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

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**Running head:** Respiratory muscle perfusion in COPD

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**News and Noteworthy**

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

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**Abstract**

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76 | The study investigated whether [high-intensity](#) exercise interferes with inspiratory and  
77 | expiratory muscle perfusion in patients with COPD. We compared respiratory local muscle  
78 | perfusion between constant-load cycling (sustained at 80% WRpeak) and voluntary  
79 | normocapnic hyperpnoea reproducing similar work of breathing (WoB) in 18 patients  
80 | (FEV<sub>1</sub>:58±24% predicted). Local muscle blood flow index (BFI), using indocyanine green dye  
81 | and fractional oxygen saturation (%StiO<sub>2</sub>) were simultaneously assessed by near-infrared  
82 | spectroscopy (NIRS) over the intercostal, scalene, rectus abdominis and vastus lateralis  
83 | muscles. Cardiac output (impedance cardiography), WoB (oesophageal/gastric balloon  
84 | catheter), and diaphragmatic and extradiaphragmatic respiratory muscle electromyographic  
85 | activity (EMG) were also assessed throughout cycling and hyperpnoea. Minute ventilation,  
86 | breathing pattern, WoB and respiratory muscle EMG were comparable between cycling and  
87 | hyperpnoea. During cycling, cardiac output and vastus lateralis BFI were significantly greater  
88 | compared to hyperpnoea [by +4.2(2.6-5.9) L/min and +4.9(2.2-7.8) nmol/s], respectively,  
89 | (p<0.01). Muscle BFI and %StiO<sub>2</sub> were respectively lower during cycling compared to  
90 | hyperpnoea in scalene [by -3.8(-6.4- -1.2) nmol/s and -6.6(-8.2- -5.1)%], intercostal [by -1.4(-  
91 | 2.4- -0.4) nmol/s and -6.0(-8.6- -3.3)%] and abdominal muscles [by -1.9(-2.9- -0.8) nmol/s and  
92 | -6.3(-9.1- -3.4)%] (p<0.001). The difference in respiratory (scalene and intercostal) muscle BFI  
93 | between cycling and hyperpnoea was associated with greater dyspnoea (Borg CR10) scores (r=  
94 | -0.54 and r= -0.49, respectively, p<0.05). These results suggest that in patients with COPD 1)  
95 | locomotor muscle work during [high-intensity](#) exercise interferes with extradiaphragmatic  
96 | respiratory muscle perfusion and that 2) insufficient adjustment in extradiaphragmatic  
97 | respiratory muscle perfusion during [high-intensity](#) exercise may partly explain the increased  
98 | sensations of dyspnoea.

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101 | **Keywords:** perfusion, exercise, NIRS, COPD, respiratory muscles

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108 | **Introduction**

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

117 However, traditional techniques for assessing respiratory muscle blood flow are highly  
118 invasive, exposing the individuals to unnecessary health risks (14). Near-Infrared Spectroscopy  
119 in conjunction with infusions in the circulation of the light-absorbing tracer indocyanine green  
120 dye, (NIRS-ICG technique) has been increasingly applied over the past decade to provide a less  
121 invasive and reliable method for assessing absolute and relative values (blood flow index) of  
122 local respiratory (and locomotor) muscle perfusion at rest and during exercise in healthy  
123 participants and in patients with Chronic Obstructive Pulmonary Disease (COPD) (14, 32, 33,  
124 36, 54).

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

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

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

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

169

## 170 | **Methods**

### 171 | ***Study group***

172 | Eighteen clinically stable patients with COPD (FEV<sub>1</sub>: 58 ± 24% predicted) according to the  
173 | Global Initiative for Chronic Obstructive Lung Diseases (GOLD) participated in the study.  
174 | Exclusion criteria included no participation in exercise-training programs in the year before, no  
175 | long-term oxygen use and not presenting cardiovascular conditions limiting exercise tolerance,  
176 | severe orthopaedic conditions, psychiatric or cognitive disorders and/or progressive  
177 | neurological or neuromuscular disorders.

