Greater exercise tolerance in COPD during acute interval, compared to equivalent constant-load, cycle exercise: physiological mechanisms

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Running title: Interval versus continuous exercise in COPD

Keywords: Respiratory muscles, limb muscles, exercise, cardiac output, NIRS, COPD

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This is an Accepted Article that has been peer-reviewed and approved for publication in The Journal of Physiology, but has yet to undergo copy-editing and proof correction. Please cite this article as an 'Accepted Article'; doi: 10.1113/JP279531.

Key points summary:

- Exercise intolerance is common in COPD patients.
- In patients with COPD, we compared an interval exercise (IE) protocol (alternating 30 sec at 100% peak work rate (WRpeak) with 30 sec at 50% WRpeak) to moderate-intensity constant-load exercise (CLE) at 75% WRpeak, which yielded the same work rate. Exercise endurance time and total work output were almost two-fold higher in IE than CLE.
- At exercise isotime (when work completed was the same between IE and CLE), IE was associated with less dynamic hyperinflation (DH), lower blood lactate concentration, and greater respiratory and locomotor muscle oxygenation, but no differences in ventilation or cardiac output.
- However, at the limit of tolerance for each modality, DH was not different between IE and CLE, while blood lactate remained lower and muscle oxygenation higher in IE.
- Taken together, these findings suggest that DH and not muscle-based factors dictate the limits of tolerance in these COPD patients.

Abstract

The relative importance of ventilatory, circulatory and peripheral muscle factors in determining tolerance to exercise in patients with COPD is not known. In twelve COPD patients (FEV_1:58±17%pred.) we measured ventilation, cardiac output, dynamic hyperinflation, local muscle oxygenation, blood lactate and time to exhaustion during a) interval exercise (IE) consisting of 30sec at 100% peak work-rate alternated with 30sec at 50% and b) constant load exercise (CLE) at 75% WRpeak, designed to produce the same average work rate. Exercise time was substantially longer during IE than CLE (19.5±4.8 versus 11.4±2.1 min, p=0.0001). Total work output was therefore greater during IE than CLE (81.3±27.7 versus 48.9±23.8 kJ, p=0.0001). Dynamic hyperinflation (assessed by changes from baseline in inspiratory capacity-ΔIC) was less during IE than CLE at CLE exhaustion time (isotime, p=0.009), but was similar at exhaustion (ΔIC_{CLE}: -0.38±0.10 versus ΔIC_{IE}: -0.33±0.12 l, p=0.102). In contrast, at isotime, minute ventilation, cardiac output and systemic oxygen delivery did not differ between protocols (p>0.05). At exhaustion in both protocols, vastus lateralis and intercostal muscle oxygen saturation were higher in IE than CLE (p=0.014 and p=0.0002, respectively) and blood lactate

concentrations were lower (4.9±2.4 mmol/l versus 6.4±2.2 mmol/l, p=0.039). These results suggest that 1) exercise tolerance in COPD is limited by dynamic hyperinflation; and 2) cyclically lower (50%) effort intervals in IE help preserve muscle oxygenation and reduce metabolic acidosis compared to CLE at the same average work rate, but these factors do not appear to determine time to exhaustion.

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Introduction

Exercise intolerance is common in patients with Chronic Obstructive Pulmonary Disease (COPD) and has been partially attributed to dyspnea from reduced ventilatory capacity leading to dynamic hyperinflation (O'Donnell & Webb, 2008). It is further known that limited ventilatory capacity of COPD will cause hypercapnia when metabolic production of CO_2 rises out of proportion to ventilation, and that this may worsen dyspnea. Therefore, respiratory abnormalities likely contribute to exercise intolerance in COPD.

In addition to these pulmonary factors, the arterial hypoxemia caused by COPD may reduce O_2 availability to the muscles, leading to early lactate release and leg discomfort, and contributing to exercise intolerance. Moreover, there is evidence of intrinsic peripheral muscle alterations, including muscle fibre atrophy, reduction in the proportion of oxidative fibres, poor oxidative enzyme capacity, mitochondrial dysfunction and reduced capillary density (Maltais et al. 2014). Myopathy may also be present in the respiratory muscles of patients with advanced COPD (Barreiro & Gea, 2015). Therefore, it is possible that the intolerance to exercise in COPD also stems, at least in part, from skeletal muscle dysfunction.

During the early 1960s, Astrand and colleagues (1960) demonstrated that when a heavy load (350 watts) was split into short periods of work and rest (each of 0.5 min duration) it yielded a comparable load on circulation and respiration to a matched work rate (175 watts) continuous exercise protocol (Astrand et al. 1960). The authors concluded that the heavy load was transformed into a submaximal load on respiration and circulation when the short work periods were interspersed with regular rest periods during interval exercise (Astrand et al. 1960). In addition, these authors postulated that the lower blood lactate concentration with interval exercise was due to a gradual vasodilation in the active muscles during the rest periods securing an improved oxygen supply. Subsequent work by Saltin and colleagues (1976), and Essen and Kaijser (1978) confirmed (via needle biopsy muscle sampling) that the smaller increase in plasma lactate during interval than equivalent constant load exercise was attributed to partial reloading of oxygen stores of myoglobin and restoration of high-energy phosphates during the recovery periods, thereby allowing a more oxidative degradation of glycogen. This body of work suggested that an exercise protocol comprised of alternating high and low intensity power levels (interval

exercise, IE) can be sustained longer than an equivalent power, constant load moderate intensity protocol (constant load exercise, CLE).

Exercise training is the cornerstone of pulmonary rehabilitation (PR) in patients with COPD, principally because it improves exercise capacity and quality of life in these patients (Spruit et al. 2013). IE, more than CLE, may well benefit patients with COPD, because patients with advanced COPD cannot sustain intense constant-load exercise for sufficiently long periods to induce true physiological training effects (Maltais et al. 1997). Thus, for the same average power output, an IE protocol might lead to greater muscle adaptation than CLE.

Indeed, in patients with COPD, acute application of IE consisting of alternating 0.5 or 1.0 min bouts of intense work with similar periods of rest or low intensity exercise, has been shown to be associated with enhanced exercise tolerance and reduced loads on respiration and circulation, exercise plasma lactate, symptoms of breathlessness and leg discomfort (Vogiatzis et al. 2004; Sabapathy et al. 2004). Improved exercise tolerance with IE as opposed to CLE was attributed to reduced dynamic hyperinflation, thereby maintaining breathlessness at sustainable levels for a prolonged period of time (Vogiatzis et al. 2004; Sabapathy et al. 2004). In both of these studies, however, the average work rate during IE was lower than that during CLE, thereby likely explaining much if not all of the lower degree of dynamic hyperinflation and extended exercise tolerance associated with the former modality.

The present study was therefore designed to compare IE and CLE in patients with COPD using protocols that led to the same average work rate. We reasoned that comparison at the same work rate was necessary to understand the mechanisms of exercise intolerance. We designed the study to measure, on several occasions throughout the duration of each exercise test, key variables that should provide mechanistic insight into mechanisms of exercise tolerance. These were inspiratory capacity (reflecting dynamic hyperinflation), ventilation, cardiac output, O_2 delivery, blood lactate and an index of muscle oxygenation. The objective was to shed light on the basis for intolerance – whether related primarily to ventilatory or skeletal muscle dysfunction – using a study design where substantial differences in exercise tolerance were expected to occur, along with likely differences in potential contributing mechanisms (pulmonary and skeletal muscle).

We hypothesized that in COPD, the greater exercise tolerance during IE (compared to CLE at the same average work rate) was enabled primarily by increased leg muscle oxygen availability, reasoning that the lower intensity work periods during IE would facilitate partial restoration of locomotor muscle oxygenation (Saltin et al. 1976; Essen & Kaijser, 1978) and thus lower metabolic acidosis.

Materials and Methods

Ethical approval

The study was approved by the University Hospital Ethics Committee, National and Kapodistrian University of Athens (Protocol ID-18367). 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, except for registration in a database.

