Effect of helium breathing on intercostal and quadriceps muscle blood flow during exercise in COPD patients

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**Running head:** Heliox breathing during exercise in COPD

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ABSTRACT

Emerging evidence indicates that, besides dyspnea relief, an improvement in locomotor muscle oxygen delivery may also contribute to enhanced exercise tolerance following normoxic heliox (replacement of inspired nitrogen by helium) administration in patients with chronic obstructive pulmonary disease (COPD). Whether blood flow redistribution from intercostal to locomotor muscles contributes to this improvement, currently remains unknown. Accordingly, the objective of this study was to investigate whether such redistribution plays a role in improving locomotor muscle oxygen delivery whilst breathing heliox at near-maximal [75% peak work rate (WR\text{peak})], maximal (100%WR\text{peak}) and supra-maximal (115%WR\text{peak}) exercise in COPD. Intercostal and vastus lateralis muscle perfusion was measured in ten COPD patients (FEV\text{1}=50.5±5.5% predicted) by near-infrared spectroscopy using indocyanine green dye. Patients undertook exercise tests at 75 and 100%WR\text{peak} breathing either air or heliox and at 115%WR\text{peak} breathing heliox only. Patients did not exhibit exercise-induced hyperinflation. Normoxic heliox reduced respiratory muscle work and relieved dyspnea across all exercise intensities. During near-maximal exercise, quadriceps and intercostal muscle blood flows were greater whilst breathing normoxic heliox compared to air [(mean±SEM) 35.8±7.0 versus 29.0±6.5, and 6.0±1.3 versus 4.9±1.2 ml/min/100gr, respectively; p<0.05]. In addition, compared to air, normoxic heliox administration increased arterial oxygen content, as well as oxygen delivery to quadriceps and intercostal muscles (from 47±9 to 60±12, and from 8±1 to 13±3 mlO\text{2}/min/100gr, respectively; p<0.05). In contrast, normoxic heliox had neither an effect on systemic nor on quadriceps or intercostal muscle blood flow and oxygen delivery during maximal or supra-maximal exercise. Since intercostal muscle blood flow did not decrease by normoxic heliox administration, blood flow redistribution from intercostal to locomotor muscles does not represent a likely mechanism of improvement in locomotor muscle oxygen delivery. Our findings might not be applicable to patients who hyperinflate during exercise.

Key words: Work of breathing, near-infrared spectroscopy, cardiac output, muscle oxygen delivery, respiratory muscle blood flow
INTRODUCTION

During exercise expiratory flow limitation is present in a significant fraction of patients with chronic obstructive pulmonary disease (COPD) and is associated with impaired exercise performance (5, 25, 29). In these patients replacement of nitrogen by helium in the inspired gas, known as normoxic heliox, decreases turbulence within medium to large airways, increases expiratory flow rate, reduces the work of breathing, as well as the degree of exercise-induced dynamic hyperinflation and the intensity of dyspnea, thereby enhancing exercise tolerance (13, 23, 30, 31, 33).

There is emerging evidence (7) indicating that enhanced exercise tolerance by heliox is also due to an increase in locomotor muscle oxygen delivery as inferred by deoxy-hemoglobin kinetics, an index of tissue oxygen extraction (7, 15), determined by near-infrared spectroscopy during constant-work rate near-maximal exercise (70-80% of peak work rate) (7). Enhanced oxygen delivery to peripheral muscles following administration of heliox during exercise in COPD may occur via a number of mechanisms, namely: i) improved cardiac output secondary to reduced intrathoracic pressures and/or pleural pressure swings (26, 27, 34), ii) improved arterial oxygen content (6, 30), and iii) blood flow redistribution from respiratory to peripheral muscles secondary to reduction in the mechanical load of the respiratory muscles (9, 17, 18). To date, the latter mechanism has never been investigated due to lack of simultaneous measurements of blood flow to respiratory and locomotor muscles. Therefore, the potential contribution of blood flow redistribution from respiratory to peripheral muscles to the improvement in leg muscle oxygen delivery with heliox during near-maximal exercise remains unknown.

Accordingly, the primary purpose of the present study was to investigate whether blood flow redistribution from respiratory (i.e., the intercostal muscles) to locomotor muscles contributes to the suggested (7) improvement in peripheral muscle oxygen delivery with heliox during near-maximal exercise in COPD patients. In addition, since the role of this mechanism during maximal exercise has not been previously examined and as heliox is expected to enhance exercise tolerance (13, 23,
31), we also aimed to investigate the contribution of this mechanism to leg muscle oxygen delivery during maximal and supra-maximal exercise. To accomplish our goal we performed simultaneous measurements of systemic, quadriceps and intercostal muscle blood flow and oxygen delivery, as well as measurements of respiratory muscle power during exercise ranging from near-maximal to supra-maximal levels in room air and during heliox breathing in COPD patients. It was reasoned that, if at the same leg exercise intensity intercostal muscle blood flow during heliox breathing is less than that during air breathing, whilst quadriceps muscle blood flow increases, there is possible redistribution of blood flow from the respiratory (i.e., intercostals) to leg muscles.

**METHODS**

**Subjects**

Ten patients (1 female) with clinically stable COPD participated in the study according to the following inclusion criteria: 1) a post-bronchodilator forced expiratory volume in one second (FEV₁) <80% predicted without significant reversibility (<12% change of the initial FEV₁ value or <200 ml); 2) optimal medical therapy according to GOLD (14); and 3) the absence of other significant diseases that could contribute to exercise limitation. Their physical characteristics and pulmonary function is given in Table 1. The same patients were also included in our previous study (39). The study was approved by the University Hospital Ethics Committee and was conducted in accordance with the guidelines of the Declaration of Helsinki. Prior to participation in the study, all patients were informed of any risks and discomforts associated with the experiments and gave written, signed, informed consent.

