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### ► To cite this version:

Quentin Bretonneau, Claire de Bisschop, Valentin Mons, Aurélien Pichon. Intercostal muscle oxygenation and expiratory loaded breathing at rest: Respiratory pattern effect. *Respiratory Physiology & Neurobiology*, 2022, 304, pp.103925. 10.1016/j.resp.2022.103925 . hal-04563409

**HAL Id: hal-04563409**

**<https://hal.science/hal-04563409>**

Submitted on 22 Jul 2024

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1 **Intercostal muscle oxygenation and expiratory loaded breathing**  
2 **at rest: respiratory pattern effect**

3

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19 **ABSTRACT**

20 In patients with airway obstruction, an increase in breathing frequency at rest is  
21 commonly associated with a dynamic hyperinflation (DH). In such a situation,  
22 intercostal muscle oxygenation may be disturbed. This hypothesis was examined in a  
23 context of simulated airway obstruction in healthy subjects.

24 After a control period of 5 minutes, twelve participants ( $20 \pm 2$  years) breathed at rest  
25 through a 20-cmH<sub>2</sub>O expiratory threshold load, either by increasing or reducing their  
26 respiratory rate ( $ETLF^+$  or  $ETLF^-$ ). Tissue saturation index (TSI) and concentration  
27 changes in oxyhaemoglobin (oxy[Hb+Mb]) were measured as well as cardiorespiratory  
28 variables.

29 Inspiratory capacity was decreased in  $ETLF^+$  ( $p < 0.001$ ) and correlated with dyspnea. An  
30 increase in oxy[Hb+Mb] occurred in  $ETLF^+$  that was higher than in  $ETLF^-$  ( $p < 0.01$ ). TSI  
31 was not different between conditions.

32 In healthy subjects at rest, an increase in respiratory rate during a simulated obstruction  
33 with an expiratory threshold load resulted in paradoxical response with DH emergence  
34 while intercostal muscle oxygenation was preserved.

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37 **KEYWORDS**

38 Intercostal muscle oxygenation, NIRS, expiratory threshold load, respiratory pattern,  
39 dynamic hyperinflation

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## 44 **1. INTRODUCTION**

45           In patients with obstructive lung disease, maximal expiratory flows are reduced  
46 due to bronchial obstruction and flows available are usually insufficient to ensure  
47 relaxation of respiratory system. In this case, as end expiratory lung volume (EELV) is  
48 increased and tidal volume is shifted at higher lung volume, dynamic hyperinflation  
49 (DH) can occur. This situation is often observed in obstructive patients, at rest, along  
50 with an increase in respiratory rate (Klooster et al., 2015; Lavorini et al., 2013). DH is  
51 also usually majored by exercise.

52           Such a context may have deleterious consequences on the perfusion of  
53 respiratory muscles because of adverse combined factors. First, ventilatory effort,  
54 which is usually modest in healthy subjects, is much more important in patients with  
55 bronchial obstruction. Thus, maintaining adequate ventilation may require greater  
56 contraction of respiratory muscles. This exaggerated effort may affect the capillary  
57 perfusion of respiratory muscles while in the same time their oxygen needs are  
58 increased (Leenaerts and Decramer, 1990). Furthermore, in a context of DH, intercostal  
59 muscles are likely to be stretched (Wilson et al., 2001). This change in length may affect  
60 the capillaries by reducing their diameter (Poole et al., 1997). Thereby, in context of  
61 high expiratory resistance at rest, blood perfusion could be particularly disturbed in  
62 intercostal muscles, especially if DH occurs. Muscle oxygen supply could become  
63 insufficient according to their majored metabolic needs, inducing a metabolic imbalance  
64 state.

65           Several studies have already imposed an external expiratory load on healthy  
66 subjects in order to induce the ventilatory disorders associated with pulmonary  
67 pathologies (Athanasopoulos et al., 2010; Barrett et al., 1994; Bretonneau et al., 2020,  
68 2019; Stark-Leyva et al., 2004; Xiao et al., 2012). In our previous study, an expiratory  
69 threshold load (ETL) had been imposed in healthy subjects at rest to assess ventilatory  
70 adaptations (Bretonneau et al., 2019). Ventilatory variables such as tidal volume and  
71 total respiratory time were increased in response to expiratory loaded breathing while  
72 intercostal muscle blood volume and concentration in oxyhaemoglobin were decreased.  
73 However, metabolic balance seemed not disturbed in these muscles. A limit of this  
74 study was nevertheless that DH was probably not induced, as inspiratory capacity was  
75 not changed in response to ETL.

76           In the current study, in order to appreciate the influence of DH on metabolic  
77 balance in intercostal muscles, two respiratory patterns were imposed on healthy  
78 subjects during simulated obstruction condition. Subjects were asked to breathe either  
79 with high respiratory rate or with low breathing frequency against an ETL. High  
80 respiratory rate associated with expiratory loaded breathing was hypothesized to induce  
81 DH that was expected to disturb importantly intercostal muscle oxygenation.