Study Group

Twelve clinically stable patients with COPD with a history of long term-cigarette smoking (>40 pack per year) were recruited for the study. The following inclusion criteria were used: 1) a post-bronchodilator forced expiratory volume/forced vital capacity volume ratio (FEV_1/FVC) < 0.7 and 2) optimal medical therapy according to GOLD (2016). Exclusion criteria included: 1) any pathologic conditions that could interfere with exercise, 2) respiratory diseases other than COPD, 3) clinical signs of acute heart failure or heart disease (i.e. arrhythmia, ischemic heart disease or cardiomyopathy), 4) long-term oxygen therapy or requirement for oxygen support during exercise, 5) engagement in any exercise-training program in the last 3 months, and 6) any hospital admission or COPD exacerbation within the previous 6 weeks.

Experimental Design

Experiments were conducted in 3 visits. On visit 1, patients underwent an incremental cardiopulmonary exercise test on an electromagnetically braked cycle ergometer (Ergoline 800, SensorMedics, Anaheim, CA) to the limit of tolerance to establish peak work rate (WR_{peak}). On different visits (i.e., visits 2 and 3, separated by 96 hours), patients performed two exercise protocols on the same electromagnetically braked cycle ergometer

to the limit of tolerance. Specifically on visit 2, patients performed a constant-load exercise test that was sustained at 75% WR_{peak} (CLE) to the limit of tolerance. On visit 3 patients performed a high-intensity interval exercise (IE) protocol consisting of 30 seconds work at 100% WR_{peak} interspersed with 30 seconds work at 50% WR_{peak}. The CLE protocol was necessarily always performed before the IE protocol, because endurance time was expected to be significantly longer during IE than CLE. In turn, this was to enable the measurement, during both IE and CLE, of key variables at what we define as isotime: the time of exhaustion during CLE. Comparing variables at isotime will be shown to be critical to the analysis of our data.

Baseline assessment

All patients underwent the following assessment prior to visit 1: anthropometrics, pulmonary function, and functional capacity. Fat tissue was estimated by a bioelectric impedance device (Maltron BF 907, Essex, UK). The 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 and post-bronchodilator static lung volumes were measured according to the American Thoracic Society (ATS) and European Respiratory Society (ERS) standards (GOLD, 2016). The six-minute walking distance test was performed according to the ATS guidelines (ATS, 2002). Quadriceps muscle strength and endurance were assessed using the maximal isometric voluntary contraction technique of the knee extensors (Allaire et al. 2004; Swallow et al. 2007). Furthermore, subjects were instructed to maintain a tension representing 60% of their maximal voluntary contraction (MVC) to the limit of tolerance. A computer screen served as a feedback mechanism to help patients 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). Physical activity in terms of steps per day was assessed by a validated for COPD activity monitor (Actigraph GT3X, Actigraph LLC Pensacola, FL, USA), (Rabinovich et al. 2013) using a standardized procedure (Demeyer et al. 2014). In brief, physical activity was assessed for seven consecutive days. Patients with a minimum of four valid days (including weekends), counting only days with at least 480 min of

wearing time during waking hours (as defined between 07:00 h–20:00 h) were contained in the analysis (Demeyer et al. 2014).

Exercise testing

The incremental exercise test was performed on an electromagnetically braked cycle ergometer (Ergo-line 800; SensorMedics, Anaheim CA) with ramp load increments of 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 60 rpm. During the IE and CLE exercise protocols (i.e., visits 2 and 3), 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. Pulmonary gas exchange, ventilatory and circulatory parameters were averaged on 60-second intervals. Blood pressure was measured by a sphygmomanometer. During the protocol, patients performed every two minutes inspiratory capacity (IC) maneuvers to identify the degree of dynamic lung hyperinflation assuming constant total lung capacity (TLC) (O'Donnell et al. 2006). The intensity of dyspnea and leg discomfort during the tests was assessed using the modified Borg scale (Borg, 1982). Lactate concentration was measured from venous blood samples by CO-oximetry (ABL 625, Radiometer, Copenhagen, Denmark). Using local anesthesia (2% lidocaine) and sterile technique, a venous catheter was introduced percutaneously into a forearm vein oriented in the proximal direction. The catheter was kept patent throughout the experiment by periodic flushing with heparinized (1 unit/ml) saline.

Central hemodynamic measurements

Cardiac output was measured noninvasively and essentially continuously during the IE and CLE exercise protocols by transthoracic impedance (PhysioFlow PF05; Manatec Biomedical, Macheren, France, PhysioFlow) validated for patients with COPD over a wide range of exercise intensities including peak exercise (Louvaris et al. 2019). Six electrodes (PhysioFlow PF5; Manatec Biomedical, Macheren, France) in total were placed according to the manufacturers' instructions: two on the neck on the left side (one vertically above the other over the carotid artery above the supraclavicular fossa); two

anteriorly in the xiphoid region; and two in locations corresponding to the V1 and V6 positions used for conventional ECG monitoring as were previously described (Louvaris et al. 2019). For optimizing the impedance signal a careful skin preparation was performed. This included shaving (in the sites of electrode placement) and the application of a mildly abrasive gel plus cleaning with alcohol. Data points were excluded when signal quality was less than 90% (Louvaris et al. 2019). Cardiac output values were recorded at 1-second intervals and averaged for offline analysis at 60-second intervals. 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] and $\%SpO_2$ (Borghi-Silva et al. 2008). The difference in arterio-venous oxygen content (a-vO_2) was calculated by dividing whole body oxygen uptake by cardiac output. The oxygen extraction ratio was calculated as the ratio of the arterio-venous 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.

Quadriceps and intercostal muscle oxygenation by Near-Infrared Spectroscopy

To measure quadriceps and intercostal muscle oxygenation, two sets of near-infrared spectroscopy (NIRS, NIRO 200 Spectrophotometer, Hamamatsu Photonics, Japan) optodes were placed on the skin over the left vastus lateralis muscle 10-12 cm above the knee, and on the 7^{th} intercostal (at the midaxillary line) and secured using double-sided adhesive tape thus minimizing the intrusion of extraneous light and loss of near-infrared light as previously described (Louvaris et al. 2018). A marker was used to draw the position of the NIRS optodes on the skin of each patient to secure an identical reposition during visit 3. The intensity of incident and transmitted light was recorded continuously along with the relevant specific extinction coefficients, and was used to record changes in the oxygenation status of hemoglobin/myoglobin (Hb/Mb). The variables assessed by NIRS (Spatially Resolved Technique at specific wavelengths 735, 810, and 850 nm) were the concentration changes oxygenated Hb+Mb [oxy(Hb+Mb)], deoxygenated Hb+Mb [deoxy(Hb+Mb)], and total Hb+Mb volume ([total(Hb+Mb)] the sum of [oxy(Hb+Mb)] and [deoxy(Hb+Mb)]) expressed in μmol/l. In the present study we presented the values of [deoxy(Hb+Mb)] as an index of muscles O_2 extraction and [total(Hb+Mb)] as an index of

blood volume reflecting changes in microvascular conductance (vasodilation or vasoconstriction responses) and/or an increased capillary hematocrit for intercostal and vastus lateralis muscle (Grassi & Quaresima, 2016). In addition, a commonly derived parameter from NIRS studies in humans is the 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). The $StiO_2$,% is an absolute index of local muscle oxygen saturation which is commonly adopted as an index of tissue oxygen availability reflecting the balance between muscle oxygen supply and demand (Grassi & Quaresima, 2016) and the tissue capacity to match oxygen supply relative to its metabolic demand (Vogiatzis et al. 2015; Louvaris et al. 2017). A path length of 18.6 cm was set up for both intercostal and vastus lateralis muscles according to the manufacturer's recommendations. Separation distance between the NIRS light transmitter and receiver probes was 40 mm, thus allowing a maximum NIRS penetration depth of 20 mm. Adipose tissue thickness measurements (fat + skin layer) were performed by measuring skinfold thickness using a Harpenden skinfold caliper on the 7^{th} intercostal space and the vastus lateralis muscle (Van Beekvelt et al. 2001). The mean values of the 12 subjects of the adipose tissue for intercostal and vastus lateralis muscles were 9.0 ± 3.8 mm and 5.3 ± 2.1 mm, respectively. NIRS values were zeroed at the start point of each exercise protocol. NIRS data were sampled at 6 Hz and exported in document file format and averaged for offline analysis at 60-second intervals.