**Experimental design**

Experiments were conducted in two visits. In visit 1, patients underwent an incremental preliminary exercise test to the limit of tolerance [peak work rate (WRpeak)] breathing room air. In visit 2, patients undertook in a balanced ordering sequence two exercise protocols where the inspired gas mixture was either room air or normoxic heliox (see data supplement, Fig. 1). The two
protocols were separated by 60 minutes of rest. In room air (Protocol 1), patients initially performed a constant-work rate near-maximal exercise bout corresponding to 75% WRpeak for 3-4 minutes and 30 minutes later a second constant-work bout of maximal exercise corresponding to 100% WRpeak for 2-3 minutes. During normoxic heliox breathing (Protocol 2), subjects exercised first at 75% WRpeak and 30 min later at 100% WRpeak. Following 30 min of rest patients performed a third constant-work rate supra-maximal exercise bout sustained at 115% WRpeak for 1-2 minutes. Prior to imposing the target load on the bicycle ergometer, patients were asked to perform unloaded cycling for 60 seconds reaching and maintaining a cadence of approximately 50 rpm. Heliox supplementation was achieved by having subjects inspire from a Douglas bag containing a gas mixture of 21% oxygen and 79% helium that was connected to the inspiratory port of a non-rebreathing two way valve by a piece of tubing. The same apparatus was utilized during room air breathing in order to ensure a blinding strategy of administration of the inspired gas mixture. During protocols, vastus lateralis and intercostal muscle blood flow [assessed by near-infrared spectroscopy (NIRS) and the light-absorbing tracer indocyanine green (ICG) dye] as well as cardiac output (assessed by the dye dilution method again using ICG), were measured during the final minute of each of the exercise bouts. End-inspiratory and end-expiratory compartmental (rib cage and abdominal) chest wall volumes (measured by optoelectronic plethysmography) as well as esophageal and gastric pressures (to assess respiratory muscle load) were continuously recorded during the exercise trials.

**Preliminary testing**

In visit 1, the incremental exercise tests were performed on an electromagnetically braked cycle ergometer (Ergoline 800; Sensor Medics, Anaheim, CA) with a ramp increase of load increments of 5 or 10 W/min to the limit of tolerance (the point at which the work rate could not be tolerated due to severe sensation of dyspnea and/or leg fatigue; peak exercise data are included in Table 1), with the patients maintaining a pedalling frequency of 40-50 rpm. Tests were preceded by a 3 min rest period, followed by 3 min of unloaded pedaling. The following pulmonary gas
exchange and ventilatory variables were recorded breath by breath (Vmax 229; Sensor Medics, Anaheim, CA): oxygen uptake, carbon dioxide elimination, minute ventilation, tidal volume, breathing frequency, and respiratory exchange ratio. Heart rate and percentage of arterial oxygen saturation (%SpO₂) were determined using the R-R interval from a 12-lead on-line electrocardiogram (Marquette Max; Marquette Hellige GmbH, Germany) and a pulse oximeter (Nonin 8600; Nonin Medical, North Plymouth, MN), respectively.

**Subject preparation**

Subjects were prepared first with radial arterial and femoral venous catheters, and then with esophageal and gastric balloons for assessment of esophageal and gastric pressures. Using local anesthesia (2% lidocaine) and sterile technique, identical catheters were introduced percutaneously into the left femoral vein and the right radial artery, both oriented in the proximal direction. The catheters were used to collect arterial and venous blood samples and also to inject ICG dye into the venous line and sample blood after each injection from the arterial line for cardiac output measurement and muscle blood flow calculation. The catheters were kept patent throughout the experiment by periodic flushing with heparinized (1 unit/ml) saline.

Esophageal and gastric pressures were assessed by thin-walled balloon catheters (Ackrad Laboratories, Inc., Crandford, NJ) coupled to differential pressure transducers (MP-45, ±250 cmH₂O; Validyne Corp., Northridge, CA). The balloons were inserted by nasal intubation following the application of 2% lidocaine anesthetic gel to the nose and with the assistance of continuous pressure monitoring. Esophageal and gastric balloons were positioned in the middle third of the esophagus and stomach, respectively.

**Exercise protocols**

During these tests recordings of pulmonary gas exchange and ventilatory variables were performed breath by breath as during the preliminary testing. Arterial blood pressure was measured by a sphygmomanometer and blood gas analysis was made in blood samples drawn from the radial artery and femoral vein during the final minute of each of the exercise bouts. Airflow was measured
with a hot wire pneumotachograph (Vmax 229; Sensor Medics) near the mouthpiece, and tidal volume changes were obtained by integrating the flow signal. Before each protocol, pneumotachograph and gas analyzers of the system (Vmax 229; Sensor Medics) were calibrated with the experimental gas mixture. Esophageal and gastric pressures and flow rates were displayed on a computer screen and digitized at 60 Hz using an analog-to-digital converter connected to the same computer used for optoelectronic plethysmography (OEP system, BTS, Milan, Italy). End-inspiratory and end-expiratory compartmental (rib cage and abdominal) chest wall volume changes during exercise were determined by optoelectronic plethysmography (37-39). In brief, the movement of 89 retro-reflective markers placed front and back over the chest wall from clavicles to pubis was recorded. Markers were tracked by six video cameras, three in front of the subject and three behind. Dedicated software recognized in real time the markers on each camera, reconstructed their 3D co-ordinates by stereophotogrammetry and calculated volume changes. Ventilatory variables (tidal volume, breathing frequency and breathing pattern parameters) reported in the manuscript were those recorded by using optoelectronic plethysmography during the final minute of each of the exercise bouts.