### 178 | ***Study design***

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

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

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#### 194 ***Preliminary assessments***

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

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#### 201 ***Experimental design***

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

206 During visit 2 (>48 hours after *visit 1*) patients underwent a constant-load cycling test at  
207 80% WR<sub>peak</sub> to the limit of tolerance (i.e., exercise duration 366 ±109 sec) aiming to record  
208 the ventilatory responses (i.e., mean tidal volume, breathing frequency and minute ventilation)  
209 that patients were requested to reproduce during the hyperpnoea trial on visit 3 (*see below*).  
210 The limit of exercise tolerance was defined as the time point at which patients signalled the  
211 inability to continue exercising or could not maintain the required pedalling rate (50 – 60  
212 revolution/min) despite being encouraged by the investigators to carry on cycling. Before and  
213 after the constant-load cycling test, assessment of isometric quadriceps strength and quadriceps  
214 muscle contractile fatigue (Magstim Co Ltd, Whitland, UK) were performed (16).

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

223 | touching the knees. Hyperpnoea was sustained to the point patients could not maintain  
224 ventilatory responses to levels comparable to those during exercise. During the hyperpnoea test  
225 investigators provided continuous verbal guidance aiming to ensure a maximum variation in  
226 minute ventilation less than 5% throughout the test. This was facilitated by visual feedback on  
227 breathing parameters that was provided real-time on a screen monitor (73). Normocapnia was  
228 maintained by having subjects inspire from a Douglas bag containing 5% CO<sub>2</sub>, 21% O<sub>2</sub> and  
229 74% N<sub>2</sub>, connected to a two-way non-rebreathing valve (model 2700, Hans Rudolph) by a  
230 piece of tubing (85, 86). Following a sufficient resting period [average 27 min (range: 25-31  
231 min)], patients performed a constant-load cycling test at 80% WR<sub>peak</sub> to the limit of tolerance.  
232 During hyperpnoea and constant-load exercise, recordings of pulmonary gas exchange and  
233 ventilatory variables were performed breath-by-breath (V<sub>max</sub> 229; Sensor Medics, San Diego,  
234 CA). Arterial oxygen saturation was measured continuously by a pulse oximeter and blood  
235 pressure was assessed every minute by an automated cuff monitor integrated to the cycle  
236 ergometer. Breathlessness and leg discomfort were measured by the modified Borg scale (11).  
237 During cycling, patients performed inspiratory capacity (IC) manoeuvres every two minutes to  
238 identify the degree of dynamic lung hyperinflation assuming constant total lung capacity (TLC)  
239 (64).

#### 240 ***Subject preparation***

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

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

#### 260 ***Respiratory muscle pressures and work of breathing***

261 The oesophageal/gastric balloon catheter was used for the assessment of Pes, Pga,  
262 transdiaphragmatic pressure ( $P_{di}=P_{ga}-P_{es}$ ) and diaphragm EMG activation via five diaphragm  
263 electromyography electrode pairs (Yinghui Medical Equipment Technology Co. Ltd.,  
264 Guangzhou, China). After optimal placement (20, 45, 58), the catheter was secured at the  
265 patient's nose with tape. The diaphragm EMG signals were sampled at 2000 Hz (Micro1401-3,  
266 Cambridge Electronic Design Limited, Cambridge, UK), amplified (Biomedical amplifier,  
267 Guangzhou, China) and then recorded and processed by a data acquisition software (Spike 2,  
268 Cambridge Electronic Design Limited, Cambridge, UK). Diaphragm EMG data were converted  
269 into root mean square (RMS) and were expressed as percentages of maximum activation  
270 (diaphragm EMG, %max) that was recorded during IC maneuvers (i.e., obtained at rest or  
271 during exercise, 20, 73). Respiratory flow signals, Pes and Pga signals were continuously  
272 sampled at 100 Hz (Micro1401-3, Cambridge Electronic Design Limited, Cambridge, UK), and  
273 then recorded and processed by the same data acquisition software (Spike 2, Cambridge  
274 Electronic Design Limited, Cambridge, UK). Maximal Pes and Pdi pressures were measured  
275 from FRC during sniff maneuvers, and maximal Pga was measured from TLC during forced  
276 expiratory capacity maneuvers. Pes, Pga and Pdi were expressed as percentages of maximal  
277 activation and were used as indices of global inspiratory, expiratory and diaphragmatic effort,  
278 respectively (45). Pes, Pga, Pdi and WoB over one-minute periods was calculated by  
279 integrating volume and pressure generated (e.g.,  $P_{es} \text{ WoB} = P_{es} \times \text{tidal volume}$ ), then  
280 multiplied by breathing frequency (e.g.,  $P_{es} \text{ WoB}/\text{min} = P_{es} \text{ WoB} \times \text{bf}$ ) and presented in  
281 L/cmH<sub>2</sub>O/min. Pes, Pga and Pdi Pressure-Time Products (PTP) – commonly considered indices  
282 of the energy of breathing (46) – were assessed by multiplying each of the pressures by the  
283 time of muscle contraction and breathing frequency and presented in cmH<sub>2</sub>O/sec/min.