Data analysis

Respiratory, cardiovascular, hemodynamic, and muscle oxygenation responses during CLE were averaged every 60 seconds. When the subject terminated exercise before a full 60 seconds had elapsed, the values were averaged over this final shorter period of less than 60 seconds. During IE, all patients cycled at 100% WR_{peak} for 30 sec alternated with 30 sec exercise at 50% WR_{peak}. Continuously monitored physiological data during IE were recorded during exercise at both 100% WR_{peak} and active recovery phases (50% WR_{peak}). Importantly, during IE, inspiratory capacity, Borg scores, lactate concentration and blood pressure measurements were performed only during the 30 sec of cycling at 100% WR_{peak}. In addition, all patients reached their limit of tolerance after the 100% WR_{peak} part of the

cycle (rather than during the 50% WR_{peak} part), and moreover, each subject was able to complete the final 30 sec at 100% WR_{peak}.

Statistical analysis.

In figures, data are presented at rest, at the first 8 minutes of exercise that all patients were able to exercise, as well as at the limit of tolerance (Tlim) for each of the two exercise protocols (Tlim_CLE and Tlim_IE). Data are reported as mean±SD. The Shapiro-Wilk test revealed that all data were normally distributed. The primary outcome of this study was the change in exercise time between the two different exercise protocols. 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 0.314 was calculated based on data from a previous study in patients with COPD (FEV_1: 52±15%predicted) (Sabapathy et al. 2004) which demonstrated significant improvements in endurance cycling time between IE and CLE exercise tests. That study (Sabapathy et al. 2004) revealed an improvement in endurance time during IE versus CLE by 17.9 minutes with a corresponding pooled standard deviation of 5.6 minutes (Sabapathy et al. 2004). In the present study the critical sample size was calculated to be 10 patients using a mixed model analysis of variance (ANOVA, Exercise protocols x Time) as the primary statistical analysis method (GPower software 3.1, Figures 1-4). When ANOVA detected a significant interaction effect, Bonferroni post-hoc test was used for identifying pairwise differences in physiological variables at exact time points of exercise between CLE and IE (Figures 1-4). Finally, a dependent samples t-tests was used to identify statistically significant pairwise differences in physiological responses between CLE and IE at Tlim (Figures 1-4 and Table 3). Data were analyzed using the SPSS statistical program, version 18 (SPSS, Chicago, IL). The level of significance was set at $p < 0.05$.

Results

Subject characteristics

Subject demographic, anthropometric and lung function characteristics as well as peak exercise and functional capacity data are shown in Tables 1 and 2. Patients exhibited resting lung hyperinflation, moderate reduction in carbon monoxide diffusion capacity, and mild reduction in arterial oxygen tension (Table 1). In addition, patients exhibited

reduced peak exercise capacity (Blackie et al. 1989) with moderate arterial oxygen desaturation and exercise-induced dynamic hyperinflation (Table 2). They also demonstrated impaired functional capacity assessed by the 6-min walk test test (Troosters et al. 1999; Allaire et al. 2004) and a sedentary lifestyle (Tudor-Locke et al. 2008) assessed by accelerometry (Table 2).

Exercise time, work rate and work output responses between IE and CLE

Exercise endurance time was greater during IE compared to CLE ($p=0.0001$). Average cycling work rate did not differ between IE and CLE (Table 3). At the limit of tolerance during IE total work output ($p=0.0001$) and total energy expenditure ($p=0.0001$) were greater compared to CLE (Table 3).

Comparisons between IE and CLE at isotime

At isotime, minute ventilation, oxygen uptake (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) did not differ between IE and CLE (Figure 1a, 2b and Table 3, $p>0.05$). The ventilatory reserve, reflected by the ratio of V_E/MVV, did not differ between IE and CLE (Table 3, $p>0.05$). However, during IE tidal volume and duty cycle were greater compared to CLE ($p=0.031$ and $p=0.041$, respectively). Furthermore, the reduction of inspiratory capacity (IC) was less ($p=0.009$) during IE compared to CLE indicating less dynamic hyperinflation (Figure 1b and Table 3). Accordingly, dyspnoea scores were lower ($p=0.001$) during IE compared to CLE (Figure 1c).

At isotime, cardiac output, heart rate and stroke volume did not differ between IE and CLE (Figure 2a, 2c and Table 3, $p>0.05$). In contrast, systemic vascular conductance was greater during IE compared to CLE ($p=0.024$, Figure 2d) due to lower mean arterial blood pressure (Table 3, $p=0.001$). In addition, systemic arterial oxygen content was greater during IE compared to CLE ($p=0.0025$, Figure 3b) secondary to less reduction in arterial oxygen saturation ($p=0.0022$, Figure 3a). No differences were found in systemic oxygen delivery or extraction between IE and CLE (Figure 3c and 3d, $p=0.761$ and $p=0.225$, respectively).

At isotime during IE, changes from baseline in vastus lateralis and intercostal muscle [deoxy(Hb+Mb)] concentration, were less ($p=0.001$ and $p=0.0025$, respectively) compared to CLE (Figure 4). Furthermore, changes from baseline in vastus lateralis and intercostal

muscle [total(Hb+Mb] concentration were greater (p=0.023 and p=0.039, respectively) and changes from baseline in fractional oxygen saturation ($StiO_2$,%) were less (p=0.011 and p=0.027, respectively) compared to CLE (Figure 4). During IE lactate concentration was lower compared to CLE (p=0.021, Table 3). Leg discomfort scores were lower (p=0.020) at isotime during IE compared to CLE (Figure 1d).

Comparisons between IE and CLE at the limit of tolerance

At the limit of tolerance, minute ventilation, oxygen uptake (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) did not differ between IE and CLE (Figure 1a, 2b and Table 3, p>0.05) but lactate concentration was lower during IE compared to CLE (p=0.039, Table 3). The changes from baseline in IC, V_E/MVV, V_T/IC, tidal volume, duty cycle (Table 3, p>0.05) and dyspnea (Figure 1c, p=0.265) were not different between IE and CLE.

At the limit of tolerance, cardiac output, stroke volume, heart rate, systemic vascular conductance and mean arterial blood pressure did not differ between IE and CLE (Figure 2 and Table 3, p>0.05). Only systemic arterial oxygen content was greater during IE compared to CLE (p=0.0042, Figure 3b) secondary to greater arterial oxygen saturation (p=0.033, Figure 3a). No differences were found in systemic oxygen delivery or extraction between IE and CLE (Figure 3c and 3d, p=0.430 and p=0.255, respectively).

At the limit of tolerance, during IE, changes from baseline in vastus lateralis and intercostal muscle [deoxy(Hb+Mb)] concentration, were less (p=0.019 and p=0.033, respectively) compared to CLE (Figure 4). Furthermore, changes from baseline in vastus lateralis and intercostal muscle [total(Hb+Mb] concentration were greater (p=0.019 and p=0.026, respectively) and changes from baseline in fractional oxygen saturation ($StiO_2$,%) were less (p=0.014 and p=0.0002, respectively) compared to CLE (Figure 4). At the limit of tolerance leg discomfort scores did not differ between IE and CLE (Figure 1d, p=0.681).

Typical examples of main responses from an individual patient

Figure 5, shows a typical example of vastus lateralis and intercostal muscle fractional oxygen saturation ($StiO_2$,%), cardiac output and minute ventilation during CLE and IE. During CLE vastus lateralis $StiO_2$,% decreased from baseline reaching a plateau within the first minute of exercise. In contrast, during IE vastus lateralis $StiO_2$,% decreased from

baseline but was partially recovered during the active recovery phases (Figure 5a). In contrast these perturbations in $StiO_2$,% were not seen for the intercostal muscles during IE (Figure 5b). During interval exercise cardiac output and minute ventilation reached a plateau 3 to 4 minutes into the exercise protocols (Figure 5c and 5d).

Discussion

Main findings

The main findings of the present study were as follows. Exercise tolerance at the same average power output was 11 minutes during CLE, but almost twice as long (20 minutes) during IE, enabling almost twice the total work. During IE, cardiac output and ventilation were similar at isotime and exhaustion, and also not different from values at exhaustion during CLE. In contrast, blood lactate was lower and quadriceps muscle oxygenation higher during IE than during CLE, both at isotime and exhaustion. Most importantly, IC was greater (indicating less hyperinflation) during IE than during CLE at isotime, but at the limits of tolerance in both protocols, IC reached the same values.