82 **2. MATERIAL AND METHODS**

83 **2.1. Participants**

84 Twelve healthy active men ( $20 \pm 2$  years,  $1,7 \pm 0.1$  m,  $70 \pm 9$  kg) were included in this  
85 study. Anthropometric and pulmonary function data are given in Table 1. All subjects  
86 had  $FEV1 \geq 80\%$  of the predicted value (Quanjer et al., 2012),  $FEV1/FVC > 70\%$  and  
87 skinfold  $< 20$  mm at the seventh left intercostal space (Grassi and Quaresima, 2016).

88 \*\*\* Table 1 \*\*\*

89 **2.2. Ethical approval**

90 The protocol was approved by the Ethics Committee in Sport Science Research  
91 (CERSTAPS 2018-25-01-22) and was presented to participants prior to the beginning of  
92 the study. All subjects gave their written informed consent.

93 **2.3. Study protocol**

94 **2.3.1 Experimental protocol**

95 Two sessions were scheduled. At each session, subjects breathed as naturally as  
96 possible for 5 minutes, seated on a chair with a nose clip (control period). Afterwards,  
97 participants breathed for 20 minutes through a 20-cmH<sub>2</sub>O expiratory threshold load  
98 (ETL, Threshold PEP, Respironics Inc., Murrysville, PA, USA). As soon as the ETL  
99 was applied at the mouth, participants had to increase their control breathing frequency  
100 by 30% or decrease it by 40% (ETLF<sup>+</sup> or ETLF<sup>-</sup> condition) according to the  
101 randomisation (Figure 1). This decrease of 40% was based on our previous study in

102 which subjects were free to adjust their respiratory pattern in response to ETL  
103 (Bretonneau et al., 2019). Sound metronome was used to regulate the respiratory rate.

104 Tidal volume was calculated to obtain minute ventilation close to that experienced  
105 spontaneously during our previous study (Bretonneau et al., 2019). Seven days  
106 separated the two sessions scheduled at the same time of day.

### 107 2.3.2 Measurements and materials

108 *2.3.2.1 Anthropometry.* Skinfold was assessed at the seventh left intercostal space with a  
109 skinfold caliper (Harpenden, British Indicators Ltd., St. Albans, England) and a whole-  
110 body bioelectrical impedance technique was used to assess body fat mass (Tanita BC-  
111 418 MA, Tanita Corporation, Tokyo, Japan).

112 *2.3.2.2 Pulmonary function tests and inspiratory capacity.* Slow and forced vital  
113 capacities (SVC and FVC, respectively) were evaluated at each session (Metalyzer-3B,  
114 Cortex Biophysik GmbH, Leipzig, Germany) according to the ATS/ERS Task Force  
115 recommendation (Miller et al., 2005).

116 Inspiratory capacity (IC) was measured several times during the protocol (Figure 1) to  
117 detect ETL-induced DH (Guenette et al., 2013; Yan et al., 1997). During control period  
118 (Ctrl), a set of two maximal inspirations were performed and the best maneuver (*i.e.* the  
119 one with the highest IC) was chosen as reference IC. During ETL period, two single  
120 maximal inspirations were performed. A second trial was realized if the inspiration had  
121 been disturbed (*e.g.* saliva swallowing during the maneuver).

122 *2.3.2.3 Maximal respiratory pressures.* Maximal expiratory and inspiratory pressures  
123 (PE<sub>max</sub> and PI<sub>max</sub>) were measured before and 30 seconds after ETL period with a non-

124 invasive manometer (MicroRPM, Micro Medical, Kent, England). Sniff nasal technique  
125 was used to assess P<sub>I</sub>max while P<sub>E</sub>max was measured at the mouth. Three attempts  
126 were performed before the protocol to familiarize with the procedure.

127 *2.3.2.4 Intercostal muscle oxygenation.* Near-infrared spectroscopy (NIRS) (Oxymon  
128 Mk III, Artinis Medical Systems, Zetten, The Netherlands) was used to assess the tissue  
129 saturation index (TSI) and the concentration changes in oxygenated, deoxygenated and  
130 total haemoglobin and myoglobin in the intercostal muscles ( $\Delta$  oxy[Hb+Mb],  $\Delta$   
131 deoxy[Hb+Mb] and  $\Delta$  total[Hb+Mb], respectively) (Barstow, 2019; Bretonneau et al.,  
132 2020, 2019; de Bisschop et al., 2014). While TSI and oxy[Hb+Mb] reflect local tissue  
133 oxygenation, deoxy[Hb+Mb] may reflect local oxygen extraction. Furthermore,  
134 total[Hb+Mb] (*i.e.* the sum of oxy[Hb+Mb] + deoxy[Hb+Mb]) can be seen as an  
135 indicator of local blood volume. The concentration changes in response to ETL were  
136 calculated from control period. The optode was placed in the seventh intercostal space,  
137 on the left side of the subject, between the midclavicular and the anterior axillary lines.  
138 As maximal penetration depth of the light is approximately half of the distance between  
139 transmitter and receiver (Chance et al., 1992), interoptode distance was adjusted  
140 between 3.3 and 4.8 cm according to adipose tissue thickness. A differential pathlength  
141 factor of 4 was chosen (van Beekvelt et al., 2002). Wavelengths of 859 and 763 nm  
142 were used. Data were recorded at 10 Hz and exported at 1 Hz after running average  
143 filtering.

