal

(p=0.054) and abdominal (p=0.033) muscles compared to cycling (Table 5). During cycling, deoxy [Hb+Mb] concentration significantly increased from rest in intercostal (p=0.009), abdominal (p=0.0027) and vastus lateralis muscle (p=0.0042) and it was found to be significantly greater for the 7th intercostal (p=0.0006) and abdominal muscles (p=0.0011) compared to hyperpnoea (Table 5). During hyperpnoea, scalene, 7th intercostal, abdominal and vastus lateralis muscle %StiO$_2$ was not different compared to that recorded at rest (p>0.05, Figure 4 and Table 5). In contrast, during cycling, a significant reduction from rest in %StiO$_2$ was observed in scalene (p<0.0001), 7th intercostal (p=0.0015), abdominal (p<0.0001) and vastus lateralis muscle (p=0.0013) (Figure 4 and Table 5). Furthermore, scalene (p<0.0001), 7th intercostal (p=0.0002) abdominal (p<0.0001) and vastus lateralis muscle (p=0.0009) %StiO$_2$ was significantly lower during cycling compared to hyperpnoea (Figure 4 and Table 5). In addition, no significant differences were found in respiratory and leg muscles %StiO$_2$ to cycling versus hyperpnoea across different stages of COPD severity (Table 6).

Associations between muscle activation, perfusion and dyspnoea during cycling and hyperpnoea

We found significant inverse relationships between the reduction in the BFI of scalene and 7th intercostal muscles and the greater dyspnoea scores in cycling compared to hyperpnoea (r=-0.54, p=0.026 and r=-0.49, p=0.043, respectively). No significant relationships were found for abdominal and vastus lateralis muscle BFI and dyspnoea scores (r=-0.32, p=0.020 and r=0.05, p=0.83, respectively). In addition, no significant relationships were found amongst differences in activation of diaphragm, scalene, parasternal, 7th intercostal and abdominal muscles and the differences in dyspnoea scores between cycling and hyperpnoea trial (p>0.1). Finally, no significant relationships were found between differences in activation of scalene, 7th intercostal and abdominal muscles and differences in their perfusion between cycling and hyperpnoea trials (p>0.1).

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Discussion

Main findings

The main findings of the present study in patients with COPD are as follows. 1) During hyperpnoea, when locomotor muscles did not compete with the respiratory muscles for the

available blood flow, intercostal, scalene, and abdominal local muscle perfusion was significantly increased from rest (Figure 2 and 3, and Table 5). However, during high-intensity exercise (i.e., 80% WRpeak), intercostal, scalene, and abdominal local muscle perfusion did not increase from rest (Figure 2 and 3, and Table 5) whilst cardiac output reached peak values (Tables 1 and 4). 2) Intercostal, scalene, and abdominal muscle oxygen extraction (inferred by deoxy [Hb+Mb]) was greater and microvascular conductance (inferred by total [Hb+Mb]) and oxygen saturation (%$StiO_2$) were lower during cycling compared to hyperpnoea (Figure 4 and Table 5). 3) Lack of increase from resting levels in respiratory muscle perfusion during exercise compared to hyperpnoea occurred at comparable levels of respiratory muscle activation and work of breathing (Table 3) and it was associated with greater dyspnoea sensations. Collectively, these results suggest that high-intensity exercise interferes with extradiaphragmatic respiratory muscle perfusion and that limitations in extradiaphragmatic respiratory muscle perfusion during cycling may, in part, explain the increased dyspnoea sensation in exercising patients with COPD.

Mechanisms of insufficient adjustments in respiratory muscle perfusion during cycling

We considered several factors that might be singly or jointly responsible for the insufficient adjustments in extradiaphragmatic respiratory muscle perfusion during cycling. First, patients across different stages of disease severity (35), exhibited a profound degree of dynamic lung hyperinflation during cycling which may have, in turn, hindered the normal increase in cardiac output (Table 2). Indeed, heart compression and intrathoracic hypovolemia consequent to exercise-induced dynamic hyperinflation, have been postulated to impede the normal increase in cardiac output (4, 51, 82) whilst reductions in dynamic lung hyperinflation by bronchodilators or Heliox administration have been shown to improve cardiac function during high-intensity exercise in patients with COPD (46, 47, 55, 87). Under these circumstances the circulatory system may be unable to meet the demands of the respiratory muscles during cycling requiring greater muscle oxygen extraction (85). Indeed, we found that for a comparable work of breathing between hyperpnoea and cycling, insufficient respiratory muscle blood flow (Figure 2 and 3, Table 5 and 6) during cycling was associated with greater respiratory muscle oxygen extraction as this was inferred by a greater increase in deoxy [Hb+Mb] compared to hyperpnoea.