Locus of exercise limitation

The purpose of the study was to use two exercise modalities with the same average power requirements, one using constant load exercise (CLE) and the other alternating bouts of higher and lower intensity exercise (IE), known to result in different endurance times, to tease out the factor(s) responsible for causing a COPD patient to stop exercise. We considered several factors that might be singly or jointly responsible and measured each over time during both exercise protocols: minute ventilation, cardiac output, systemic oxygen delivery, local locomotor and respiratory muscle oxygenation, blood lactate levels and dynamic hyperinflation. In the following discussion, deductions are made from two comparisons of the IE and CLE data: a) comparisons at what we define as "isotime" – which is the time patients quit exercise in the CLE modality (11 minutes on average, Table 3). Here we compared data from the two modalities at the same absolute time; and b) Comparisons at the limit of tolerance in each modality, meaning data at 20 minutes in IE and at 11 minutes in CLE (Table 3).

Dynamic hyperinflation was significantly less at isotime in IE than CLE, but at the limit of tolerance, dynamic hyperinflation was the same in the two modalities. Of all of the

outcome variables we assessed, only dynamic hyperinflation displayed this temporal pattern, and this is consistent with a determining role in time to exhaustion (O'Donnell et al 2001a). On the other hand, minute ventilation was not different between IE and CLE at isotime. However, during CLE, subjects quit (definition of isotime) but with IE they continued another 9 minutes. This means that the very same patients could maintain minute ventilation for 20 minutes (during IE) even when they had to quit at 11 minutes during CLE. This suggests that having reached a particular level of minute ventilation is not the critical factor determining time to exhaustion. Exactly the same reasoning applies to systemic oxygen delivery and to cardiac function (as reflected by cardiac output).

Local muscle oxygenation measures of both the vastus lateralis and the intercostal muscles (determined by NIRS) showed higher oxygen saturation ($StiO_2$,%) at isotime in IE than in CLE. At first sight, this might implicate muscle oxygen availability as important to determining exhaustion time, but the key observation was that comparing muscle oxygenation at exhaustion in both modalities, local muscle oxygen saturation remained higher in IE than CLE. If muscle oxygenation were the critical factor, we would have expected similar values at the limit of tolerance in both modalities. Exactly the same reasoning applies to blood lactate levels, which were lower in IE than CLE at isotime, and which remained lower at exercise termination in IE than at exhaustion in CLE. It may be true that better muscle oxygenation and less lactate accumulation play a role in enabling greater exercise tolerance, but the point is that these factors do not appear to play a role in determining the time point at which the patient becomes exhausted and has to quit exercise (for the reasons presented above).

These observations do however highlight the need to understand the reasons for, and effects of, better muscle oxygenation and lower blood lactate in IE than CLE. The data show that systemic oxygen delivery was on average the same between the two modalities and thus do not account for different oxygenation and lactate responses. We would reason that the less perturbed oxygenation and lactate responses at exhaustion in IE compared to CLE were the result of performing sub-lactate-generating exercise intensity for 50% of the exercise time, plus the likely insufficient time (30 seconds) for the muscles to respond to the 100% effort part of the cycle in terms of oxygen utilization and lactate production kinetics (Saltin et al. 1976; Essen & Kaijser 1978).

This in turn suggests that repeating our study using longer periods of 100% effort balanced by similarly prolonged periods at 50% effort, to still maintain average power output at 75%, might reduce the difference in time to exhaustion as previously shown in healthy participants by Astrand and colleagues (1960). Indeed, extrapolating to the obvious, the IE protocol must result in shorter endurance time than the CLE protocol were the periods of 100% effort increased to target 11 minutes with then 11 minutes at 50%. Given that our patients could sustain 75% CLE for only 11 minutes, they would not even be able to sustain 100% effort for this period of time and would thus reach the limit of tolerance in less than 11 minutes. The overarching hypothesized principle, however, would be that whatever cycle time period (between 50% and 100% effort) was used in IE, exercise limitation would occur when dynamic hyperinflation reached the same critical value.

Differences in physiological responses between IE and CLE

The present study shows that IE yielding matched work rate to CLE was effective in terms of prolonging endurance time and simultaneously enhancing total work output in patients with COPD. Average systemic oxygen delivery was not different between IE and CLE. It is therefore likely that the repeated low work rate periods during IE allowed greater systemic vascular and microvascular conductance during these periods, and thus improved local locomotor and respiratory muscle oxygen supply inferred by greater total(Hb+Mb)] and lower miscovascular oxygen extraction (deoxy Hb+Mb)], respectively. This in turn should have promoted partial restoration of local muscle oxygen stores as previously described in healthy individuals (Saltin et al. 1976; Essen et al. 1978). Systemic vascular conductance at isotime during IE was greater compared to CLE secondary to the lower mean arterial pressure during IE, thereby suggesting reduced vascular resistance most likely attributable to the repeated moderate intensity cycling periods (Kriel et al. 2016; 2019). Increased perfusion and muscle re-oxygenation would be expected to enhance performance in locomotor muscles, potentially due to increased ATP and phosphocreatine resynthesis (Spencer et al. 2006) and/or via centralized neuromuscular downregulation in response to the increased rate of biochemical changes (Amann & Calbet, 2008).

Improved respiratory and leg muscle oxygenation during IE was accompanied, at isotime, by reduced leg discomfort compared to CLE. The increased local muscle oxygenation would be expected to reduce muscle metabolic acidosis and sensory afferent traffic in type III-IV fibres (innervating both respiratory & locomotor muscles) to the somatosensory cortex, thereby mitigating motor command and perceived sensations during interval exercise (Laviolette & Laveneziana, 2014). Previous work from our group has shown that improved local intercostal and quadriceps muscle oxygen delivery via oxygen or heliox breathing is associated with reduced dyspnea and leg discomfort sensations in patients with COPD (Louvaris et al. 2012; 2014; 2018).

At isotime during IE we found that for a comparable level of minute ventilation patients exhibited less dynamic hyperinflation (Figure 1) and thus restrictions to tidal volume expansion, thereby contributing to significantly less dyspnea (Laveneziana et al. 2011). Less dynamic hyperinflation at isotime during IE was accompanied by a larger tidal volume. This was accompanied by significantly greater inspiratory time and a trend for a lower breathing frequency compared to CLE despite comparable levels of minute ventilation during both exercise modalities (Figure 1). This type of breathing pattern has been described following administration of bronchodilators, oxygen and heliox and is associated with increased inspiratory reserve volume and reduced dyspnea (Palange et al. 2004; O'Donnell et al. 2001b; 2004). It also improves alveolar ventilation through reduction in dead space/tidal volume ratio.

Previous work from our group (Vogiatzis et al. 2005; Nasis et al. 2009) in patients with advanced COPD highlighted the superiority of interval exercise training compared to constant load exercise during exercise training in enhancing exercise tolerance by lessening the ventilatory constraints when total training volume was matched between the two exercise modalities. The results of the present study expand these findings by showing that single interval exercise bouts alternating maximal and moderately intense pedaling periods yield an approximately two-fold increase in total work output and endurance time. Furthermore, the present work shows that despite the comparable ventilatory and circulatory loads with interval exercise, both respiratory and locomotor muscle oxygenation and microvascular conductance were greater, whilst miscovascular oxygen

extraction and blood lactate concentration were lower with IE compared to CLE. These findings fit with the alleviated respiratory and locomotor muscle discomfort with IE.

Novelty

To our knowledge this is the first study that simultaneously assessed the load imposed on respiration and circulation along with measurements of locomotor and respiratory muscle oxygen availability during two different (but load-equivalent) exercise modalities that are recommended by the American and British Thoracic Societies and the European Respiratory Society in the setting of pulmonary rehabilitation (Spruit et al. 2013; Bolton et al. 2013). Using non-invasive and validated (for patients with COPD) technologies (impedance cardiography and NIRS) (Louvaris et al. 2018; 2019), we examined the response of various physiological systems during exercise to the limit of tolerance. Our work provides the physiological rationale for the implementation of interval exercise alternating maximal intensity work periods with periods of moderate exercise loads, maintaining reduced dynamic hyperinflation and breathing discomfort and extending endurance time compared to constant load exercise at the same average power output.