144 *2.3.2.5 Ventilatory variables and gas exchanges.* Inspiratory and expiratory times (T<sub>i</sub>  
145 and T<sub>e</sub>, respectively) and tidal volume (V<sub>t</sub>) were measured breath-by-breath using the  
146 Metalyzer device (Cortex Biophysik GmbH, Leipzig, Germany). Total respiratory time  
147 (T<sub>tot</sub>), inspiratory duty cycle (T<sub>i</sub>/T<sub>tot</sub>), breathing frequency (BF) and ventilatory flow



148  $(\dot{V}E)$  were calculated, as mean inspiratory and expiratory flow rates ( $V_t/T_i$  and  $V_t/T_e$ ).  
149 Oxygen consumption ( $\dot{V}O_2$ ), carbon dioxide production ( $\dot{V}CO_2$ ) and end-tidal  $CO_2$   
150 pressure ( $P_{et}CO_2$ ) were likewise recorded. Respiratory exchange ratio (RER) and  
151 respiratory equivalents in oxygen ( $\dot{V}E/\dot{V}O_2$ ) and carbon dioxide ( $\dot{V}E/\dot{V}CO_2$ ) were  
152 calculated.

153 *2.3.2.6 Dyspnea.* Perception of dyspnea was assessed using the modified 0-10 Borg-  
154 scale, before each IC measurement (Kendrick et al., 2000). Subjects were required to  
155 point on the scale the level of their respiratory discomfort.

156 *2.3.2.7 Pulse oximetry.* A pulse oximeter (Wrist Ox2 3150, Nonin Medical Inc.,  
157 Plymouth, MN, USA) was placed at the right index to continuously record pulse  
158 haemoglobin oxygen saturation ( $SpO_2$ ).

159 *2.3.2.8 Cardiovascular parameters.* A sphygmomanometer (Omron M3, Health Care  
160 CO. Ltd, Kyoto, Japan) was used to measure systolic and diastolic blood pressure (SBP  
161 and DBP) before each IC measurement. Mean arterial pressure (MAP) was calculated as  
162 follows:  $MAP = DBP + 1/3 (SBP - DBP)$ . A PhysioFlow device was used to measure  
163 stroke volume (SV), heart rate (HR) and cardiac output ( $\dot{Q}_c$ ) non-invasively and beat-  
164 by-beat (PF-05, Manatec Medical, Poissy, France). This recording was triggered  
165 synchronously with the recordings of the other variables measured continuously.

#### 166 ***2.4. Statistical analysis***

167 Statistical analysis was performed with Statistica (StatSoft Inc., Tulsa, OK, USA). For  
168 variables recorded continuously, mean values were calculated from the last 30 seconds  
169 preceding IC measurements. Following the verification of parametric conditions, a first

170 two-way repeated measures ANOVA was performed to evaluate the responses to ETL  
171 (control period vs. ETL10') according to the respiratory patterns (ETLF<sup>+</sup> vs. ETLF<sup>-</sup>). A  
172 second ANOVA was conducted to test the effect of ETL duration (ETL10' vs. ETL20')  
173 according to the respiratory patterns. A two-way repeated measures ANOVA was also  
174 conducted to compare maximal respiratory pressures before and after ETL period. A  
175 Tukey post-hoc analysis was performed when ANOVA was significant. Furthermore, in  
176 order to detect DH induced by ETL, IC measured at ETL10' and ETL20' were compared  
177 with the corresponding one measured during control period. Hedges' g was calculated  
178 to define effect size (0.2: small; 0.5: medium; 0.8: large; 1.2: very large; 2.0: huge).  
179 Correlation coefficients were assessed using the Pearson or Spearman test, according to  
180 Gaussian distribution. Values were expressed as mean  $\pm$  standard deviation (SD) and  
181 results were considered as significant for a p-value < 0.05.

## 182 3. RESULTS

### 183 3.1. Ventilation

184  $\dot{V}_E$  was not different between the two experimental conditions and  $V_t$  was higher in  
185  $ETLF^-$  as compared to  $ETLF^+$  ( $p < 0.001$ ).  $T_i/T_{tot}$ ,  $V_t/T_i$  and  $V_t/T_e$  were not different  
186 between conditions (Table 2 and Figure 2).

187 In  $ETLF^+$ , IC was decreased from Ctrl (2.8 L at 20'  $ETLF^+$  vs. 3.5 L during Ctrl;  
188  $p < 0.001$ , Hedges'  $g = 0.8$ ) (Figure 3).

189 Dyspnea was not different between conditions and increased significantly during  $ETLF^+$   
190 (from 2.5/10 on Borg scale at 10' to 4.1/10 at 20') (Figure 3).