#### 284 ***Respiratory muscle activation***

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

290 vastus lateralis [(vl), left vastus lateralis muscle 10-12 cm above the knee] was measured by  
291 surface electromyography (EMG) (Desktop Direct Transmission (DTS), NORAXON,  
292 Scottsdale, USA) (73), sampled at 2000 Hz (Micro1401-3, Cambridge Electronic Design  
293 Limited, Cambridge, UK), and then recorded and processed by a data acquisition software  
294 (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK). For EMGsca, EMGscm,  
295 EMGpicm, EMG7<sup>th</sup>icm data were expressed as percentages of maximum activation during IC  
296 maneuvers (i.e., obtained at rest or during exercise, 20, 73) and for EMGabd data were  
297 expressed as percentages of maximum activation during forced expiratory capacity maneuvers  
298 (73). EMGvl data of maximum activation were recorded during a maximal voluntary isometric  
299 contraction of the knee extensors (40). All ventilatory and respiratory pressures, WoB,  
300 respiratory and locomotor muscle activation signals used for comparisons at rest, during  
301 hyperpnoea and cycling were the average of all values recorded over the last 60 seconds at rest  
302 and during the last 30 seconds of hyperpnoea and cycling.

### 303 ***Central hemodynamic responses***

304 Cardiac output was measured continuously during hyperpnoea and cycling by an impedance  
305 cardiography device (PhysioFlow PF05; Manatec Biomedical, Macheren, France, PhysioFlow).  
306 Six electrodes were placed according to the manufacturers' instructions (53). Data points were  
307 excluded when signal quality was less than 90% (53). Cardiac output values were recorded at  
308 1-second intervals and were the average over the last 60 seconds during rest and during the last  
309 30 seconds of hyperpnoea and cycling trials. Systemic oxygen delivery was calculated as the  
310 product of cardiac output and arterial oxygen content; the latter was calculated using the  
311 following formula:  $[1.39 \times \text{hemoglobin concentration [Hb]} \times \%SpO_2]$  (12). Arterio-venous  
312 oxygen content (a-vO<sub>2</sub>) difference was calculated by dividing whole-body oxygen uptake by  
313 cardiac output (73). The oxygen extraction ratio was calculated as the ratio of the arteriovenous  
314 oxygen content (a-vO<sub>2</sub>) difference to arterial oxygen content and expressed in percentage.  
315 Systemic vascular conductance was calculated by dividing cardiac output by the mean arterial  
316 blood pressure (73).