Study limitations

In the present study we employed specific CLE and IE protocols without exploring the effectiveness of other interval modalities presenting various format of work and recovery phases. Hence the physiological and symptom responses may have been influenced by the choice of these protocols. CLE sustained at 75% WRpeak is adopted to assess the efficacy of pharmacological and non-pharmacological interventions on exercise tolerance in patients with COPD (Puente-Maestu et al. 2005). Therefore, the IE protocol alternating 100% WRpeak with 50% WRpeak every half minute was chosen to produce matched mechanical work to CLE. Nevertheless, we cannot exclude the possibility that different IE protocols may give different results as previously shown by Astand and colleagues (1960) employing lengthier bouts of interval exercise and recovery periods. Recent work in healthy sedentary individuals assessing vastus lateralis muscle oxygenation by NIRS during high-intensity IE revealed no difference in muscle oxygen availability to that achieved during a work matched session of moderate CLE (Kriel et al. 2019). Given the evidence of peripheral and respiratory muscle dysfunction in COPD (Maltais et al. 2014; Barreiro & Gea, 2015) as well as the prevalence of circulatory, ventilatory and gas

exchange limitations to exercise in COPD (Neder et al. 2000), it is likely that partial alleviation of these constraints may have importantly contributed to enhanced locomotor muscle oxygen availability.

Methodological considerations

Cardiac output, arterial oxygen saturation and locomotor muscle oxygenation responses were all assessed using non-invasive methodologies at the possible expense of reduced absolute accuracy compared with gold standard methodologies. However, this study is based on a repeated-measures design, with the main purpose being to perform within-subject comparisons of the physiological data obtained by these techniques A) between IE and CLE at the time of tolerance in CLE and B) between IE and CLE at their respective exhaustion times. Therefore, we reasoned that any systematic errors from the use of these non-invasive methodologies would not contribute to uncertainty in these within-subject comparisons, while random errors would be handled by statistical analysis. A study by Bougault et al. (2005) compared the PhysioFlow method with the direct Fick method in patients with COPD during exercise. The study revealed that the PhysioFlow overestimated cardiac output compared to the direct Fick method and precluded the use of impedance cardiography in clinical routine in hyperinflated patients during intense exercise. Nevertheless, authors did not find any correlation between cardiac output measured by PhysioFlow or the direct Fick method and hyperinflation estimated by RV/TLC. Furthermore, the study by Charloux et al. (2000) comparing the PhysioFlow method with the direct Fick method in patients with COPD during exercise concluded that that the PhysioFlow provides a clinically acceptable and non-invasive evaluation of cardiac output in hyperinflated patients. Our own study (Louvaris et al. 2019) in patients with COPD during exercise showed that PhysioFlow yields systematically higher absolute values compared to the dye dilution method by ≈ 1.0 L/min or 18%. However, this overestimation of ≈ 1 L/min remained unchanged from rest to peak exercise in patients with COPD and less than 20%, in alignment with the clinically acceptable difference between two cardiac output evaluation methods (Stetz et al. 1982; LaMantia et al. 1990). NIRS-derived [deoxy(Hb+Mb)] concentration represents an index of oxygen extraction, whereas [total(Hb+Mb)] concentration is used as an index of blood volume representing only a proxy of muscle tissue oxygenation. Both parameters are expressed in relative

terms and the proportional contributions of arterial and venous blood to the overall signal are unknown (Grassi & Quaresima, 2016; Barstow, 2019).

Conclusively the non-invasive methods we used in the present study may show systematic differences in absolute values from accepted invasive measurements (Bougault et al. 2005 Asha et al. 2018; Grassi & Quaresima, 2016; Barstow, 2019). We recognise that differences between invasive and non-invasive methodologies continue to be debated in the literature (Bougault et al. 2005 Asha et al. 2018; Grassi & Quaresima, 2016; Barstow, 2019). Accordingly, the use of these measurements in our study was not to establish absolute values but to compare values across times and exercise modalities within each patient, meaning that the importance of absolute accuracy is considerably lessened.

Implications in the setting of Pulmonary Rehabilitation

Systematic reviews (Beauchamp et al. 2010; Zainlundin et al. 2011) comparing the effects of IE versus CLE training modalities during pulmonary rehabilitation in patients with COPD do not show superiority of IE compared to moderate-intensity CLE training (typically sustained 60-75% of PWR) in the magnitude of improvement in exercise tolerance, physiological responses or peripheral muscle phenotypic and genotypic adaptations (Vogiatzis et al. 2002; 2005; Puhan et al. 2006; Arnardóttir et al. 2007; Varga et al. 2007; Nasis et al. 2009; Mador et al. 2009; Vogiatzis et al. 2011). However, in these studies the relative cardiorespiratory load with IE training was lower compared to CLE training. This would have limited the magnitude of the physiological training effects. The present study indicates that acute alteration of maximal and moderate intensity IE, compared to CLE at the same average power output, places comparable loads on respiration and circulation to CLE, with lower exertional symptoms leading to a two-fold increase in total work output and endurance time. Hence, alternating maximal with moderate loads during IE may be physiologically more effective to alternating periods of loaded and unloaded cycling. Furthermore, IE is deemed safer to CLE as systemic arterial oxygen de-saturation is only mild compared to continuous exercise.

Conclusions

When COPD patients exercise at 75% of maximal power via: a) CLE at 75% and b) IE alternating 100% and 50% every 30 sec, time to exhaustion and total work performed are

almost twice as long with IE than CLE. The present study suggests that dynamic hyperinflation is the main determinant of exercise limitation and that slower hyperinflation with IE than CLE explains the differences in endurance time. On the other hand, minute ventilation, cardiac output, systemic oxygen delivery, muscle oxygen availability and lactate accumulation do not explain the ability of patients to exercise longer with IE than CLE.

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Additional information

- Data availability statement

Data available on request due to privacy/ethical restrictions. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

- Competing interests

The authors declare that they have no competing interests.

- Authors Contribution

ZL contributed to the conception of the work. ZL, IV, AA and SZ contributed to the study design. ZL, NC, SS, AA contributed to data acquisition and analysis. ZL, SZ, PDW and IV contributed to the interpretation of data. ZL, NC, SS, AA, SZ, PDW and IV contributed to the drafting of the manuscript. All authors approved the final and revised version of the manuscript.

- Funding Statement

This work was supported by the Thorax Research Foundation, Athens Greece. Dr Zafeiris Louvaris is a postdoctoral research fellow of the FWO- Flanders (12U5618N).

- Acknowledgements

We thank all patients for their considerable motivation during this investigation.

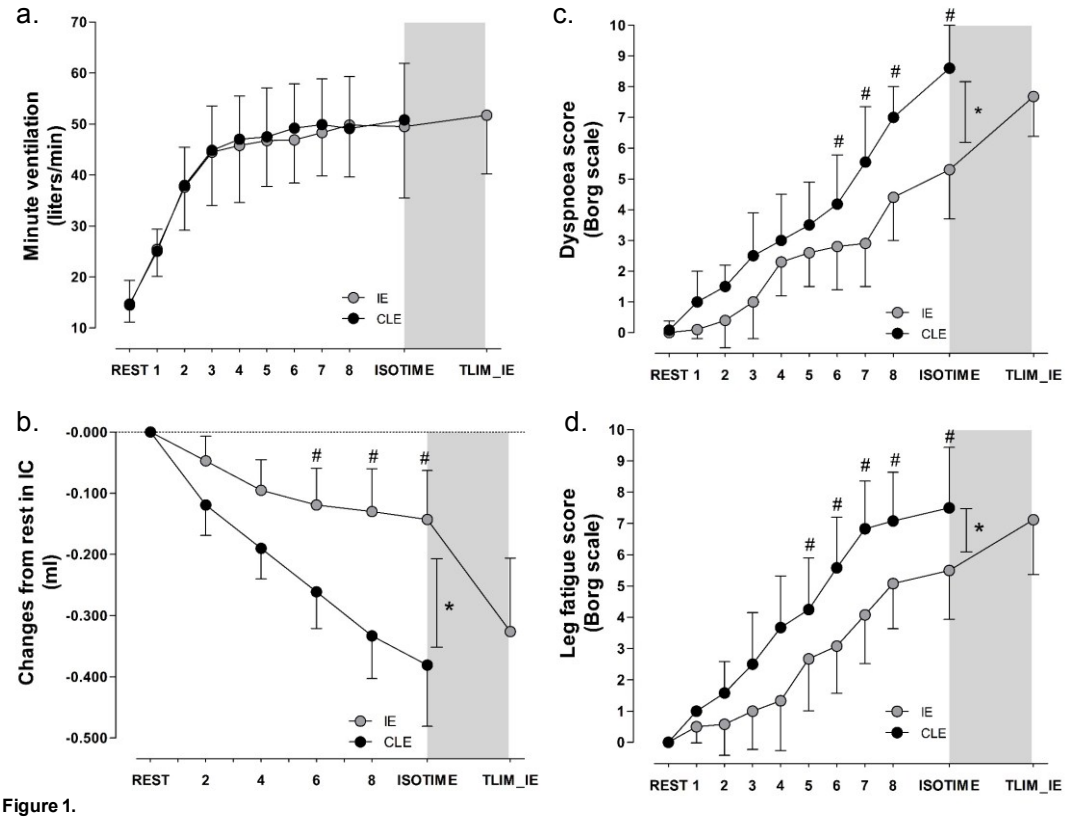

Figure 1.