191 \*\*\* Table 2 \*\*\*

### 192 3.2. Intercostal muscle oxygenation

193 NIRS values are shown in Figure 4. In response to ETL, oxy[Hb+Mb] was significantly  
194 increased in  $ETLF^+$  but not in  $ETLF^-$  (at 20': +4.40  $\mu\text{M}$  vs. +0.44  $\mu\text{M}$ ;  $p < 0.01$ ,  
195 Hedges'  $g = 0.9$ ). These changes were significantly different between breathing  
196 patterns. Total[Hb+Mb] was increased in  $ETLF^+$  while it was decreased after a few  
197 minutes in  $ETLF^-$  (at 20': +2.3  $\mu\text{M}$  vs. -1.5  $\mu\text{M}$ ;  $p < 0.05$ ). These changes were  
198 significantly different between breathing patterns. Deoxy[Hb+Mb] decreased identically  
199 in both conditions after a few minutes of loaded breathing. TSI was not different  
200 between conditions and did not vary from control period, neither in  $ETLF^+$  nor in  $ETLF^-$ .

### 201 3.3. Maximal respiratory pressures

202 P<sub>E</sub>max and P<sub>I</sub>max were not different between conditions and did not change after ETL  
203 period (from 150 cmH<sub>2</sub>O to 147 cmH<sub>2</sub>O for P<sub>E</sub>max and from 114 cmH<sub>2</sub>O to 110  
204 cmH<sub>2</sub>O for P<sub>I</sub>max).

#### 205 **3.4. Gas exchanges**

206 As shown in Table 2,  $\dot{V}E/\dot{V}O_2$  and  $\dot{V}E/\dot{V}CO_2$  were increased from Ctrl while PetCO<sub>2</sub>  
207 was decreased. RER,  $\dot{V}E/\dot{V}O_2$  and  $\dot{V}E/\dot{V}CO_2$  were lower in ETLF<sup>+</sup> than in ETLF<sup>-</sup>.  
208 Conversely, PetCO<sub>2</sub> was higher in ETLF<sup>+</sup>.  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and SpO<sub>2</sub> were not different  
209 between conditions. At 20', SpO<sub>2</sub> was 98% in the two conditions.

#### 210 **3.5. Cardiovascular variables**

211 Neither cardiac parameters (HR, SV and  $\dot{Q}_c$ ; Table 2) nor arterial pressures (SBP, DBP,  
212 and MAP) were significantly different between conditions. At 20', MAP was 99 mmHg  
213 in ETLF<sup>+</sup> and 100 mmHg in ETLF<sup>-</sup>.

#### 214 **3.6. Correlations**

215 In ETLF<sup>+</sup>, changes in IC ( $\Delta = \text{ETL20}' - \text{control period}$ ) were correlated with changes in  
216 dyspnea ( $r = -0.67$ ,  $p = 0.02$ ).

## 217 **4. DISCUSSION**

218           This study aimed to investigate intercostal muscle oxygenation in a context of  
219 high expiratory resistance that frequently induces DH. In such a situation in which  
220 intercostal muscles are likely to be stretched, blood perfusion could be impaired and  
221 oxygenation disturbed. In order to appreciate the influence of DH on metabolic balance  
222 in intercostal muscles, two respiratory patterns were imposed on healthy subjects by  
223 simulating obstruction with an ETL. We found that at rest, increasing respiratory rate  
224 resulted in paradoxical response with DH and dyspnea onset while blood perfusion was  
225 enhanced and oxygenation of intercostal muscles preserved.

### 226 ***4.1. Methodological aspects – Breathing frequency***

227           In  $_{ETLF}^+$  condition, control breathing frequency was increased by 30% in order  
228 to induce DH (*i.e.* to increase end-expiratory lung volume) (Gelb et al., 2004). Initially,  
229 a rise of 40% was planned but during our pre-experimentations, several participants  
230 expressed difficulties to maintain the rhythm during 20 minutes.

231           In  $_{ETLF}^-$  condition, control breathing frequency was decreased by 40% as soon  
232 as the ETL was applied. This drop was imposed in order to reproduce the spontaneous  
233 change previously observed in healthy subjects in similar context (Bretonneau et al.,  
234 2019). Furthermore, it was imposed with the aim of maintaining (or decreasing) the  
235 end-expiratory lung volume with the ETL (Bretonneau et al., 2019). Thus, in the current  
236 study,  $_{ETLF}^-$  condition can be seen as a control condition.

### 237 ***4.2. Respiratory patterns, hyperinflation and intercostal muscle oxygenation***

238 In obstructive lung diseases, worsening of DH is usually demonstrated by a  
239 decrease in IC from steady-state period (Guenette et al., 2013; Yan et al., 1997). In  
240  $ETLF^-$  condition, IC did not vary from control period. In contrast, in  $ETLF^+$  condition, IC  
241 decreased to a significant and large extent according to the Hedges'  $g$  (-19%, reduction  
242  $> 150$  ml in 9 participants). These results confirm that in a context of high expiratory  
243 resistance, DH is favoured by rapid and shallow breathing. Our results agree with those  
244 reported by Gelb et al. (2004) as in their study performed in obstructive patients, a 18%  
245 decreased in inspiratory capacity was induced by a two-fold increase in respiratory rate.

246 When an external expiratory resistance is applied at the mouth, healthy subjects  
247 usually increase spontaneously their tidal volume (Barrett et al., 1994; Bretonneau et al.,  
248 2019; Suzuki et al., 1991; Xiao et al., 2012), probably to minimize unconsciously the  
249 work of expiratory muscles. Indeed, by increasing tidal volume, a greater elastic recoil  
250 of lung may facilitate expiration. In  $ETLF^+$  condition, as tidal volume is decreased, only  
251 a shift in tidal breathing to higher lung volumes seems able to increase the elastic recoil.  
252 These suggestions could explain the occurrence of DH with a rapid and shallow  
253 breathing but not with a deep and slow ventilation in healthy population with ETL.

