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NEWCASTLE

1 **Differences in respiratory muscle responses to hyperpnea or loaded breathing in COPD**

2

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23 **Abstract**

24 **Introduction:** To compare acute mechanical and metabolic responses of the diaphragm and rib  
25 cage inspiratory muscle during two different types of respiratory loading in patients with COPD.

26 **Methods:** In 16 patients (age:65±13, 56% male, FEV<sub>1</sub>:60±6%pred, Pimax:82±5%pred)  
27 assessments of respiratory muscle electromyography (EMG), esophageal (Pes) and gastric (Pga)  
28 pressures, breathing pattern, and noninvasive assessments of systemic (VO<sub>2</sub>, cardiac output,  
29 oxygen delivery and extraction) and respiratory muscle hemodynamic and oxygenation  
30 responses (blood flow index [BFI], oxygen delivery index, deoxyhemoglobin concentration  
31 [HHb] and tissues oxygen saturation [StiO<sub>2</sub>]), were performed under two different conditions of  
32 respiratory muscle loading (hyperpnea and loaded breathing).

33 **Results:** During hyperpnea, breathing frequency, minute ventilation, esophageal and diaphragm  
34 pressure-time product (PTP)/min, cardiac output and VO<sub>2</sub> were higher than during loaded  
35 breathing ( $P<0.05$ ). Average inspiratory Pes and Pdi per breath scalene (SCA),  
36 sternocleidomastoid (SCM), and intercostal muscle activation was higher during loading  
37 breathing ( $P<0.05$ ). Higher Pdi during loaded breathing compared to hyperpnea was due to  
38 higher Pes ( $P<0.05$ ). Diaphragm activation, inspiratory and expiratory Pga and expiratory  
39 abdominal muscle activation did not differ between the two conditions ( $P>0.05$ ). SCA-BFI and  
40 oxygen delivery index were lower and SCA-HHb was higher during loaded breathing.  
41 Furthermore, SCA and intercostal muscle StiO<sub>2</sub> were lower during loaded breathing compared to  
42 hyperpnea ( $P<0.05$ ).

43 **Conclusion:** Greater inspiratory muscle effort during loaded breathing evoked larger ribcage and  
44 neck muscle activation compared to hyperpnea. In addition, lower SCA and intercostal muscles

45  $\text{StiO}_2$  during loading breathing than during hyperpnea might indicates a mismatch between  
46 inspiratory muscle oxygen delivery and utilization.

47 **Key Words:** RESPIRATORY MUSCLE ACTIVATION, RESPIRATORY MUSCLE  
48 LOADING, RESPIRATORY MUSCLE METABOLISM, RESPIRATORY MUSCLE  
49 TRAINING.

## 50 INTRODUCTION

51 Improvements in both respiratory muscle endurance and strength can be observed in  
52 patients with COPD after either whole-body, or specific respiratory muscle endurance  
53 training.(1-3) The improvements in respiratory muscle function in response to endurance training  
54 are probably mainly due to the increased ventilatory demands induced by (exercise) hyperpnea.  
55 Hyperpneic training provides a high respiratory flow / low resistance stimulus to the respiratory  
56 muscles during a high number of consecutive repetitions.(2, 3) It has also been demonstrated that  
57 adding specific hyperpneic (i.e. endurance) respiratory muscle training can enhance the effects of  
58 whole body endurance training on respiratory muscle endurance. However, larger improvements  
59 in inspiratory muscle strength (i.e., pressure generating capacity) have been reported after  
60 specific inspiratory muscle strength training (IMT) in comparison with whole body endurance  
61 training (i.e. average increases of 16 vs 6 cmH<sub>2</sub>O in maximal inspiratory mouth pressure [MIP]  
62 respectively).(4, 5) During inspiratory muscle strength training loading is induced by  
63 overcoming a “high external resistance” during a limited number of breathing cycles per session  
64 (e.g. 30-40 full vital capacity breaths against loads equaling about 30-50% of MIP).(4)  
65 Therefore, as much as limb muscles respond distinctively to endurance and strengthening  
66 stimuli,(6, 7) it can also be expected that different responses are induced when the respiratory  
67 muscles are exposed to “endurance” (i.e., hyperpnea) or “strengthening” (i.e., loaded breathing)  
68 stimuli. Differences in acute responses to either endurance or strengthening stimuli imposed on  
69 the respiratory muscle in terms of muscle recruitment and activation patterns as well as local and  
70 systemic oxygenation responses have however, to the best of our knowledge, never been  
71 comprehensively characterized. Therefore, we aimed to explore and compare the acute responses

72 of a number of physiological variables during these two different types of inspiratory muscle  
73 loading in patients with COPD..