Figure 1. Ventilatory responses and symptoms during CLE and IE. a: minute ventilation, b: changes from rest in inspiratory capacity, c: Borg dyspnea scores and d: Borg leg fatigue scores recorded at rest, during the first 8 minutes for each exercise protocol and at the limit of tolerance (Tlim) for each exercise protocol. Additional measures were performed during IE at the time point where CLE was terminated (i.e at isotime). Values are means ± SD for n=12 patients with COPD. * denotes significant difference between CLE and IE throughout exercise. # denote significant difference between CLE and IE at specific time points of exercise. [Mixed model analysis of variance (ANOVA, Exercise protocols x Time) followed by pairwise comparisons, $p<0.05$].

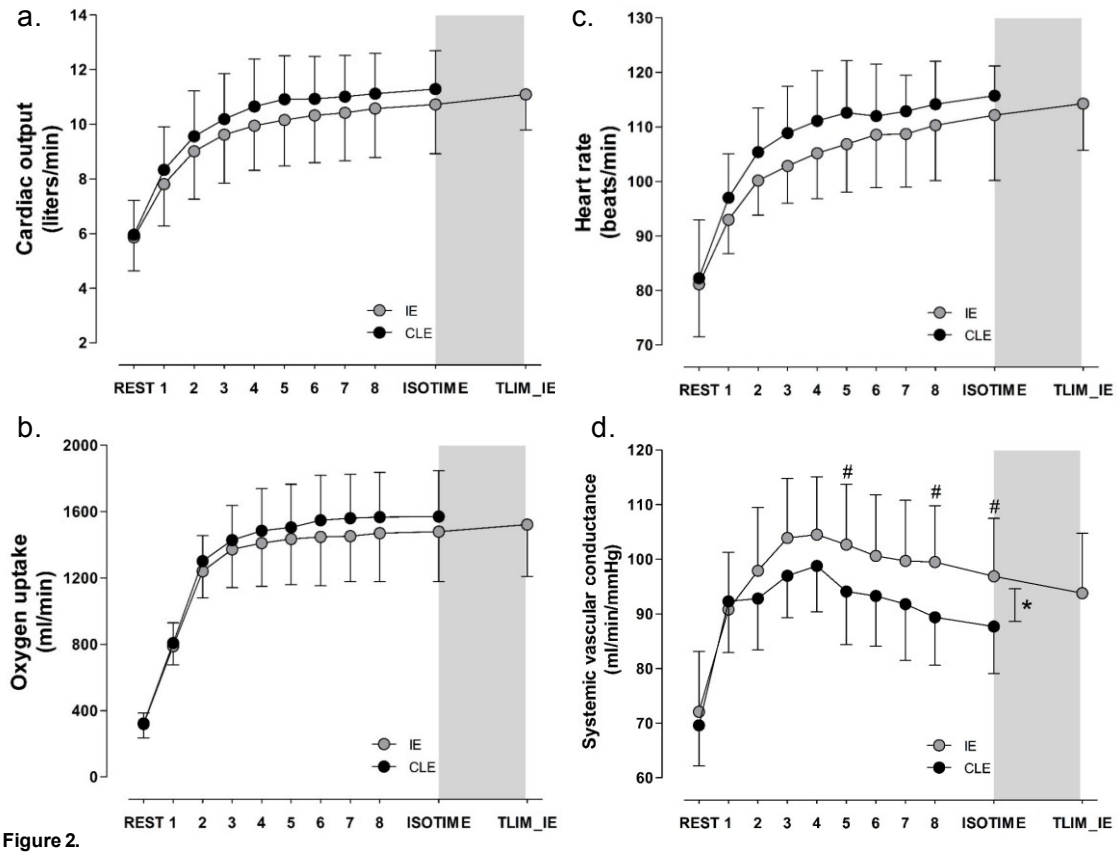

Figure 2.

Figure 2. Cardiovascular responses during CLE and IE. a: cardiac output, b: oxygen uptake, c: heart rate, d: systemic vascular conductance recorded at rest, during the first 8 minutes for each exercise protocol and at the limit of tolerance (Tlim) for each exercise protocol. Additional measures were performed during IE at the time point where CLE was terminated (i.e.: at isotime). Values are means ± SD for n=12 patients with COPD. * denotes significant difference between CLE and IE throughout exercise. # denote significant difference between CLE and IE at specific time points of exercise. [Mixed model analysis of variance (ANOVA, Exercise protocols x Time) followed by pairwise comparisons, $p<0.05$].

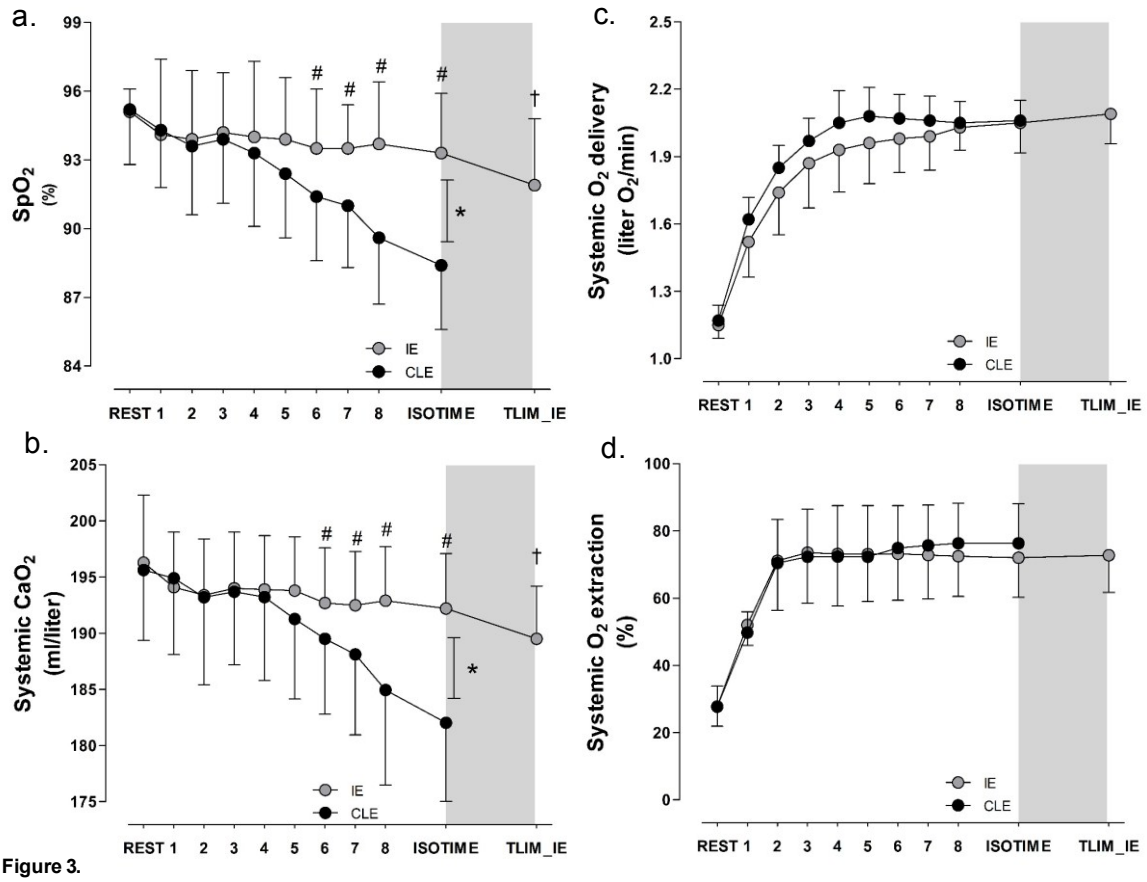

Figure 3.