317

318

### 319 ***Muscle blood flow index by NIRS***

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

322 commercial Near-Infrared Spectroscopy (NIRS, Continuous Wave, Spatially Resolved  
323 Technique, NIRO-200 and a NIRO-200NX; HAMAMATSU Photonics KK) devices were used  
324 in combination with the light-absorbing indocyanine green dye (ICG). The four NIRS probes  
325 were placed at scalene (right posterior triangle of the neck), 7<sup>th</sup> intercostal (midaxillary line, left  
326 7<sup>th</sup> intercostal space) and rectus abdominis (upper left 1/3 of rectus abdominis below the costal  
327 cartilage) muscles (73). The fourth NIRS probe was placed over the left vastus lateralis muscle  
328 | 10-12 cm above the knee (next to EMG electrode) (86). NIRS-ICG derived BFI was calculated  
329 by dividing the ICG peak concentration of the muscle by the rise time from 10 to 90% of peak  
330 according to established methods and expressed in nanomoles/sec (nmol/sec) (32, 36, 54, 73).  
331 In addition, BFI data were adjusted for resting values and expressed as fold change from rest  
332 during cycling and hyperpnoea (54). ICG injections for calculating BFI were performed at rest  
333 and during the last 30 seconds of hyperpnoea and cycling trials. ICG concentration curves data  
334 were exported by NIRS in document file format (i.e., filename extension 'txt') and stored on  
335 disk for off-line analysis. ICG concentration curves in 'txt' format were analyzed by using the  
336 Chart5 version 5.4.2 (ADInstruments) program. Low-pass filtering with a cutoff frequency of  
337 0.5 Hz and smoothing window width (by using the triangular Bartlett window function) of nine  
338 points produced the smoothed curve that was used for BFI calculation (36, 54, 73).

### 339 ***Muscle oxygenation by NIRS***

340 For respiratory and vastus lateralis muscle oxygenation, the same NIRS devices were used.  
341 Concentration changes in deoxy (Hb+Mb) were used as an index of muscles oxygen extraction  
342 and total (Hb+Mb) as an index of blood volume reflecting changes in microvascular  
343 conductance (vasodilation or vasoconstriction responses) for respiratory and vastus lateralis  
344 muscles (31). In addition, absolute values of NIRS derived fractional tissue O<sub>2</sub> saturation index  
345 (%StiO<sub>2</sub>; i.e., the ratio of [oxy(Hb+Mb)] to [total(Hb+Mb)] expressed as a fraction  
346 ([oxy(Hb+Mb)]/[total(Hb+Mb)]\*100) that reflect the tissue capacity to match oxygen supply  
347 | relative to its metabolic demand (31, 52, 84) were also recorded. A path length of 18.6 cm was  
348 set up for all respiratory and vastus lateralis muscles. Separation distance between the NIRS  
349 light transmitter and receiver probes was 40 mm, thus allowing a maximum NIRS penetration  
350 depth of 20 mm. NIRS oxygenation data were sampled at 5 or 6 Hz and averaged during the  
351 last 60 seconds at rest and during the last 30 seconds for hyperpnoea and cycling. Adipose  
352 tissue thickness (fat + skin layer) were performed by a Harpenden 10-skinfold caliper on the  
353 scalene, 7<sup>th</sup> intercostal space, rectus abdominis and the vastus lateralis muscle (80). The mean

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

### 359 *Assessment of quadriceps muscle strength and fatigue*

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

### 373 *Statistical analysis*

374 Data are expressed as mean  $\pm$  SD at rest, cycling and hyperpnoea or as mean difference with  
375 95% confidence interval (lower and upper limit) for comparisons between the three conditions  
376 (i.e., at rest, cycling and hyperpnoea). The normality of all the data was examined by the  
377 Shapiro-Wilk test. Ventilatory and breathing pattern parameters, respiratory muscle pressures  
378 and WoB, respiratory and locomotor muscle activation, central hemodynamic and respiratory  
379 and locomotor blood flow and oxygenation variables recorded at rest, during hyperpnoea, and  
380 cycling were compared using repeated-measures ANOVA or by the Friedman test when normal  
381 distribution was violated. When ANOVA (or Friedman test) detected significant differences  
382 between rest, hyperpnoea, and cycling, pairwise comparisons with Bonferroni correction (for  
383 ANOVA) or using Dunn's Multiple Comparison Test (for Friedman test) were performed as  
384 pos-hoc analysis. Changes from rest in respiratory and vastus lateralis muscle BFI and  
385 oxygenation variables between cycling and hyperpnoea tests were compared by paired t-tests  
386 when normally distributed, or by Wilcoxon signed-rank tests if normal distribution assumptions  
387 were not met. Changes in respiratory and vastus lateralis muscle BFI and oxygenation variables