254 During expiratory loaded period, total[Hb+Mb] and oxy[Hb+Mb] were not  
255 different from baseline in  $ETLF^-$  while it increased in  $ETLF^+$ . In parallel, change in  
256 deoxy[Hb+Mb] was not different between conditions. These results suggest that oxygen  
257 extraction was likely similar between conditions despite a higher blood volume and  
258 oxyhaemoglobin concentration in  $ETLF^+$ . These "over-oxygenation" could be explained  
259 by a pump effect of respiratory muscles resulting from the increase in respiratory  
260 frequency.

261 During ETL period, TSI was not different between conditions and was not  
262 modified from control period, neither in  $ETLF^+$  nor in  $ETLF^-$ , which is in agreement with  
263 our previous results (Bretonneau et al., 2019). As intercostal muscles were probably  
264 recruited during ETL period (de Bisschop et al., 2017), these results suggest that higher  
265 metabolic needs of intercostal muscles were adequately satisfied in the two conditions  
266 (Ferrari et al., 2011). Maximal respiratory pressures being not changed after expiratory  
267 loaded breathing, the preservation of metabolic balance in respiratory muscles could  
268 explain the lack of respiratory muscle fatigue at the end of the two conditions (Verges et  
269 al., 2010).

270 According to our present results, obtained at rest, DH did not seem to have an  
271 effect on the oxygen saturation of intercostal muscles but this might be somewhat  
272 different in exercise conditions. Indeed, Vogiatzis et al. (2010, 2011) recorded a  
273 decrease in intercostal muscle blood flow and oxygenation in obstructive patients when  
274 their end-expiratory volume increased in response to exercise, while conversely a better  
275 tissue oxygenation was observed when end-expiratory volume normalized (Vogiatzis et  
276 al., 2010, 2011). In agreement, in a previous study performed at exercise, our team had  
277 observed an increase in oxy[Hb+Mb] and deoxy[Hb+Mb] that stopped when DH  
278 occurred (Bretonneau et al., 2020). Besides, in the study from Louvaris et al. (2018), in  
279 which obstructive patients were asked to reproduce different levels of exercise-induced  
280 hyperpnea, both intercostal muscles blood flow and DH leveled off from an hyperpnea  
281 associated with 75% of maximal work rate. Finally, the causal links between DH and  
282 respiratory muscle oxygenation are still to be clarified.

### 283 ***4.3. Dyspnea***

284           Dyspnea was not different between conditions at  $ETL_{10'}$  and increased thereafter  
285 only in  $ETL_{F^+}$  condition (up until 4 on the modified Borg scale, dyspnea somewhat  
286 severe). Furthermore, a negative correlation between IC and dyspnea was observed in  
287 this condition. In agreement, Lavorini et al. (2013) showed that dyspnea was more  
288 intense for asthmatic patients who increased their breathing frequency in response to  
289 induced-bronchoconstriction, as compared to those who decreased it. Likewise, these  
290 patients were those who showed the greatest DH.

291           Paradoxical effects on respiratory mechanics appears associated to DH. On one  
292 side, DH increases inspiratory muscle efferent due to higher work of breathing and on  
293 the other side, DH reduces the ability of inspiratory muscles to produce strength by  
294 disrupting their tension-length relationship (Braun et al., 1982). Hence, due to the shift  
295 in tidal breathing to higher lung volumes, mismatch between inspiratory muscle efferent  
296 and mechanical/muscular response (*i.e.* neuromuscular dissociation) may occur, which  
297 could worsen dyspnea (Lougheed et al., 1993; O'Donnell et al., 1997). The correlation  
298 between IC and dyspnea highlighted in the current study, is consistent with the  
299 hypothesis that subject who reported the higher dyspnea in  $ETL_{F^+}$  could be those who  
300 experienced the higher neuromuscular dissociation.

301           It could be also hypothesized that dyspnea is linked to oxygenation state of the  
302 respiratory muscles. Indeed, a correlation between dyspnea and deoxygenation of the  
303 serratus muscle has been observed in patients with heart failure (Mancini et al., 1992).  
304 Moreover, in obstructive patients, a reduction of dyspnea has been observed during  
305 exercise while oxygen supply to the intercostal muscles was improved by oxygen



306 supplementation (Louvaris et al., 2016). Unfortunately, in our study, we did not find  
307 any association between dyspnea and intercostal muscle oxygenation.

#### 308 *4.4. Limits of the study*

309         Some points which can be considered as limits are presented below. First,  
310 respiratory muscle oxygenation was investigated in intercostal muscles by NIRS.  
311 However, the exact depth of the intercostal muscles under the skin was unknown, which  
312 represents a limit for NIRS investigations. The depth of measurement was chosen  
313 according to a previous estimation of external muscle thickness by De Troyer et al.  
314 (2003) at 7-10 mm, which seems reasonable.