Figure 3. Systemic hemodynamic responses during CLE and IE. a: arterial oxygen saturation (SpO_2) measured by pulse oximetry b: systemic arterial oxygen content, c: systemic oxygen delivery and d: systemic oxygen extraction recorded at rest, during the first 8 minutes for each exercise protocol and at the limit of tolerance (Tlim) for each exercise protocol. Additional measures were performed during IE at the time point where CLE was terminated (i.e.: at isotime). Values are means ± SD for n=12 patients with COPD. * denotes significant difference between CLE and IE throughout exercise. # denote significant difference between CLE and IE at specific time points of exercise † denote significant difference at Tlim between CLE and IE [Mixed model analysis of variance (ANOVA, Exercise protocols x Time) followed by pairwise comparisons, $p<0.05$].

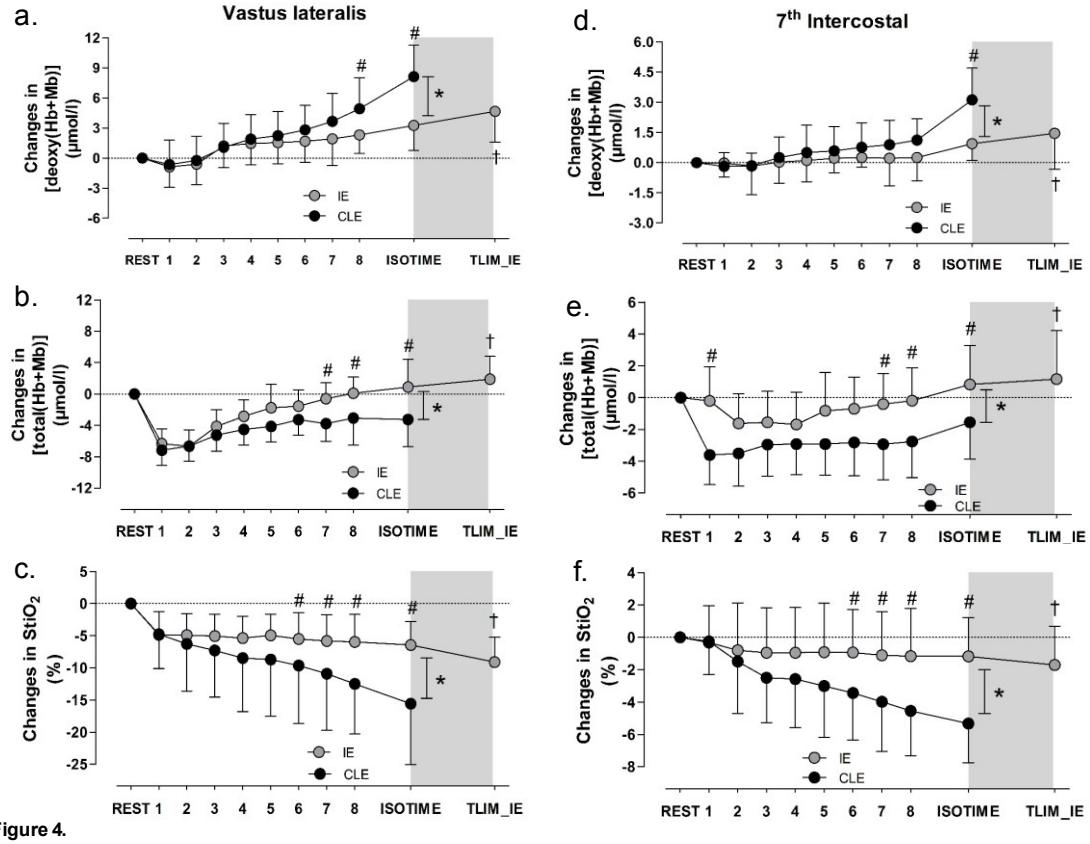

Figure 4.

Figure 4. Vastus lateralis and intercostal and local muscle oxygenation responses during CLE and IE. a: changes from rest in vastus lateralis muscle [deoxy(Hb+Mb)], b: changes from rest in vastus lateralis muscle [total(Hb+Mb)], c: changes from rest in vastus lateralis muscle fractional oxygen saturation ($StiO_2$) d: changes from rest in intercostal muscle [deoxy(Hb+Mb)], e: changes from rest in intercostal muscle [total(Hb+Mb)] and f: changes from rest in intercostal muscle fractional oxygen saturation ($StiO_2$%) during the first 8 minutes for each exercise protocol and at the limit of tolerance (Tlim) for each exercise protocol. Additional measures were performed during IE at the time point where CLE was terminated (i.e. at isotime). Values are means± SD for n=12 patients with COPD. * denotes significant difference between CLE and IE throughout exercise. # denote significant difference between CLE and IE at specific time points of exercise † denote significant difference at Tlim between CLE and IE [Mixed model analysis of variance (ANOVA, Exercise protocols x Time) followed by pairwise comparisons, $p<0.05$].

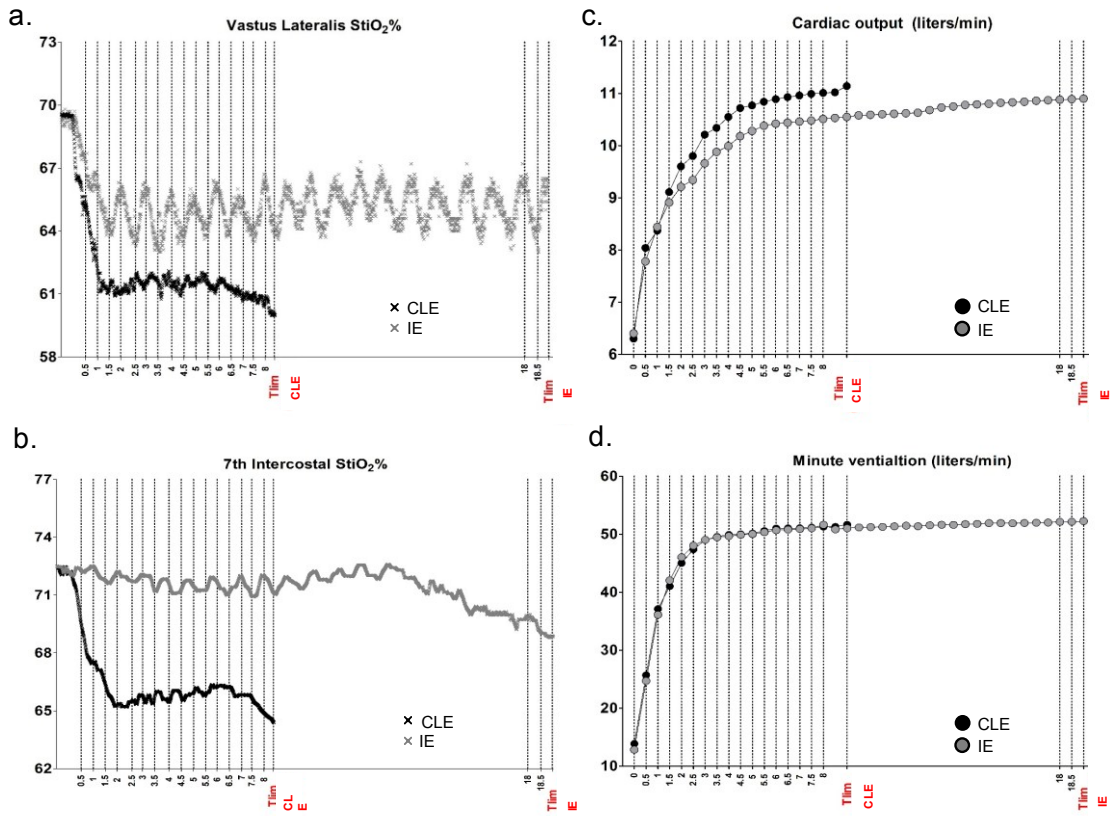

Figure 5.