388 between cycling and hyperpnoea tests among patients with different stages of diseases severity  
389 were compared by unpaired t-tests when normally distributed, or by Welch's Test if normal  
390 distribution assumptions were not met. Pearson's correlation coefficient (r) was used to  
391 establish associations between BFI (expressed as the difference between cycling and  
392 hyperpnoea) and dyspnoea (expressed as the difference between cycling and hyperpnoea) for  
393 intercostal, scalene, rectus abdominis, and vastus lateralis muscles. The minimum sample size  
394 was calculated based on 80% power and a two-sided 0.05 significance level. An expected effect  
395 size [Cohens d] of 0.497 was calculated based on data from a previous study in patients with  
396 COPD (FEV<sub>1</sub>: 51±18%predicted) (85), which demonstrated a significant decrease in intercostal  
397 muscle %StiO<sub>2</sub> during cycling compared to voluntary normocapnic hyperpnoea. Specifically,  
398 that study (85) revealed a mean difference in intercostal muscle %StiO<sub>2</sub> of -2.00% with a  
399 corresponding pooled SD of 4.02% between cycling (at 75% of peak work rate, ~60 watts) and  
400 hyperpnoea sustained at levels of minute ventilation similar to those recorded during cycling  
401 (~45 litres/min). The critical sample size was calculated to be 9 patients using repeated-  
402 measures ANOVA as the primary statistical analysis method. Taking into consideration the  
403 challenges imposed on patients by the invasive procedures, we decided to recruit 18 patients for  
404 obtaining a full dataset for the minimum required number of patients. Data were analyzed using  
405 the GraphPad Prism statistical software. The level of significance was set at p<0.05.

406

## 407 **Results**

### 408 ***Subject characteristics.***

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

### 414 ***Breathing pattern, symptoms and locomotor muscle fatigue***

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

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421 2). Specifically, the decrease from rest in inspiratory capacity during cycling did not  
422 significantly differ among patients with different stages of disease severity (GOLD I:  $-0.394 \pm$   
423  $0.407$ , GOLD II:  $-0.441 \pm -0.480$ , GOLD III-IV:  $-0.493 \pm -0.395$  L,  $p>0.1$ ). Dyspnoea at end of  
424 cycling was significantly higher compared to hyperpnoea ( $p=0.0008$ , Table 2). Leg discomfort  
425 at end of cycling was  $7.0 \pm 2.8$  on the 10-Borg scale. The primary reason for stopping cycling  
426 was dyspnoea ( $n=7$ ), leg discomfort alone ( $n=4$ ) and the combination of both leg discomfort  
427 and dyspnoea ( $n=7$ ). Compared to resting values of potentiated quadriceps twitch contraction  
428 force ( $11.5 \pm 4.3$  kg), this was significantly decreased on average by  $22 \pm 21\%$  and  $23 \pm 17\%$ ,  
429 10 and 35 min after the end of the constant-load exercise test (visit 2), respectively (*10 min*:  $9.2$   
430  $\pm 4.5$  kg and *40 min*:  $9.1 \pm 4.2$  kg, both  $p<0.001$ ).

#### 431 ***Respiratory pressures and work of breathing***

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

446

447

#### 448 ***Activation of respiratory and locomotor muscles***

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

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

#### 454 ***Central hemodynamic and metabolic responses***

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

#### 466 ***Perfusion responses of respiratory and locomotor muscles***

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

479

#### 480 ***Oxygenation responses of respiratory and locomotor muscles***

481 During hyperpnoea, total [Hb+Mb] concentration increased from rest in scalene (p=0.0027),  
482 7<sup>th</sup> intercostal (p=0.079) and abdominal (p=0.028) muscles (Table 5). In addition, during  
483 hyperpnoea, total [Hb+Mb] concentration was greater for the scalene (p=0.0061), 7<sup>th</sup> intercostal