Esophageal and gastric pressures were averaged over 30 s breath samples in every minute of the exercise tests. The mechanical power of breathing (total respiratory muscle power) was determined at each exercise level by ensemble-averaging several breaths to integrate the average tidal volume - esophageal pressure loop multiplied by the breathing frequency and expressed in Cal/min (39). More specifically, respiratory muscle power was calculated as the area enclosed by the tidal chest wall volume change - esophageal pressure dynamic loops multiplied by respiratory frequency. We considered the entire loops (i.e., both inspiration and expiration), therefore our respiratory muscle power represents the total power developed by all (both inspiratory and expiratory) respiratory muscle groups. The lung-apposed (41) rib cage muscle power was calculated as the area enclosed by the tidal lung-apposed rib cage volume change - esophageal pressure dynamic loops multiplied by respiratory frequency. We also considered the entire loops (i.e., both
inspiration and expiration), therefore our rib cage muscle power represents the total power developed by all (both inspiratory and expiratory) rib cage muscle groups. Transdiaphragmatic pressure (Pdi) was obtained by subtracting esophageal from gastric pressure. Tidal excursion in Pdi (ΔPdi) was taken as peak Pdi during inspiration minus baseline Pdi. The pressure-time products for the diaphragm (PTPdi) and expiratory abdominal muscles (PTPab) were obtained by multiplying the area subtended by each trace (i.e., the integral of Pdi and gastric pressure, respectively, over time) by the respiratory frequency and had units of cmH₂O•s/min (22). The baseline for PTPdi and PTPab was determined for each breath as the level observed at the start of inspiration and expiration, respectively.

**Cardiac output**

Cardiac output was determined by the dye dilution method (11) using known volumes of ICG (1.0 ml at 5 mg/ml) injected into the left femoral vein followed by a rapid 10 ml flush of isotonic saline. Blood was withdrawn from the right radial artery using an automated pump (Harvard Apparatus, USA) at 20 ml/min through a linear photodensitometer (Pulsion ICG; ViCare Medical, Denmark) connected to a cardiac output computer (Waters CO-10; Rochester, MN) through a closed loop, sterile tubing system, as previously described (37-39).

**Intercostal and quadriceps muscle blood flow by NIRS**

In order to measure intercostal and quadriceps muscle blood flow, two sets of NIRS optodes were placed, one on the skin over the left 7th intercostal space at the mid-axillary line and the other over the left vastus lateralis muscle 10-12 cm above the knee, both secured using double sided adhesive tape. The optode separation distance was 4 cm, corresponding to a penetration depth of ~2 cm. The left intercostal space was used in order to avoid potential blood flow contributions from the liver on the right side of the body. Optodes were connected to a NIRO 200 spectrophotometer (Hamamatsu Photonics KK, Hamamatsu, Japan), which was used to measure ICG concentration following the same 5 mg bolus injection of ICG in the left femoral vein used for cardiac output assessment. Tissue microcirculatory ICG was detected transcutaneously by measuring light
attenuation with NIRS at 775-, 813-, and 850-nm wavelengths and analysed using an algorithm incorporating the Modified Beer-Lambert Law (3, 12, 21, 36), as previously described (16, 38, 39).

Blood flow was calculated from the rate of tissue ICG accumulation over time measured by NIRS according to the Sapirstein principle (35). Accordingly, for any time interval less than the time to reach peak tissue accumulation of tracer, the tissue receives the same fraction of the ICG bolus as quantified in arterial blood (input function). Two separate time points within the first half of the curve were used to calculate flow, and the average value was taken to represent the tissue ICG accumulation. Total blood flow was then calculated, as previously reported (3).

Systemic, and quadriceps and intercostal muscle vascular conductance was calculated by dividing the cardiac output, and the quadriceps and intercostal muscle blood flow by the mean arterial blood pressure, respectively. Systemic, and quadriceps and intercostal muscle oxygen delivery was calculated as the product of the cardiac output, and the quadriceps and intercostal muscle blood flow and arterial oxygen content, respectively.

**Blood analysis and calculations**

Radial arterial and femoral venous tensions of O₂ and CO₂, pH, haemoglobin concentration, lactate concentration, and percentage of arterial and venous oxygen saturation were measured from 2 ml blood samples using a blood gas analyser combined with a co-oximeter (ABL 625; Radiometer, Copenhagen, Denmark) within 10 s of collection. Arterial and venous O₂ content were computed from standard equations (39). Arterio-venous O₂ content difference was divided by arterial O₂ content to give leg O₂ extraction.

**Statistical analysis**

Data are reported as means ± SEM, unless otherwise stated. The minimum sample size was calculated based on 80% power and a two-sided 0.05 significance level. Sample size capable of detecting between-condition (i.e., air, heliox) difference of 30% was estimated for the change in intercostal muscle blood flow at WRpeak using the standard deviations from our previous study (38).
The critical sample size was estimated to be nine patients. To compare responses between air and heliox at different fractions of WR_{peak}, paired t-tests were carried out and followed by Bonferroni adjustment for multiple comparisons. The level of significance for all analyses was set at p < 0.05.

**RESULTS**

**Subject characteristics and baseline exercise capacity**

Patients had moderate to severe airflow obstruction with increased static lung volumes, moderate reductions in carbon monoxide diffusion capacity, and mildly reduced arterial oxygen tension (Table 1). Four patients were GOLD stage II, three were GOLD stage III and the remaining three were GOLD stage IV (14). Subjects exhibited reduced maximal exercise capacity with moderate hemoglobin desaturation (Table 1).