315         Secondly, in the present study, the activity of the intercostal muscles was not  
316 measured electromyographically. However, the activation of intercostal muscles has  
317 previously been demonstrated under similar conditions in humans (de Bisschop et al.,  
318 2017; Sears and Newsom Davis, 1968). In addition, a two-fold increase in  $V_t/T_e$  from  
319 baseline was observed in the two conditions, which indicates the recruitment of  
320 intercostal muscles.

321         Thirdly, cardiac values were obtained with a Physioflow device, whose accuracy  
322 is still debated in obstructive patients, especially during exercise (Bougault et al., 2005;  
323 Louvaris et al., 2019). In our study however, cardiac parameters were measured only at  
324 rest, and in view of carrying out a general survey of cardiac adjustments alongside DH  
325 and respiratory muscle activity.

326         Finally, the study was undertaken on healthy subjects using simulation  
327 conditions. Thus, the physiological responses described in this study may differ from  
328 the real-condition responses that occur in patients with obstructive pulmonary diseases.

329 ***4.5. Strength, clinical implication and perspective of the study***

330           In the present study, we demonstrated that DH was induced by breathing rapidly  
331 against an expiratory load and that the shift in tidal breathing to higher lung volumes  
332 was associated with dyspnea. Thus, our observations carried out on healthy subjects  
333 suggest to advise slow and deep breathing to patients during exacerbation of bronchial  
334 obstruction in order to limit DH and dyspnea. Nevertheless, these recommendations  
335 should be confirmed on patients to be clinically relevant.

336           In addition, this study confirms that the use of a PEP can be an excellent way to  
337 increase EELV in healthy subjects. This model seems particularly interesting for  
338 studying the isolated effects of DH without including the others potential effects  
339 associated with obstructive pulmonary diseases (Erram et al., 2021).

340 **5. CONCLUSION**

341           In conclusion, this study performed in healthy subjects demonstrated that during  
342 expiratory loaded breathing at rest, increased respiratory rate results in paradoxical  
343 adaptations as DH and dyspnea happen without negative change neither in blood  
344 perfusion nor in oxygenation variables in intercostal muscles. Thus, shift in tidal  
345 breathing to higher lung volume at rest probably does not induce mismatch between  
346 oxygen need and supply in intercostal muscles.

347 **ACKNOWLEDGEMENTS**

348 This study was supported in part by the European Union and the New Aquitaine region  
349 through the Habisan program (CPER-FEDER).

350

351 **DECLARATION OF INTEREST**

352 None.

353

354 **AUTHORS' CONTRIBUTIONS**

355 All authors have made substantial contributions to all of the following: (1) the  
356 conception and design of the study, or acquisition of data, or analysis and interpretation  
357 of data, (2) drafting the article or revising it critically for important intellectual content,  
358 (3) final approval of the version to be submitted.

359

360 **FUNDING**

361 This research did not receive any specific grant from funding agencies in the public,  
362 commercial, or not-for-profit sectors.

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- 509



510 **FIGURE LEGENDS**

511 Figure 1. Study design and time measurements. With AP, arterial pressure; Ctrl, control;  
512 ETL, expiratory threshold load; IC inspiratory capacity; PEmax, maximal expiratory  
513 pressure and PImax, maximal inspiratory pressure.

514 Figure 2. Respiratory times and ventilatory flows during control (Ctrl) and expiratory  
515 threshold load (ETL) periods. In  $ETLF^-$  condition, the breathing frequency should be  
516 decreased as soon as the ETL was applied (panel A). In  $ETLF^+$  condition, the breathing  
517 frequency should be increased (panel B). During ETL periods, data were analysed at the  
518 10<sup>th</sup> and the 20<sup>th</sup> minute ( $ETL10'$  and  $ETL20'$ ). Values are mean  $\pm$  SD. With Te, expiratory  
519 time; Ti, inspiratory time; Ttot, total respiratory time; Vt/Te, spontaneous expiratory  
520 flow and Vt/Ti, spontaneous inspiratory flow.

521 Figure 3. Inspiratory capacity (IC, panel A) and dyspnea (panel B) during control (Ctrl)  
522 and expiratory threshold load (ETL) periods. In  $ETLF^-$  condition, the breathing  
523 frequency should be decreased as soon as the ETL was applied. In  $ETLF^+$  condition, the  
524 breathing frequency should be increased. During ETL periods, data were analysed at the  
525 10<sup>th</sup> and the 20<sup>th</sup> minute ( $ETL10'$  and  $ETL20'$ ). Values are mean  $\pm$  SD. \$, difference  
526 between Ctrl and  $ETL10'$  regardless of condition or #, for the corresponding condition ( $p$   
527  $< 0.05$ ). †, difference between  $ETL10'$  and  $ETL20'$  for the corresponding condition ( $p$   $<$   
528  $0.05$ ). \*, difference between conditions during ETL period ( $p$   $< 0.05$ ).