Secondly, a potential mechanical impediment to extradiaphragmatic respiratory muscle perfusion might be due to intense muscle contraction and the development of high

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intramuscular pressures (19, 50, 75). Actually, the decrease in the operational capacity and potential deformation of vessels (squeezing or extension) within the inspiratory muscles resulting from the elevation of the ribs and sternum due to dynamic lung hyperinflation and/or strong recruitment of the abdominal muscles in the face of expiratory flow limitation may compromise extradiaphragmatic respiratory muscle perfusion (54, 55, 57, 60, 87, 90). In support of this mechanism, we previously demonstrated in patients with COPD that a reduction in dynamic lung hyperinflation and inspiratory and expiratory pressures during cycling by Heliox administration lead to an increase in both intercostal and abdominal muscle blood flow compared to room air breathing (55, 87).

Thirdly, an increased sympathetic vasoconstrictor outflow to the respiratory muscles upon activation of the respiratory muscle metaboreflex may also provide a possible explanation for the insufficient adjustment in respiratory muscle perfusion during cycling (76). In this context, recently, it was suggested that muscle contractions of the respiratory muscles during high-intensity exercise can cause increased group III and IV afferent activity leading to a sympathetically mediated vasoconstriction, thereby contributing to limitations in respiratory muscle blood flow and O_2 transport (76). The proposed greater development of intramuscular pressures and increased sympathetically mediated vasoconstriction to the respiratory muscles during cycling compared to hyperpnoea are supported by the findings (Table 5) showing lower microvascular conductance, inferred by lower total [Hb+Mb] during cycling compared to hyperpnoea, for all measured by NIRS extradiaphragmatic respiratory muscles. Therefore, the results of the present study do not provide evidence that insufficient adjustment in respiratory muscle perfusion during exercise is attributed to blood flow redistribution from the respiratory to the locomotor muscles but support the notion that central hemodynamic and local muscle mechanical impairments may contribute to the impediment of respiratory muscle perfusion during exercise in patients with COPD.

Association between respiratory muscle perfusion and dyspnoea

We found that at comparable levels of global respiratory muscle work, dyspnoea sensations were significantly greater during cycling compared to hyperpnoea (Tables 2 and 3). Furthermore, we demonstrated that the lower respiratory (intercostal and scalene) muscle BFI during cycling compared to hyperpnoea was associated with greater dyspnoea sensations during cycling. A potential explanation is that the lower local respiratory muscle BFI and microvascular oxygen supply during cycling compared to hyperpnoea would be expected to

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increase respiratory muscle metabolic acidosis and sensory afferent traffic in type III-IV fibres (innervating respiratory muscles) to the somatosensory cortex, thereby increasing the sensory intensity of unsatisfied inspiration during the cycling trial (49, 65, 66). Our findings are in line with previous and more recent studies in healthy individuals and in patients with chronic diseases (39, 41, 88) showing that skeletal muscle fatigue resistance is closely coupled with functional microvascular circulation for supporting adequate gas exchange, delivery of nutrients and removal of metabolites. Furthermore, our results corroborate with previous findings showing that improvements in intercostal and abdominal muscle oxygen delivery by Oxygen or Heliox supplementation are associated with reduced dyspnoea in patients with COPD (86, 54-57) (Table 2). However, part of the greater dyspnoea that patients demonstrated during cycling compared to hyperpnoea may be explained by ventilatory constraints (Table 2) and by the increase in peripheral locomotor muscle metabolic acidosis (leading to quadriceps muscle fatigue, *see* results section) and the greater sensory afferent traffic in type III-IV fibres to the somatosensory cortex as previously described by O´Donnell et al. (65, 66**).** Nevertheless, despite the association between diminished extradiaphragmatic respiratory muscle perfusion and greater dyspnoea levels, the mechanism(s) underlying this association remains not clear and future studies need to investigate the contributing role of impaired respiratory muscle perfusion during exercise on dyspnoea levels in these patients.