Effect of heliox on respiratory muscle loading and kinematics

Whilst breathing normoxic heliox, total respiratory muscle power was lower compared to room air during exercise sustained at 75, 100 and 115% WR_{peak} (Fig. 1A). This was due to unloading of both inspiratory and expiratory muscles. Indeed, tidal excursion in transdiaphragmatic pressure (ΔP_{di}) and pressure-time product for the diaphragm (PTP_{di}) (Fig. 1, E and C), as well as peak expiratory gastric pressure and pressure-time product for the expiratory abdominal muscles (PTP_{ab}) (Fig. 1, D and F) were lower during heliox compared to air breathing. Similarly, power produced by the rib cage muscles, both inspiratory and expiratory (reflecting in part, due to the contribution of other respiratory muscles, the activity of the external and internal intercostals), was lower whilst breathing heliox than air at 100% WR_{peak} (Fig. 1B). During heliox breathing, improvements in peak expiratory gastric pressure and PTP_{ab} were larger than those in ΔP_{di} and PTP_{di}, respectively. In fact, mean peak expiratory gastric pressure during exercise bouts was reduced by 22.5%, whereas mean ΔP_{di} was reduced by only 8.9%. Mean PTP_{ab} during exercise bouts was reduced by 32.3%, whereas mean PTP_{di} was reduced by only 6.3%. Heliox breathing compared to air reduced the degree of exercise-induced hyperinflation of the rib cage compartment.
as indicated by the decrease in both end-expiratory and end-inspiratory rib cage volumes (Fig. 2A), but had no effect on the abdominal or total chest wall volume regulation (Fig. 2, B and C). Notably, our patients did not exhibit exercise-induced hyperinflation at any percentage of WRpeak as total chest wall end-expiratory volume did not increase during exercise while breathing room air (Fig. 2C). At 75% WRpeak heliox breathing increased arterial oxygen partial pressure and minute ventilation due to an increase in tidal volume (Table 2). Dyspnea and leg effort sensation scores were lower during exercise sustained at 75 and 100% WRpeak whilst breathing heliox compared to room air. In addition, at 115% WRpeak during heliox breathing intensity of dyspnea remained lower compared to 100% WRpeak in room air (Table 2).

**Effect of heliox on central hemodynamic responses**

Whilst breathing normoxic heliox compared to room air, stroke volume increased during exercise sustained at 75 and 100% WRpeak (Fig. 3B), but due to inverse change in heart rate (Fig. 3D), the increase in cardiac output (absolute by 1 L/min, relative by ~ 10%) during heliox breathing failed to reach the level of statistical significance (p=0.08) (Fig. 3A). Cardiac output demonstrated a plateau at all exercise intensities examined, either during heliox or air breathing. As mean arterial pressure was not different between interventions, systemic vascular conductance exhibited trends identical to cardiac output (Fig 3E). Arterial oxygen content was significantly greater whilst breathing heliox compared to room air both at rest and during exercise sustained at 75% WRpeak (Fig 3C). Systemic oxygen delivery was significantly improved by heliox administration only during near-maximal exercise (i.e., 75% WRpeak) (Fig. 3F).

**Effect of heliox on leg and respiratory muscle hemodynamic responses**

Quadriceps muscle vascular conductance, blood flow and oxygen delivery during exercise sustained at 75% WRpeak were greater whilst receiving normoxic heliox compared to room air. However, there was no difference in any of these variables either between heliox and air breathing at 100% WRpeak or between heliox at 115% WRpeak and air at 100% WRpeak (Fig. 4, A-C). Similarly, intercostal muscle vascular conductance, blood flow and oxygen delivery were greater with heliox
breathing compared to room air during exercise sustained at 75 % WRpeak, whereas there was no
difference in any of these variables either between heliox and air breathing at 100% WRpeak or
between heliox at 115% WRpeak and air at 100% WRpeak (Fig. 4, D-F). No difference between
interventions was detected in leg arterio-venous oxygen content difference, oxygen extraction and
venous lactate (see data supplement, Table 1).

**DISCUSSION**

It is documented that lightening the work of breathing by the administration of normoxic
heliox enhances exercise tolerance in patients with COPD (1, 7, 13, 30, 31, 33), and that besides its
respiratory effects which ultimately reduce the intensity of dyspnea (1, 13, 30, 31, 33), heliox
enhances exercise tolerance by increasing locomotor muscle oxygen delivery (1, 7). The present
study investigated whether the potential increase in locomotor muscle oxygen delivery with heliox
breathing is due to an improvement in cardiac output and/or in arterial oxygen content or
alternatively is due to blood flow redistribution from intercostal to locomotor muscles. We
demonstrated that intercostal muscle blood flow did not decrease with heliox during exercise
intensities ranging from near-maximal to supra-maximal levels and therefore, according to our
reasoning, blood flow redistribution from the intercostal to locomotor muscles did not represent a
probable mechanism of leg muscle hemodynamic improvement during exercise in COPD patients.
More specifically, the novel findings of the study were the following. Firstly, during near-maximal
exercise (i.e., at 75% WRpeak), both quadriceps and intercostal muscle blood flow were greater
whilst breathing heliox compared to room air. As expected, normoxic heliox reduced total
respiratory muscle power by unloading both inspiratory and expiratory muscles. These changes in
lung mechanics most likely improved stroke volume and cardiac output, and combined with
increased arterial oxygen content, increased systemic oxygen delivery. We also confirmed the
increase in locomotor muscle oxygen delivery (7) during heliox breathing that should be the result
of improvements in both systemic oxygen delivery and locomotor muscle blood flow. Secondly,
during maximal and supra-maximal exercise (i.e., at 100 and 115% WR_{peak}), quadriceps and intercostal muscle blood flow were not different during heliox compared to room air breathing. Despite the decrease in total respiratory muscle loads by heliox administration, neither systemic oxygen delivery nor quadriceps or intercostal muscle oxygen delivery was improved. It is thus suggested that in patients with COPD the beneficial effects of heliox on respiratory and peripheral muscle haemodynamics and oxygen delivery are limited to near-maximal exercise.