484 (p=0.054) and abdominal (p=0.033) muscles compared to cycling (Table 5). During cycling,  
485 deoxy [Hb+Mb] concentration significantly increased from rest in intercostal (p=0.009),  
486 abdominal (p=0.0027) and vastus lateralis muscle (p=0.0042) and it was found to be  
487 significantly greater for the 7<sup>th</sup> intercostal (p=0.0006) and abdominal muscles (p=0.0011)  
488 compared to hyperpnoea (Table 5). During hyperpnoea, scalene, 7<sup>th</sup> intercostal, abdominal and  
489 vastus lateralis muscle %StiO<sub>2</sub> was not different compared to that recorded at rest (p>0.05,  
490 Figure 4 and Table 5). In contrast, during cycling, a significant reduction from rest in %StiO<sub>2</sub>  
491 was observed in scalene (p<0.0001), 7<sup>th</sup> intercostal (p=0.0015), abdominal (p<0.0001) and  
492 vastus lateralis muscle (p=0.0013) (Figure 4 and Table 5). Furthermore, scalene (p<0.0001), 7<sup>th</sup>  
493 intercostal (p=0.0002) abdominal (p<0.0001) and vastus lateralis muscle (p=0.0009) %StiO<sub>2</sub>  
494 was significantly lower during cycling compared to hyperpnoea (Figure 4 and Table 5). In  
495 addition, no significant differences were found in respiratory and leg muscles %StiO<sub>2</sub> to  
496 cycling versus hyperpnoea across different stages of COPD severity (Table 6).

#### 497 *Associations between muscle activation, perfusion and dyspnoea during cycling and* 498 *hyperpnoea*

499 We found significant inverse relationships between the reduction in the BFI of scalene and  
500 7<sup>th</sup> intercostal muscles and the greater dyspnoea scores in cycling compared to hyperpnoea (r=-  
501 0.54, p=0.026 and r=-0.49, p=0.043, respectively). No significant relationships were found for  
502 abdominal and vastus lateralis muscle BFI and dyspnoea scores (r=-0.32, p=0.020 and r=0.05,  
503 p=0.83, respectively). In addition, no significant relationships were found amongst differences  
504 in activation of diaphragm, scalene, parasternal, 7<sup>th</sup> intercostal and abdominal muscles and the  
505 differences in dyspnoea scores between cycling and hyperpnoea trial (p>0.1). Finally, no  
506 significant relationships were found between differences in activation of scalene, 7<sup>th</sup> intercostal  
507 and abdominal muscles and differences in their perfusion between cycling and hyperpnoea  
508 trials (p>0.1).

509

510

## 511 **Discussion**

### 512 *Main findings*

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

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

### 531 *Mechanisms of insufficient adjustments in respiratory muscle perfusion during cycling*

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

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

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

573 Thirdly, an increased sympathetic vasoconstrictor outflow to the respiratory muscles upon  
574 activation of the respiratory muscle metaboreflex may also provide a possible explanation for  
575 the insufficient adjustment in respiratory muscle perfusion during cycling (76). In this context,  
576 recently, it was suggested that muscle contractions of the respiratory muscles during [high-](#)  
577 [intensity](#) exercise can cause increased group III and IV afferent activity leading to a  
578 sympathetically mediated vasoconstriction, thereby contributing to limitations in respiratory  
579 muscle blood flow and O<sub>2</sub> transport (76). The proposed greater development of intramuscular  
580 pressures and increased sympathetically mediated vasoconstriction [to the respiratory muscles](#)  
581 during cycling compared to hyperpnoea are supported by the findings (Table 5) showing lower  
582 microvascular conductance, inferred by lower total [Hb+Mb] during cycling compared to  
583 hyperpnoea, for all measured by NIRS extradiaphragmatic respiratory muscles. [Therefore, the](#)  
584 [results of the present study do not provide evidence that insufficient adjustment in respiratory](#)  
585 [muscle perfusion during exercise is attributed to blood flow redistribution from the respiratory](#)  
586 [to the locomotor muscles but support the notion that central hemodynamic and local muscle](#)  
587 [mechanical impairments may contribute to the impediment of respiratory muscle perfusion](#)  
588 [during exercise in patients with COPD.](#)