Strength and methodological considerations

Unique to our investigation is the simultaneous assessment of inspiratory, expiratory, and leg muscle perfusion whilst concomitantly assessing central haemodynamics and ensuring comparable work of breathing during hyperpnoea and exercise. Complementary to our study were the measures of the neural respiratory drive (diaphragm and extradiaphragmatic respiratory muscles activation by EMG) during hyperpnoea and cycling to better understand whether differences in respiratory muscle perfusion partly account for the greater dyspnoea levels during high-intensity exercise. To the best of our knowledge previous studies in patients with COPD focused on the perfusion of the 7th intercostal space, acknowledging the potential technical limitation of this site measurement (76, 83). We opted to investigate -besides intercostal muscles- the perfusion of the scalene muscle as it represents a superficial primary muscle of inspiration (27) with high activity at rest and during high-intensity exercise in patients with COPD (25, 92). Furthermore, the abdominal muscles are the major muscles of expiration and are activated by patients with COPD even during quiet breathing (63), whereas

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their efficiency is not affected as much by the occurrence of lung hyperinflation during exercise compared with the diaphragm (24).

To avoid arterial cannulation we assessed relative muscle perfusion using the NIRS-derived BFI method (33, 36, 54). We observed that during cycling, four patients demonstrated a modest increase in BFI of vastus lateralis as their values fall outside the lower limits of confidence interval whilst two patients demonstrated a decrease in vastus lateralis BFI compared to rest (*BFI responses of the six patients are marked with open symbols in Figure 3*). Nevertheless, the insufficient increase in leg muscle BFI of these six patients was not associated with a concomitant increase in their respiratory muscle BFI, to support that in these patients high-intensity exercise did not impair their extradiaphragmatic respiratory muscle perfusion (*Figure 3 A, B and C, open symbols*). Furthermore, the large inter-subject variability observed in BFI of respiratory and vastus lateralis muscles (Figure 2) could be attributed to inter-subject variability in subcutaneous tissue, muscle vasculature and capillary density and/or in the large variation in work rate (range, min: 25 watts /max: 100 watts) and minute ventilation (range, min: ~12 liters/min / max: ~71 liters/min) patients exhibited during the trials. Therefore, the aforementioned parameters had to be taken into account when using the NIRS-ICG methodology for comparing BFI data on an individual level (36, 54).

During cycling, patients exhibited moderate arterial oxygen desaturation (Table 4) that could have contributed to the greater respiratory (and locomotor) muscle hypoxemia compared to hyperpnoea (Table 5 and Figure 4). However, it is challenging to appreciate the effects of arterial hypoxemia on muscle perfusion and oxygenation responses during cycling compared to hyperpnoea for two reasons. First, this would have required an experimental condition where patients would cycle under hyperoxia (aiming to prevent arterial oxygen desaturation) and second, because during cycling cardiac output and systemic oxygen delivery were two-fold greater compared to hyperpnoea.

In the present study, we employed a single bout of cycling corresponding to a high-intensity load (80%WRpeak) causing profound ventilatory, respiratory, and circulatory responses. Hence the physiological and symptom responses described in this context are pertinent only to high-intensity, sustained exercise that is commonly adopted to assess the efficacy of pharmacological and non-pharmacological interventions in patients with COPD (69). However, the results of the present study may have limited external validity and practical significance during activities of daily living where it has been shown that the average energy requirement

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corresponds to a moderate intensity of physical activity (i.e. approximately 50% of VO_2peak , 81).

We deliberately chose non-invasive procedures for assessing central hemodynamic and respiratory and locomotor muscle oxygenation responses to cause minimal stress and pain to the patients and recognize the debate that exists in the literature for their absolute accuracy compared with gold standard methodologies (7, 12, 13, 31, 53, 54). Nevertheless, this study is based on a repeated-measures design, with the main purpose being to measure the same participants under different conditions (i.e., rest, hyperpnoea and cycling). Therefore, any systematic errors from the use of these non-invasive methodologies would not contribute to uncertainty in these repeated measures comparisons of the same group of patients.