Normoxic heliox administration enhances exercise tolerance in patients with COPD mainly owing to its effects on reducing the degree of dynamic hyperinflation and the intensity of dyspnea sensations (13, 31). However, the effect of heliox on systemic and peripheral muscle blood flow and oxygen delivery remains less well understood as there are conflicting lines of evidence, either suggesting no effects on cardiac output and systemic oxygen extraction (30), or demonstrating acceleration of on-exercise kinetic responses of cardiac output and noninvasive indices of peripheral muscle oxygenation (7) that have been interpreted to indicate improvements in central haemodynamics as well as in peripheral muscle oxygen delivery and extraction. In addition, helium breathing attenuated end-exercise peripheral locomotor muscle fatigue by about one-third in all patients with COPD, presumably by improving leg muscle oxygen delivery (1). The disparity of findings may stem from differences in the applied experimental protocols, as the findings by Oelberg et al (30) were obtained at peak exercise workloads, whereas the studies by Chiappa and colleagues (7) and Amann et al (1) were conducted during sub-maximal or near-maximal constant-work rate exercise where the effect on central haemodynamics would be expected to be different compared to maximal exercise (4, 24, 28). Accordingly, our study was designed to deal with this discrepancy by exercising patients at near-maximal, maximal and supra-maximal intensities. Moreover, to the best of our knowledge, this is the first study to test the respiratory muscle unloading effects of heliox breathing on peripheral muscle oxygen delivery with direct measures of cardiac output, and limb blood flow, vascular conductance and oxygen delivery across a wide range of workloads and respiratory pressures in patients with COPD.
Central hemodynamic responses during near-maximal exercise

Our results clearly confirm the positive effects of heliox breathing on reducing the total power of the respiratory muscles during exercise sustained at 75% WRpeak (Fig. 1A), thus corroborating previous findings by Eves and co-workers (13). This was due to unloading of inspiratory (as indicated by decreased ΔPdi and PTPdi) and mainly expiratory muscles (as indicated by the greater than inspiratory pressures decrease in peak expiratory gastric pressure and PTPab) (Fig. 1). Reduced inspiratory and mainly expiratory pressures by heliox administration may justify the finding of increased stroke volume (Fig. 3B) by decreasing ventricular afterload and mainly increasing venous return, respectively (24, 26, 27, 34). However, the numerical increase in cardiac output by ~ 10% failed to reach statistical significance (Fig. 3A). Nevertheless, our findings suggest that unloading the respiratory muscles by heliox administration during near-maximal exercise alleviates disturbances in central haemodynamics imposed by the mechanics of breathing in patients with COPD, thereby corroborating previous findings obtained during constant-load sub-maximal or near-maximal exercise in COPD (1, 7).

Intercostal and peripheral muscle hemodynamic responses during near-maximal exercise

More importantly we observed improved quadriceps and intercostal muscle oxygen delivery during near-maximal exercise whilst breathing normoxic heliox (Fig. 4, C and F). This result can be attributed to the increase in both arterial oxygen content (Fig. 3C) and quadriceps and intercostal muscle blood flow (Fig. 4, B and E). The finding that unloading the respiratory muscles improved peripheral muscle perfusion directly confirms previous suggestions that reductions in the work of breathing cause an increase in locomotor muscle blood flow and oxygen delivery in healthy individuals (17, 18) and in patients with COPD (1, 2, 7).

An additional novelty of the present study is that we were able to directly assess respiratory muscle blood flow during exercise by measuring the perfusion of the internal and external intercostal muscles over the 7th intercostal space using NIRS and ICG (16). In COPD, monitoring
intercostal muscle blood flow during exercise has additional important implications as there is evidence to indicate that chronic hyperinflation makes these patients use their intercostal muscles more vigorously than normal subjects during exercise, whilst the diaphragm makes a relatively limited contribution to the generation of maximal levels of ventilation (10, 32), likely due to its flattened shape. By measuring intercostal muscle blood flow in the present study, we were able to demonstrate that intercostal muscle blood flow not only did not decrease with heliox during submaximal exercise, but in fact increased, thus challenging the possibility of blood flow redistribution from the intercostal to the locomotor muscles as a mechanism of increase in locomotor muscle blood flow during heliox breathing. However, it should be emphasized that our findings do not necessarily rule out the likelihood of blood flow redistribution from the diaphragm to locomotor muscles during heliox breathing. Previous investigations point out for an inspiratory metaboreflex which is thought to be initiated by fatiguing diaphragmatic contractions with a consequent increase in sympathetic outflow to the working limbs (9, 17, 18), and heliox breathing could potentially decrease this sympathetic outflow and increase locomotor muscle blood flow by decreasing diaphragmatic workload and perfusion. Indeed, strong inferences about diaphragmatic perfusion cannot be made from intercostal muscle blood flow measurements, especially in patients with COPD. It has been shown in healthy humans that as ventilation rose, intercostal muscle blood flow was linearly correlated with the work of breathing and transdiaphragmatic pressure (16), but we are unaware of similar findings in patients with COPD, where blood flow to the diaphragm and intercostals might even change in opposite directions as intercostals try to compensate for a functionally impaired diaphragm (10, 32).