### 589 ***Association between respiratory muscle perfusion and dyspnoea***

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

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

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

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

641 To avoid arterial cannulation we assessed relative muscle perfusion using the NIRS-derived  
642 BFI method (33, 36, 54). We observed that during cycling, four patients demonstrated a modest  
643 increase in BFI of vastus lateralis as their values fall outside the lower limits of confidence  
644 interval whilst two patients demonstrated a decrease in vastus lateralis BFI compared to rest  
645 (*BFI responses of the six patients are marked with open symbols in Figure 3*). Nevertheless, the  
646 insufficient increase in leg muscle BFI of these six patients was not associated with a  
647 concomitant increase in their respiratory muscle BFI, to support that in these patients high-  
648 intensity exercise did not impair their extradiaphragmatic respiratory muscle perfusion (*Figure*  
649 *3 A, B and C, open symbols*). Furthermore, the large inter-subject variability observed in BFI of  
650 respiratory and vastus lateralis muscles (Figure 2) could be attributed to inter-subject variability  
651 in subcutaneous tissue, muscle vasculature and capillary density and/or in the large variation in  
652 work rate (range, min: 25 watts /max: 100 watts) and minute ventilation (range, min: ~12  
653 liters/min / max: ~71 liters/min) patients exhibited during the trials. Therefore, the  
654 mentioned parameters had to be taken into account when using the NIRS-ICG  
655 methodology for comparing BFI data on an individual level (36, 54).

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

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

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683 [corresponds to a moderate intensity of physical activity \(i.e. approximately 50% of VO<sub>2</sub>peak ,](#)  
684 [81\).](#)

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

### 693 **Study limitation**

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

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

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

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**Deleted:** Nevertheless, this study is based on a repeated-measures design, with the main purpose being to perform within-subject comparisons of the physiological data obtained during hyperpnoea and cycling. Therefore, any systematic errors from the use of these non-invasive methodologies would not contribute to uncertainty in these within-subject comparisons.

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

726 Performing the hyperpnoea trial first and the cycling protocol afterwards (on the same day)  
727 may have influenced respiratory muscle oxygen delivery and uptake kinetic responses during  
728 the cycling test owing to muscle warm-up (1, 2, 15). However, the findings that before cycling,  
729 baseline values of heart rate ( $79 \pm 15$  beats/min), cardiac output ( $5.5 \pm 1.4$  litres/min) and %StiO<sub>2</sub>  
730 in scalene ( $68 \pm 9\%$ ), 7<sup>th</sup> intercostal ( $76 \pm 11\%$ ) and abdominal muscles ( $81 \pm 17\%$ ) did not differ  
731 compared to resting values (Figure 4 and Table 3 and 4,  $p > 0.01$ ) suggest that the time elapsed  
732 between the two protocols eliminated any effect of prior exercise on respiratory muscle blood  
733 flow regulation during cycling. Besides, patients' dyspnoea sensations prior to the cycling test  
734 ( $0.9 \pm 1.0$ ) returned to resting levels (Table 2,  $p > 0.05$ ). Inspiratory capacity manoeuvres for  
735 evaluating dynamic lung hyperinflation were not performed during hyperpnoea [thereby](#)  
736 [enabling patients to better focus on reaching the targeted breathing pattern and minute](#)  
737 [ventilation](#), (34, 85, 86). However, whether dynamic lung hyperinflation, if any, compromised  
738 [diaphragm and extradiaphragmatic](#) respiratory muscle perfusion during hyperpnoea was not  
739 evaluated in the present study.

740 Respiratory muscle pressures and work of breathing could not be measured in 7 out of 18  
741 patients. We argue that this limitation did not affect the findings of the present investigation.

742 Similar studies in healthy individuals and in patients with COPD (85, 86) demonstrated that  
743 manipulation of the breathing pattern is sufficient to lead to similar respiratory muscle  
744 pressures and work of breathing between hyperpnoea and cycling trials as seen in this study.

745 [Finally, measures of respiratory muscle twitch force assessed by magnetic stimulation of](#)  
746 [phrenic nerves to evaluate potential respiratory muscle fatigue during cycling and hyperpnoea](#)  
747 [would have further strengthened our study.](#) ***Clinical implications and future perspectives***

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

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

### 777 **Conclusions**

778 The results of the present study suggest that in patients with COPD [high-intensity](#),  
779 locomotor muscle work during exercise interferes with extradiaphragmatic respiratory muscle  
780 perfusion despite a two-fold increase in cardiac output. Insufficient respiratory muscle  
781 perfusion during [high-intensity](#) exercise has a profound effect on extradiaphragmatic  
782 respiratory muscle oxygen availability and it is associated with greater dyspnoea sensations in  
783 patients with COPD.