529 Figure 4. Intercostal muscle oxygenation during control (Ctrl) and expiratory threshold  
530 load (ETL) periods. In  $ETLF^-$  condition, the breathing frequency should be decreased as  
531 soon as the ETL was applied. In  $ETLF^+$  condition, the breathing frequency should be

532 increased. During ETL periods, data were analysed at the 10<sup>th</sup> and the 20<sup>th</sup> minute  
533 (ETL10' and ETL20'). Values are mean  $\pm$  SD. Tissue saturation index (TSI, panel A).  
534 Concentration changes in oxyhaemoglobin ( $\Delta$  oxy[Hb+Mb], panel B),  
535 deoxyhaemoglobin ( $\Delta$  deoxy[Hb+Mb], panel C), and total haemoglobin ( $\Delta$   
536 total[Hb+Mb], panel D). <sup>#</sup>, difference between Ctrl and ETL10' for the corresponding  
537 condition (p < 0.05). <sup>‡</sup>, difference between ETL10' and ETL20' (p < 0.05). <sup>\*</sup>, difference  
538 between conditions during ETL period (p < 0.05).

## 539 TABLES

540 **Table 1.** Anthropometric and pulmonary function characteristics.

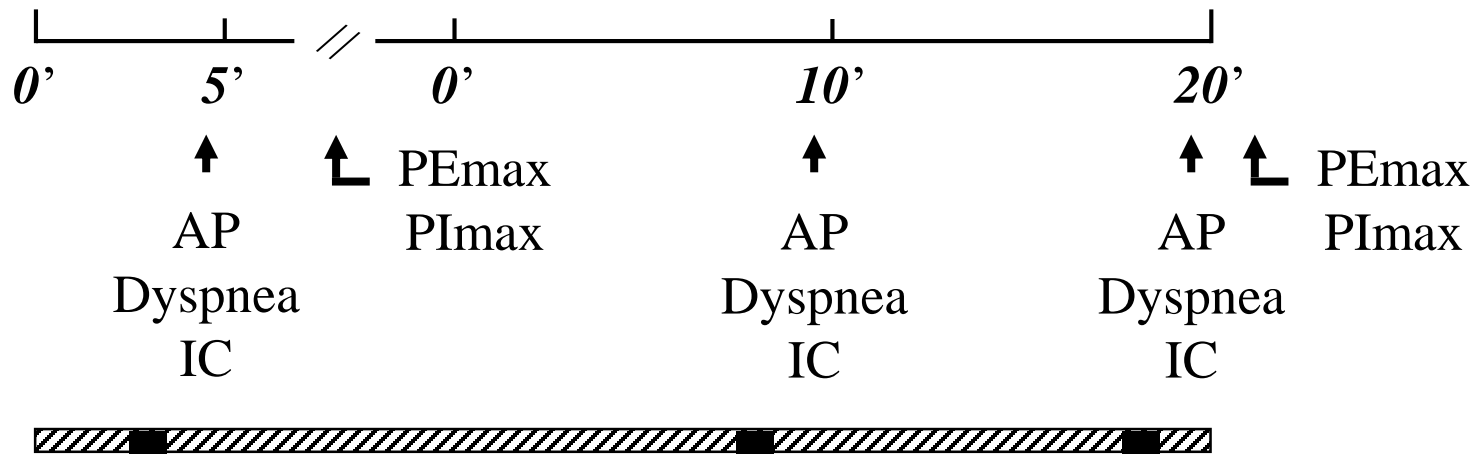
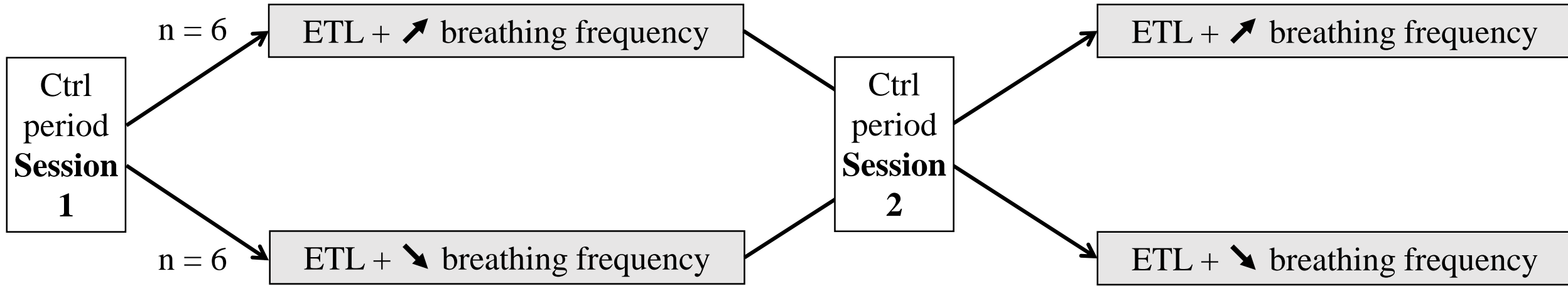
	Absolute values			Predicted values (%)		
BMI (kg/m <sup>2</sup> )	23	±	2	-		
Body fat mass (%)	14	±	4	-		
Left 7 <sup>th</sup> IS ATT (mm)	4.7	±	2.0	-		
ERV (L)	1.5	±	0.4	-		
IRV (L)	3.1	±	0.4	-		
IC (L)	3.8	±	0.3	-		
SVC (L)	5.3	±	0.4	99	±	7
FEV1 (L)	4.6	±	0.5	112	±	5
FVC (L)	5.3	±	0.5	113	±	6
FEV1/FVC (%)	86	±	4	99	±	5
MMEF 25-75% (L/sec)	5.0	±	0.9	109	±	15

541 Values are mean ± SD. With BMI, body mass index; ERV, expiratory reserve volume;  
542 FEV1, forced expiratory volume during the first second; FVC, forced vital capacity; IC,  
543 inspiratory capacity; IRV, inspiratory reserve volume; IS ATT, intercostal space  
544 adipose tissue thickness; MMEF, mean median expiratory flow between 25% and 75%  
545 of FVC and SVC, slow vital capacity.