The increase in intercostal muscle vascular conductance during heliox breathing (Fig. 4D) could be attributed to decreased sympathetic nerve activation due to the increased arterial oxygen partial pressure (Table 2) and the mitigation on the strain placed on central haemodynamics (1, 7) (Fig. 3A) during helium breathing. In addition, increased expiratory muscle activity per se has been found to enhance sympathetic outflow (8), and heliox breathing by decreasing this activity (Fig. 1,
D and F) might have lessened sympathetic activation. All these factors, by increasing intercostal muscle vascular conductance could potentially contribute to the increase in intercostal muscle blood flow during near-maximal exercise. Moreover, direct mechanical factors could have allowed better perfusion of the intercostals muscles during heliox breathing. Indeed, intercostal muscle blood flow may be reduced by local compressive factors associated with increased intra-muscular tension and changes in intercostal muscle fibers orientation, and heliox breathing may have diminished intra-muscular tension (Fig. 1, B and D) and changed rib cage geometry and/or operational volumes (Fig. 2A), thus decreasing intercostal muscle vascular compression and increasing blood flow.

**Intercostal and peripheral muscle hemodynamic responses during maximal and supra-maximal exercise**

During maximal exercise, total respiratory muscle power remained lower during heliox breathing compared to room air as a result of unloading of both inspiratory and expiratory muscles (Fig. 1). Stroke volume increased with heliox compared to air breathing, most probably as a result of the same reasons reported above for near-maximal exercise (24, 26, 27, 34) but the numerical increase in cardiac output by ~ 10% failed to reach statistical significance (Fig. 3). In addition, neither quadriceps nor intercostal muscle vascular conductance, blood flow or oxygen delivery improved by heliox administration as they reached similar values to those seen at maximal exercise in room air (Fig. 4), most likely reflecting the upper limits by which sympathetic and cardiovascular responses are matched to the highest exercise intensities in humans (19).

Based on the findings by Richardson et al (33) showing that heliox administration improved exercise tolerance by 15%, we decided to subject our patients to a short bout of supra-maximal exercise sustained at 115% of peak work capacity in order to identify the mechanisms by which exercise tolerance is enhanced in COPD. We were surprised to find that although total respiratory muscle power remained lower during supra-maximal exercise with heliox breathing compared to room air (Fig. 1), neither central [i.e., cardiac output, systemic oxygen delivery (Fig. 3)] nor
Peripheral and intercostal muscle haemodynamic responses [i.e., quadriceps and intercostal muscle blood flow and oxygen delivery (Fig. 4), leg muscle oxygen extraction (see data supplement, Table 1)] improved. Conversely, dyspnea sensation during supra-maximal exercise with heliox was lower compared to maximal exercise in room air (Table 2), thereby confirming previous suggestions that the major improvement seen by heliox administration during exercise in patients with COPD is primarily due to reduced intensity of dyspnea sensations (7, 13, 23, 31).

Dyspnea and heliox breathing

Heliox breathing reduced dyspnea perception for the same or even higher workload (Table 2). Heliox reduces the pressure required to overcome frictional resistance by decreasing the degree of turbulence, and this effect is more important in flow-limited COPD patients at high flow rates and during expiration with consequent increases in maximal expiratory flow (31). Therefore, the most likely explanation for our finding is that heliox by reducing turbulent airway resistances and respiratory impedance decreased the total power of the respiratory muscles because of unloading of inspiratory and mainly expiratory muscles (Fig. 1). It is notable that heliox decreased the power of the respiratory muscles during exercise sustained at 75% WRpeak despite an increment in minute ventilation (Table 2). This increment in minute ventilation was due to an increase in tidal volume, finding compatible with that of other studies using heliox during sub-maximal or near-maximal exercise in COPD patients (1, 7).

Perception of leg discomfort and dyspnea are recognized as two major contributors to exercise limitation in COPD (29), and their relief by heliox administration (Table 2) is expected to improve exercise performance (1). Although we did not measure this effect in the present study, several investigations utilizing heliox breathing in patients with COPD have reported prolongation in time to exhaustion (e.g., Refs. 7, 23, 31). Therefore, clinical implications may be derived by using heliox in ventilatory-limited COPD patients, allowing them to sustain a high-power output for a period of time long enough to induce a significant training effect during rehabilitation (31) and/or
to have a significant impact on daily exercise performance and health status beyond that possible with ambulatory oxygen alone, provided that heliox can be administered in a way patients find acceptable (23).