Figure 5. A typical example from an individual patient with COPD for vastus lateralis (a) and 7th intercostal space (b) local muscle fractional oxygen saturation $StiO_2$ raw data presented every 30 seconds for CLE and IE during the first 8 minutes and at the limit of tolerance (Tlim) for each exercise protocol. c: a typical example from the same individual for cardiac output (c) and minute ventilation (d) averaged and presented every 30 seconds for CLE and IE during the first 8 minutes and at the limit of tolerance (Tlim) for each exercise protocol.

Table 1. Demographic, anthropometric and clinical characteristics

	Demographics/Anthropometrics
Age, yr	64 ± 10
Height, cm	172 ± 6
Weight, kg	83.5 ± 11.2
BMI, kg/m^2	28.2 ± 2.9
FFMI, kg/m^2	19.1 ± 2.2
	Pulmonary function data
FEV_1, % predicted	58.4 ± 17.2
FVC, % predicted	81.6 ± 15.7
FEV_1 / FVC	52.4 ± 11.1
RV, % predicted	158 ± 33
FRC, % predicted	144 ± 24
TLC, % predicted	112 ± 19
IC, % predicted	74 ± 16
RV/TLC, %	47 ± 6
IC/TLC, %	32 ± 9
TL_{CO}, % predicted	64.6 ± 13.1
MVV, l/min	71.5 ± 22.3
SaO_2, %	95.3 ± 1.3
Hb, g/dL	14.84 ± 0.66
mMRC	2.00 ± 0.71

Values are expressed as means ± SD for n=12 subjects. BMI, body mass index; FFMI, fat free mass index; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; RV, residual volume; FRC, functional residual capacity; TLC, total lung capacity; IC, inspiratory capacity; TL_{CO}, diffusing capacity of the lung for carbon monoxide; MVV: maximal voluntary ventilation, SaO_2, arterial oxygen saturation, Hb, Haemoglobin concentration; mMRC, modified Medical Research Council dyspnea score.

Table 2. Peak exercise and functional capacity data

	Peak exercise capacity
WR_{peak}, W	92 ± 19
WR_{peak}, % predicted	56 ± 23
VO_{2peak}, l/min	1.511 ± 0.378
VO_{2peak}, % predicted	65 ± 19
VCO_{2peak}, l/min	1.620 ± 0.546
AT, l/min	1.091 ± 0.453
$RER_{peak,}$	1.08 ± 0.06
V_{Epeak}, l/min	53.3 ± 8.2
VE/VCO_{2nadir}	33.2 ± 9.3
$PETCO_{2peak,}$ mmHg	36.9 ± 4.8
V_E/MVV, %	73.9 ± 18.1
$V_{T peak,}$ l/min	1.83 ± 0.22
Bf, breaths/min	29.1 ± 3.9
$HR_{peak,}$ beats/min	121 ± 10
MAP, mmHg	122 ± 12
ΔIC, l	-0.363 ± 0.165
$SpO_{2\ peak}$, %	89 ± 5
Borg dyspnoea scores	8.3 ± 2.9
Borg leg effort scores	7.1 ± 3.2
	Functional capacity
6-minute walking test, meters	466 ± 44
6-minute walking test, % predicted	70 ± 9
Quadriceps muscle force, kg	43 ± 13
Quadriceps muscle force, % predicted	52 ± 14
Quadriceps muscle endurance, sec	40 ± 24

Quadriceps muscle endurance, % predicted	50 ± 32
Physical activity levels, steps per day	5722 ± 2132

Values are expressed as means ± SD for n=12 subjects. WRpeak, peak work rate; VO_2peak, peak oxygen uptake; VCO_2peak, carbon dioxide output; AT, anaerobic threshold; RER, respiratory exchange ratio; VEpeak, peak minute ventilation; VE/VCO_2, ventilatory equivalent for VCO_2; V_T, tidal volume; $PETCO_2$, end-tidal pressure of carbon dioxide output; Bf, breathing frequency; HR, heart rate; MAP, mean arterial blood pressure, ΔIC, changes in inspiratory capacity from rest; MVV: maximal voluntary ventilation; SpO_2, arterial oxygen saturation assessed by pulse oximetry.

Table 3. Responses to constant-load exercise (CLE) and two interval exercise protocols (IE)

	Tlim_CLE	Isotime	Tlim_IE
Cycling responses			
Work rate, watts	69.8 ± 18.6	-	69.5 ± 16.6
Cadence, rpm	60 ± 6.9	-	59 ± 6.9
Tlim, min	11.4 ± 2.1	-	19.5 ± 4.8 *
Total work, kJ	48.9 ± 23.8	-	81.3 ± 27.7 *
Metabolic and Ventilatory responses			
E.E., kcals	83 ± 28	81 ± 21	147 ± 44 *
VO_2, ml/min/kg	18.1 ± 4.0	17.2 ± 3.6	17.7 ± 3.6
VCO_2, l /min	1492 ± 331	1378 ± 355	1449 ± 342
RER	0.95 ± 0.03	0.93 ± 0.03	0.95 ± 0.07
Lactate, mmol/l	6.43 ± 2.24	3.96 ± 1.76*	4.87 ± 2.39 *
V_E, l/min	50.3 ± 10.8	49.5 ± 15.0	51.8 ± 11.5
V_E/MVV, %	70.4 ± 16.9	69.7 ± 15.2	72.8 ± 16.6
V_E/VCO_2, %	34.2 ± 6.6	34.5 ± 6.0	35.4 ± 6.6
$V_{Tpeak,}$ l/min	1.66 ± 0.29	1.74 ± 0.38*	1.67 ± 0.34
Ti, sec	0.78 ± 0.14	0.93 ± 0.17*	0.80 ± 0.26
Ti/Ttot	37.3 ± 5.4	44.4 ± 5.5*	38.0 ± 4.5
Bf, breaths/min	30.6 ± 5.4	28.4 ± 4.9	31.0 ± 5.0
ΔIC, l	-0.381 ± 0.10	-0.143 ± 0.08*	-0.326 ± 0.12

VT/IC, %	84.3 ± 19.9	78.3 ± 12.9	82.8 ± 18.9
Cardiovascular and muscle oxygenation responses			
CO, l/min	11.3 ± 1.3	10.7 ± 1.9	11.1 ± 1.3
Stroke volume, ml/min	98.8 ± 13.5	95.3 ± 12.7	97.1 ± 13.9
Syst. O_2 del., liters O_2/min	2.05 ± 0.14	2.04 ± 0.29	2.09 ± 0.30
Syst. a-vO_2 dif, ml/dL	13.92 ± 0.33	13.79 ± 0.28	13.85 ± 0.25
SBP, mmHg	173 ± 21	152 ± 14 *	162 ± 17
DBP, mmHg	104 ± 17	96 ± 14	103 ± 19
MAP, mmHg	129 ± 12	113 ± 9 *	122 ± 17
$StiO_2$ Vastus Lateralis, %	-15.55 ± 9.5	-6.38 ± 3.6 *	-9.10 ± 3.8 *
$StiO_2$ Intercostal, %	-5.33 ± 2.2	-1.18 ± 2.2 *	-1.71 ± 2.3 *

Values are expressed as means ± SD for n=12 subjects. * denotes significant differences from CLE by pairwise comparisons ($p<0.05$). Tlim, time to limit of tolerance; CLE, constant-load exercise; IE, interval exercise, E.E, energy expenditure, VCO_2peak, carbon dioxide output; RER, respiratory exchange ratio, VE minute ventilation; MVV, maximal voluntary ventilation; VE/VCO_2, ventilatory equivalent for VCO_2; $PETCO_2$, end-tidal pressure of carbon dioxide output; V_T, tidal volume; Ti, time of inspiration; Ti/Ttot, duty cycle of inspiration; Bf, breathing frequency; ΔIC, changes in inspiratory capacity from rest; CO, cardiac output; Syst. O_2 del, systemic oxygen delivery; a-vO_2 dif, systemic arteriovenous oxygen content difference; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; $StiO_2$, fractional oxygen saturation. Isotime data are those obtained on IE at the same time-point as at Tlim on CLE.