Study limitation

The study could have been benefited from the inclusion of an elderly, age-matched healthy control group to determine whether the insufficient adjustment in respiratory muscle perfusion associated with greater dyspnoea levels during exercise was due to COPD, age, inactivity or other factors. However, as this study is part of a larger randomized clinical trial in patients with COPD, the recruitment of a healthy group was not feasible.

We used continuous wave (CW) near-infrared spectrometers (spatial resolved spectroscopy [SRS], Hamamatsu photonics), where the light source is of constant intensity, and providing changes in superficial muscle haeme components from an arbitrary baseline (10). Recently more advanced near-infrared spectrometers incorporating time-domain technology can provide deeper muscle NIRS readings and absolute concentrations of the heme components in tissues of interest (10, 30, 43, 67). However, NIRS devices based on CW technology are the only commercial instruments with the capacity to simultaneously measure tissue haeme variables and ICG concentrations for the calculation of tissue perfusion.

Due to limitations in the number of NIRS probes, measures were performed on a single muscle site for both respiratory and leg muscles acknowledging the substantial heterogeneity evident especially within the locomotor muscles (42, 44, 52, 84). Besides, we did not assess the reproducibility of the BFI measures during hyperpnoea and cycling assuming minimal variation due to steady state exercise (cycling and hyperpnoea) which in turn might cause insignificant variation in central hemodynamic, metabolic and ventilatory variables. In support of this, a recent study by Dominelli et al. (26), found reproducible BFI values (assessed by NIRS) in

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both vastus lateralis and sternocleidomastoid muscles when ventilation, oxygen uptake, and WoB was consistent between repeated inspiratory muscle loading trials.

Performing the hyperpnoea trial first and the cycling protocol afterwards (on the same day) may have influenced respiratory muscle oxygen delivery and uptake kinetic responses during the cycling test owing to muscle warm-up (1, 2, 15). However, the findings that before cycling, baseline values of heart rate (79±15 beats/min), cardiac output (5.5±1.4 litres/min) and $\%StiO_2$ in scalene (68±9%), 7th intercostal (76±11%) and abdominal muscles (81±17%) did not differ compared to resting values (Figure 4 and Table 3 and 4, $p>0.01$) suggest that the time elapsed between the two protocols eliminated any effect of prior exercise on respiratory muscle blood flow regulation during cycling. Besides, patients´ dyspnoea sensations prior to the cycling test (0.9±1.0) returned to resting levels (Table 2, $p>0.05$). Inspiratory capacity manoeuvres for evaluating dynamic lung hyperinflation were not performed during hyperpnoea thereby enabling patients to better focus on reaching the targeted breathing pattern and minute ventilation (34, 85, 86). However, whether dynamic lung hyperinflation, if any, compromised diaphragm and extradiaphragmatic respiratory muscle perfusion during hyperpnoea was not evaluated in the present study.

Respiratory muscle pressures and work of breathing could not be measured in 7 out of 18 patients. We argue that this limitation did not affect the findings of the present investigation. Similar studies in healthy individuals and in patients with COPD (85, 86) demonstrated that manipulation of the breathing pattern is sufficient to lead to similar respiratory muscle pressures and work of breathing between hyperpnoea and cycling trials as seen in this study. Finally, measures of respiratory muscle twitch force assessed by magnetic stimulation of phrenic nerves to evaluate potential respiratory muscle fatigue during cycling and hyperpnoea would have further strengthened our study. ***Clinical implications and future perspectives***

Randomised controlled trials in patients with COPD (17, 29, 45) have demonstrated that specific inspiratory muscle strength training (IMT) alone or as an adjunct intervention to an aerobic exercise training program may induce significant improvements in exercise capacity and dyspnoea sensations. Besides, evidence showing that implementation of high-intensity IMT in patients with chronic heart failure may improve the perfusion of exercising muscles (during upper limb muscle exercise) (18) potentially by attenuating the respiratory muscle metabaroreflex (91). In this context, studies in patients with COPD and in healthy individuals have shown an increase in the proportion of type I fibres and the size of type II fibres in the