**Study limitations**

Firstly, due to the intensive and exhausting nature of the study, and for patient safety, repeated testing across the different work rates so as to establish reproducibility of cardiac output and regional muscle blood flow measurements in patients with COPD was not performed. Secondly, because our patients did not exhibit exercise-induced hyperinflation, as assessed by optoelectronic plethysmography, at any percentage of WRpeak (Fig. 2C), most of them should belong to those who strongly recruit their abdominal muscles (the so-called non-hyperinflators) (40) and the present results might not be so pertinent to patients who progressively hyperinflate during exercise (the so-called hyperinflators), when hyperinflation is also evaluated by optoelectronic plethysmography. Indeed, unloading the expiratory abdominal muscles during heliox breathing in the so-called non-hyperinflators of the present study had potentially major advantage on central hemodynamic responses (e.g., increase in stroke volume and cardiac output), and such an advantage may not be so prominent in the so-called hyperinflators who do not recruit strongly their expiratory abdominal muscles. On the other hand, dynamic hyperinflation has adverse effects on central hemodynamic responses (26, 27, 34), and reduction of the degree of exercise-induced dynamic hyperinflation during heliox breathing could still improve cardiac output and muscle blood flow. Nevertheless, this reservation may only be relevant when optoelectronic plethysmography is used to evaluate the exercise-induced dynamic hyperinflation, because many of our patients, who were deemed as not exhibiting exercise-induced dynamic hyperinflation with optoelectronic plethysmography, would be considered as exhibiting hyperinflation if the degree of dynamic hyperinflation was assessed by decreases in inspiratory capacity during exercise. Indeed, different results of the degree of exercise-induced dynamic hyperinflation in COPD patients are reported by authors (29) who measure inspiratory capacity by integrating flow at the mouth (spirometry) as an
index of hyperinflation and those authors who use optoelectronic plethysmography to measure chest wall volumes (25). This difference can be explained by the discrepancy between the methods used to measure dynamic hyperinflation, as optoelectronic plethysmography can potentially underestimate and/or spirometry can potentially overestimate dynamic hyperinflation (25). Spirometry measures the volume of gas entering or leaving the lungs at the mouth, whereas optoelectronic plethysmography measures the volume of the trunk, which includes volume changes at the mouth, but also two other variables—gas compression and decompression in the lungs, and blood shifts between the trunk and extremities (25). It has been calculated (25) that for peak expiratory abdominal (or pleural, as during expiration the diaphragm is relaxed and abdominal pressure is freely transmitted into the pleural space) pressures of about 22 cmH_2O at maximal exercise workload [our peak expiratory abdominal pressures at the end of exercise bouts were even higher reaching approximately 30 cmH_2O during air breathing (Fig. 1D)], optoelectronic plethysmography would measure an end-expiratory chest wall volume that would be 330 ml less than that measured by spirometry, and thus optoelectronic plethysmography would not detect 89% of the reduction in inspiratory capacity (mean value = 370 ml) measured by spirometry (29). Finally, since some of our patients were neither particularly obstructed nor nutritionally depleted (Table 1), the findings of the present study might not be so applicable in other COPD populations.

**Perspectives and significance**

The present study constitutes the first experimental demonstration that intercostal muscle blood flow does not decrease with heliox during exercise intensities ranging from near-maximal to supra-maximal levels, and therefore blood flow redistribution from the intercostal to locomotor muscles does not represent a likely mechanism of leg muscle hemodynamic improvement with heliox during exercise in COPD patients. We confirm that heliox reduces respiratory muscle power and relieves dyspnea sensations during near-maximal, maximal and supra-maximal exercise. However, only during near-maximal exercise does heliox improve peripheral and respiratory muscle blood flow and oxygen delivery as well as systemic oxygen delivery, whereas during
maximal and supra-maximal exercise heliox has no effect on central and peripheral muscle haemodynamics. Because our patients did not exhibit exercise-induced hyperinflation, as assessed by optoelectronic plethysmography, the present results might not be applicable to patients who hyperinflate during exercise, when hyperinflation is also measured with the same technique. Nevertheless, since heliox breathing relieved the perception of leg discomfort and dyspnea, two recognized major determinants of exercise limitation in COPD (29), heliox administration is anticipated to improve exercise performance and prolong the time to exhaustion, thus enhancing the training effect during rehabilitation (31) and/or improve daily exercise performance and health status (23).

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REFERENCES


FIGURE LEGENDS

Fig. 1. Respiratory muscle load and power. A: total respiratory muscle power, B: rib cage muscle power, C: pressure-time product for the diaphragm (PTPdi), D: peak expiratory gastric pressure, E: tidal excursion in transdiaphragmatic pressure (ΔPdi), and F: pressure-time product for expiratory abdominal muscles (PTPab) recorded at different fractions of peak work rate (WRpeak) during exercise whilst breathing normoxic heliox (open triangles) or room air (filled triangles). Values are means ± SE for 10 subjects. Asterisks denote significant differences (p<0.05) between exercise whilst breathing heliox versus exercise in room air at an identical fraction of peak work rate, whereas crosses denote significant differences (p<0.05) compared to exercise at 100% WRpeak in room air.

Fig. 2. Chest wall volume regulation. A: rib cage, B: abdominal, and C: total chest wall volume regulation at the end of inspiration and expiration recorded at different fractions of peak work rate (WRpeak) during exercise whilst breathing normoxic heliox (open triangles) or room air (filled triangles). Values are means ± SE for 10 subjects. Asterisks denote significant differences (p<0.05) between exercise whilst breathing heliox versus exercise in room air at an identical fraction of peak work rate, whereas crosses denote significant differences (p<0.05) compared to exercise at 100% WRpeak in room air.

Fig. 3. Central hemodynamic responses. A: cardiac output, B: stroke volume, C: arterial oxygen content (CaO2), D: heart rate, E: systemic vascular conductance, and F: systemic oxygen delivery measured at different fractions of peak work rate (WRpeak) during exercise whilst breathing normoxic heliox (open triangles) or room air (filled triangles). Values are means ± SE for 10 subjects. Asterisks denote significant differences (p<0.05) between exercise whilst breathing heliox versus exercise in room air at an identical fraction of WRpeak.