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### **Disclosure**

The authors declare that they have no competing interests.



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1230 **Table 1.** Subjects characteristics, pulmonary function and peak exercise and  
1231 functional, quadriceps and respiratory muscle capacity data.  
1232

<b>Demographics / Anthropometrics</b>	
<b>Sex, male/female</b>	10/8
<b>Age, years</b>	66±6
<b>BMI, kg/m<sup>2</sup></b>	27±6
<b>Pulmonary function</b>	
<b>FEV<sub>1</sub>, L</b>	1.4±0.6
<b>FEV<sub>1</sub>, %pred.</b>	58±24
<b>FVC, L</b>	3.2±0.8
<b>FVC, %pred.</b>	99±30

<b>FEV<sub>1</sub>/FVC, %</b>	46±13
<b>MVV, L/min</b>	53±16
<b>MVV, %pred.</b>	68±24
<b>TLC, L</b>	6.5±1.5
<b>TLC, %pred.</b>	116±20
<b>RV, L</b>	3.4±1.25
<b>RV, %pred.</b>	151±45
<b>RV/TLC, %</b>	51±10
<b>TL<sub>CO</sub>, mmol/min/kpa</b>	4.2±1.4
<b>TL<sub>CO</sub>, %pred.</b>	55±16
<b>SpO<sub>2</sub>, %</b>	94 ± 1
<b>Hb, g/dl</b>	14.5 ± 1.3

#### Peak exercise data

<b>Work rate, watts</b>	79±26
<b>Work rate, %pred.</b>	70±20*
<b>VE, L</b>	46±14
<b>VE/MVV, %</b>	87±15
<b>Δ Insp. capacity, L</b>	-0.52±0.36
<b>Tidal volume/ Insp. Capacity, %</b>	78±14
<b>VO<sub>2</sub>, L/min</b>	1.3±0.5

<b>VO<sub>2</sub>, %pred.</b>	86±31
<b>Heart rate, beats/min</b>	120±20
<b>Cardiac output, L/min</b>	11.8±2.3
<b>SpO<sub>2</sub>, %</b>	88±4
<b>Dyspnoea, 10-Borg scale</b>	7.2±2.0
<b>Leg discomfort, 10-Borg scale</b>	6.6±2.0

#### Functional, quadriceps and respiratory muscle capacity data

<b>6-minute walking test, meters.</b>	496±52
<b>6-minute walking test, %pred.</b>	87±12 <sup>**</sup>
<b>Quadriceps muscle strength, kg</b>	37±10
<b>Quadriceps muscle strength, %pred.</b>	81±23 <sup>***</sup>
<b>MIP, cmH<sub>2</sub>O</b>	73±15
<b>MIP, %pred</b>	82±21 <sup>****</sup>
<b>MEP, cmH<sub>2</sub>O</b>	157±12
<b>MEP, %pred</b>	171±12 <sup>****</sup>
<b>Physical activity levels, steps per day</b>	6663±3618

1233

1234 Data are presented as mean ± SD for n=18 patients with COPD. FEV<sub>1</sub>: forced  
 1235 expiratory volume in the first second; FVC: forced vital capacity; MVV: maximum  
 1236 voluntary ventilation; TLC: total lung capacity; RV: residual volume; TL<sub>CO</sub>: transfer  
 1237 factor for carbon monoxide; Hb: haemoglobin; VE: minute ventilation; Δ: changes in  
 1238 inspiratory capacity from rest; VO<sub>2</sub>: oxygen consumption; SpO<sub>2</sub>: arterial oxygen

1239 saturation by pulse oximeter; MIP: maximal inspiratory pressure; MEP: maximal  
1240 inspiratory pressure. **Reference values calculated by:**  
1241 \*Blackie et al. 1989, \*\*Troosters et al. 1999, \*\*\*Allaire et al. 2004, \*\*\*\*Neder et al.  
1242 1999