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external intercostal muscles along with improvements in respiratory muscle energy efficiency, following implementation of a high-intensity IMT intervention (72, 79). Furthermore, Rodrigues et al. (73) recently demonstrated that the stimuli imposed on the extradiaphragmatic muscles during high-intensity IMT by tapered flow resistive loading yielded a considerable increase in extradiaphragmatic muscle recruitment and metabolism, thus expecting substantial training adaptations to extradiaphragmatic muscles following several weeks of IMT. Yet, the effects of several weeks of IMT on perfusion, oxygenation, and activation pattern of extradiaphragmatic respiratory muscles during high-intensity exercise remain unknown in patients with COPD (45). In addition, whether potential improvements in these physiological responses following IMT are associated with lower degrees of respiratory muscle fatigue and reduced dyspnoea sensations during whole-body exercise would be of specific interest to be investigated in patients with COPD.

Conclusions

The results of the present study suggest that in patients with COPD high-intensity locomotor muscle work during exercise interferes with extradiaphragmatic respiratory muscle perfusion despite a two-fold increase in cardiac output. Insufficient respiratory muscle perfusion during high-intensity exercise has a profound effect on extradiaphragmatic respiratory muscle oxygen availability and it is associated with greater dyspnoea sensations in patients with COPD.

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Disclosure

The authors declare that they have no competing interests.

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Table 1. Subjects characteristics, pulmonary function and peak exercise and functional, quadriceps and respiratory muscle capacity data.

Demographics / Anthropometrics	
Sex, male/female	10/8
Age, years	66 ± 6
BMI, kg/m^2	27 ± 6
Pulmonary function	
FEV_1, L	1.4 ± 0.6
FEV_1, %pred.	58 ± 24
FVC, L	3.2 ± 0.8
FVC, %pred.	99 ± 30

FEV_1/FVC, %	46 ± 13
MVV, L/min	53 ± 16
MVV, %pred.	68 ± 24
TLC, L	6.5 ± 1.5
TLC, %pred.	116 ± 20
RV, L	3.4 ± 1.25
RV, %pred.	151 ± 45
RV/TLC, %	51 ± 10
TL_{CO}, mmol/min/kpa	4.2 ± 1.4
TL_{CO}, %pred.	55 ± 16
SpO_2 ,%	94 ± 1
Hb, g/dl	14.5 ± 1.3
Peak exercise data	
Work rate, watts	79 ± 26
Work rate, %pred.	70 ± 20 *
VE, L	46 ± 14
VE/MVV, %	87 ± 15
Δ Insp. capacity, L	-0.52 ± 0.36
Tidal volume/ Insp. Capacity, %	78 ± 14
VO_2, L/min	1.3 ± 0.5

$VO_{2,}$, %pred.	86 ± 31
Heart rate, beats/min	120 ± 20
Cardiac output, L/min	11.8 ± 2.3
SpO_2 ,%	88 ± 4
Dyspnoea, 10-Borg scale	7.2 ± 2.0
Leg discomfort, 10-Borg scale	6.6 ± 2.0
Functional, quadriceps and respiratory muscle capacity data	
6-minute walking test, meters.	496 ± 52
6-minute walking test, %pred.	87 ± 12 **
Quadriceps muscle strength, kg	37 ± 10
Quadriceps muscle strength, %pred.	81 ± 23 ***
MIP, cmH_2O	73 ± 15
MIP, %pred	82 ± 21****
MEP, cmH_2O	157 ± 12
MEP, %pred	171 ± 12****
Physical activity levels, steps per day	6663 ± 3618

Data are presented as mean ± SD for n=18 patients with COPD. FEV_1: forced expiratory volume in the first second; FVC: forced vital capacity; MVV: maximum voluntary ventilation; TLC: total lung capacity; RV: residual volume; TL_{CO}: transfer factor for carbon monoxide; Hb: haemoglobin; VE: minute ventilation; Δ: changes in inspiratory capacity from rest; VO_2: oxygen consumption; SpO_2: arterial oxygen

saturation by pulse oximeter; MIP: maximal inspiratory pressure; MEP: maximal inspiratory pressure. **Reference values calculated by:**

*Blackie et al. 1989, **Troosters et al. 1999, ***Allaire et al. 2004, ****Neder et al. 1999