Fig. 4. Quadriceps and intercostal muscle blood flow and oxygen delivery. A: quadriceps muscle vascular conductance, B: quadriceps muscle blood flow, C: quadriceps muscle oxygen delivery, D: intercostal muscle vascular conductance, E: intercostal muscle blood flow, and F: intercostal muscle oxygen delivery measured at different fractions of peak work rate (WRpeak) during exercise whilst
breathing normoxic heliox (open triangles) or room air (filled triangles). Values are means ± SE for 10 subjects. Asterisks denote significant differences (p<0.05) between exercise whilst breathing heliox versus exercise in room air at an identical fraction of WRpeak.
<table>
<thead>
<tr>
<th>Demographic/anthropometric</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>60 ± 7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>172 ± 6</td>
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<tr>
<td>Weight, kg</td>
<td>77 ± 18</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>26.1 ± 5.7</td>
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<tr>
<td>Fat free mass index, kg/m²</td>
<td>18.4 ± 1.8</td>
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<tr>
<td>Pulmonary function</td>
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<tr>
<td>FEV₁, liters</td>
<td>1.6 ± 0.6</td>
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<td>FEV₁, % predicted</td>
<td>50.5 ± 17.5</td>
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<tr>
<td>FVC, liters</td>
<td>3.1 ± 0.5</td>
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<td>FVC, % predicted</td>
<td>75 ± 8</td>
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<tr>
<td>TLC, liters</td>
<td>8.6 ± 1.0</td>
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<tr>
<td>TLC, % predicted</td>
<td>135 ± 12</td>
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<tr>
<td>RV, liters</td>
<td>4.9 ± 0.9</td>
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<tr>
<td>RV, % predicted</td>
<td>215 ± 35</td>
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<tr>
<td>FRC, liters</td>
<td>6.1 ± 1.1</td>
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<tr>
<td>FRC, % predicted</td>
<td>179 ± 21</td>
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<tr>
<td>DL-CO₂, % predicted</td>
<td>39 ± 13</td>
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<tr>
<td>PaO₂, Torr</td>
<td>80 ± 5</td>
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<tr>
<td>PaCO₂, Torr</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>SaO₂, %</td>
<td>93 ± 4</td>
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<tr>
<td>pH</td>
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<tr>
<td>Peak exercise data</td>
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<tr>
<td>W_Rpeak, W</td>
<td>73 ± 42</td>
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<tr>
<td>V_O₂peak, ml/min</td>
<td>1155 ± 101</td>
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<tr>
<td>H_Rpeak, beats/min</td>
<td>117 ± 14</td>
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<tr>
<td>V_Epeak, l/min</td>
<td>49 ± 16</td>
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<td>V_Tpeak, liters</td>
<td>1.6 ± 0.4</td>
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<tr>
<td>f_peak, breaths/min</td>
<td>32 ± 8</td>
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<tr>
<td>SpO₂, %</td>
<td>92 ± 3</td>
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<tr>
<td>Borg dyspnea scores</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Borg leg effort scores</td>
<td>5 ± 2</td>
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Values are means ± SD for 10 subjects. FEV$_1$, forced expiratory volume in one second; FVC, forced vital capacity; TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; DL$_{CO}$, diffusing capacity of the lung for carbon monoxide; PaO$_2$, partial pressure of arterial oxygen; PaCO$_2$, partial pressure of arterial carbon dioxide; SaO$_2$, arterial oxygen saturation; WR$_{peak}$, peak work rate; VO$_2$peak, peak oxygen uptake; HR$_{peak}$, peak heart rate; VE$_{peak}$, peak minute ventilation; VT$_{peak}$, peak tidal volume; f$_{peak}$, peak breathing frequency; SpO$_2$, arterial oxygen saturation measured by pulse oximetry.
### Table 2. Ventilatory and gas exchange responses during exercise in room air and heliox

<table>
<thead>
<tr>
<th>Work rate (% peak) in room air</th>
<th>AIR</th>
<th>HELIOX</th>
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<tbody>
<tr>
<td></td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>VE, l/min</td>
<td>47.8 ± 6.7</td>
<td>49.6 ± 6.7</td>
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<tr>
<td>VT, liters</td>
<td>1.57 ± 0.2</td>
<td>1.57 ± 0.18</td>
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<tr>
<td>Ti, s</td>
<td>0.84 ± 0.04</td>
<td>0.85 ± 0.04</td>
</tr>
<tr>
<td>Te, s</td>
<td>1.19 ± 0.10</td>
<td>1.23 ± 0.09</td>
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<tr>
<td>Ti/Ttot, %</td>
<td>42.0 ± 1.6</td>
<td>43.0 ± 1.4</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>31.0 ± 2.0</td>
<td>32.0 ± 2.0</td>
</tr>
<tr>
<td>VO2, ml/min</td>
<td>1005 ± 174</td>
<td>1069 ± 167</td>
</tr>
<tr>
<td>PaO2, Torr</td>
<td>80.5 ± 5.6</td>
<td>78.6 ± 4.3</td>
</tr>
<tr>
<td>PaCO2, Torr</td>
<td>41.0 ± 1.0</td>
<td>39.9 ± 1.2</td>
</tr>
<tr>
<td>SaO2, %</td>
<td>93.3 ± 1.2</td>
<td>93.2 ± 0.8</td>
</tr>
<tr>
<td>Arterial lactate, mmol/l</td>
<td>3.6 ± 0.5</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>Dyspnea scores</td>
<td>7 ± 2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>Leg effort scores</td>
<td>6 ± 2</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE for 10 subjects. Exercise data depict the results of the exercise tests during air and heliox at specific percentage of peak work rate breathing in room air measured during the final minute of each of the exercise bouts. VE, minute ventilation; VT, tidal volume; Ti, time of inspiration; Te, time of expiration; Ti/Ttot, duty cycle; f, breathing frequency; VO2, oxygen uptake; PaO2, partial pressure of arterial oxygen; PaCO2, partial pressure of arterial carbon dioxide; SaO2, arterial oxygen saturation. † p < 0.05 versus exercise at 100% of peak
work rate in air; ‡ p < 0.05 versus exercise in air at same percentage of peak work rate.
Figure 1.
Figure 2.
**Figure 3.**

- **A** Cardiac output (L/min)
- **B** Stroke volume (ml/beat)
- **C** $\text{CaO}_2$ (ml/L)
- **D** Heart rate (beats/min)
- **E** Systemic vascular conductance (ml/min/mm Hg)
- **F** Systemic O$_2$ delivery (L/min)

Comparisons shown at REST, 75%, 100%, and 115% of WR$_{peak}$.

*Indicates statistical significance.
Figure 4.