**Table 2.** Respiratory, metabolic and cardiac variables during control period (Ctrl) and with expiratory threshold load when the breathing frequency was decreased ( $ETLF^-$ ) or increased ( $ETLF^+$ ).

	$ETLF^-$			$ETLF^+$			Difference between...	
	<i>Ctrl</i>	<i>ETL10'</i>	<i>ETL20'</i>	<i>Ctrl</i>	<i>ETL10'</i>	<i>ETL20'</i>	<i>Ctrl</i> and <i>ETL10'</i>	<i>ETL10'</i> and <i>ETL20'</i>
<b>Ventilatory variables</b>								
Vt (L)	0.7 (0.1)	1.7 (0.3)	1.8 (0.3)	0.7 (0.1)	0.8 (0.1)	0.8 (0.1)*	#	
$\dot{V}E$ (L/min)	11.8 (2.4)	17.4 (2.6)	18.8 (2.4)	11.8 (2.0)	17.6 (2.4)	17.9 (2.5)	\$	
Ti/Ttot (%)	41.6 (4.1)	50.0 (7.3)	48.9 (6.8)	41.8 (4.3)	51.6 (4.7)	51.3 (2.5)	\$	
<b>Metabolic variables</b>								
$\dot{V}O_2$ (ml/min/kg)	0.38 (0.06)	0.35 (0.06)	0.38 (0.05)	0.36 (0.05)	0.39 (0.04)	0.38 (0.04)		
$\dot{V}CO_2$ (ml/min/kg)	0.32 (0.05)	0.36 (0.06)	0.38 (0.05)	0.31 (0.03)	0.35 (0.04)	0.35 (0.03)		
<b>Gas exchanges</b>								
RER	0.84 (0.05)	1.04 (0.08)	0.99 (0.06)	0.86 (0.07)	0.91 (0.07)	0.91 (0.07)*	#	
$\dot{V}E/\dot{V}O_2$	19.5 (2.2)	43.2 (8.8)	43.0 (6.0)	20.3 (4.4)	30.6 (4.8)	31.5 (5.4)*	\$	
$\dot{V}E/\dot{V}CO_2$	23.2 (2.7)	41.4 (6.1)	43.4 (5.2)	23.5 (3.4)	33.7 (4.1)	34.5 (4.5)*	\$	
PetCO <sub>2</sub> (mmHg)	39.6 (2.4)	26.5 (2.9)	24.7 (2.2)	39.1 (3.5)	31.7 (3.3)	30.7 (3.8)*	\$	‡
<b>Cardiac variables</b>								
HR (bpm)	74.0 (10.5)	83.4 (8.7)	80.2 (9.0)	73.6 (10.9)	83.0 (8.6)	80.7 (7.8)	\$	‡
SV (ml)	75.0 (17.5)	68.6 (14.9)	69.9 (15.0)	83.5 (8.7)	78.2 (11.3)	72.9 (12.5)	\$	
$\dot{Q}c$ (L/min)	5.5 (1.1)	5.7 (1.2)	5.6 (1.4)	6.1 (0.6)	6.5 (1.0)	5.9 (1.1)		

Values are mean (SD). During ETL periods, data were analysed at the 10<sup>th</sup> and the 20<sup>th</sup> minute (ETL10' and ETL20'). With HR, heart rate; PetCO<sub>2</sub>, end-tidal CO<sub>2</sub> pressure; RER, respiratory exchange ratio; SV, stroke volume; Ti/Ttot, inspiratory duty cycle; Vt, tidal volume; Q<sub>c</sub>, cardiac output; V̇CO<sub>2</sub>, carbon dioxide exhalation per unit of time; V̇E, ventilatory flow; V̇O<sub>2</sub>, oxygen consumption per unit of time; V̇E/V̇CO<sub>2</sub>, ventilatory equivalent for CO<sub>2</sub> and V̇E/V̇O<sub>2</sub>, ventilatory equivalent for O<sub>2</sub>. \$, difference between Ctrl and ETL10' regardless of condition (p < 0.05). #, difference between Ctrl and ETL10' in ETLF<sup>-</sup> only (p < 0.05). ‡, difference between ETL10' and ETL20' regardless of condition (p < 0.05). \*, difference between conditions during ETL period, regardless of time (p < 0.05).



▨ Continuous measurement:

- Intercostal muscle oxygenation
- Ventilatory, metabolic and cardiac parameters

■ Mean of continuous measurement (30 sec)