## 74 **METHODS**

75 **Subjects.** Sixteen symptomatic patients (Baseline Dyspnea Index  $6 \pm 1$ ),(8) with a clinical  
76 diagnosis of COPD according to the Global Initiative for Chronic Obstructive Pulmonary  
77 Disease (GOLD),(9) aged between 55 and 74 years (see online supplement) were included in the  
78 study. The study was approved by the local hospital ethics committee (reference number:  
79 S58513). Before participation in the study, all patients were informed about potential risks and  
80 discomforts associated with performing the experiments and provided written informed consent.

81 **Study design.** Experiments were performed on two visits. During the first visit (i.e.,  
82 initial testing) patients performed comprehensive pulmonary function testing.(10, 11) Maximal  
83 inspiratory muscle strength was measured by maximum inspiratory mouth pressures (MIP).(12,  
84 13) An incremental cardiopulmonary exercise test (CPET),(14) and a constant work rate cycle  
85 endurance test (CWRT),(14) were also performed during this visit (see supplemental online  
86 material for more details). During the second visit, patients performed, in random order, both a  
87 Normocapnic Hyperpnea trial (hyperpnea),(13, 15) reproducing the ventilatory responses (i.e.,  
88 mean tidal volume, breathing frequency and minute ventilation) recorded for each patient during  
89 the CWRT,(15) and a Tapered Flow Resistive Loading task (loaded breathing) reproducing  
90 ventilator loading during a high-intensity IMT session.(13, 16) Both tasks were performed for  
91 five minutes. Breathlessness was measured by the modified Borg scale at rest and at the end of  
92 each task.(17) Additionally, during the final 60 seconds of both the hyperpnea and loaded  
93 breathing tasks, respiratory muscle perfusion,(18) oxygen delivery,(19) respiratory muscle  
94 activation (root mean square EMG%max) and respiratory effort were assessed.(13, 20-23)

95 Metabolic and ventilatory variables were also assessed breath by breath during both tasks by a  
96 metabolic cart (Vmax 229; Sensor Medics, Anaheim, CA, USA).

97 **Hyperpnea.** Patients were requested to maintain tidal volume, breathing frequency and  
98 minute ventilation reproducing their own breathing responses recorded during the CWRT for  
99 five minutes.(15) Thus, during the test investigators provided continuous verbal guidance aiming  
100 to maintain a maximum variation in minute ventilation of 5% throughout the test.(15) Visual  
101 feedback on breathing parameters was also provided on a screen displayed in front of the patient  
102 so as to adjust his/her breathing frequency and tidal volume to the level required by the  
103 investigator. Normocapnia was maintained by having subjects inspire from a Douglas bag  
104 containing 5% CO<sub>2</sub>, 21% O<sub>2</sub> and 74% N<sub>2</sub> for balance, connected to a two-way non-rebreathing  
105 valve (model 2700, Hans Rudolph) by a piece of tubing.(15)

106 **Loaded breathing.** The loaded breathing training session was performed in accordance  
107 with previously published protocols of IMT using the electronic POWERbreathe KH2  
108 device.(16) Subjects were requested to breathe out completely (i.e., until residual volume)  
109 through a loaded breathing device (POWERbreathe KH2) followed by full vital capacity  
110 inspirations against an external resistance of ~50% of patients MIP for 30 breaths or for a  
111 minimum of five minutes.(16) Thereby loading the inspiratory muscles throughout their full  
112 range of motion in accordance with a previously published method.(16)

113 **Respiratory muscle pressures, work of breathing and activation during hyperpnea**  
114 **and loaded breathing.** Respiratory muscle pressures and diaphragm activation (EMGdi) were  
115 measured by a combined multipair esophageal electrode catheter with esophageal- and gastric-  
116 balloons (Yinghui Medical Equipment Technology Co. Ltd., Guangzhou, China) nasally inserted  
117 after topical anesthesia. Procedures for optimal positioning of the catheter and signal processing



118 have already been published.(20) EMGdi was converted into root mean square (RMS),  
119 normalized by its maximum activation during inspiratory capacity maneuvers (ICs) and reported  
120 as percentage of maximum activation (EMGdi, %max). Continuous measurements of esophageal  
121 (Pes), gastric (Pga) and transdiaphragmatic (Pdi, i.e., Pga - Pes) pressures and its derivatives  
122 were performed. Inspiratory Pes, Pga and Pdi max were obtained during inspiratory sniff  
123 maneuvers.(20) Expiratory Pga max, however, was obtained during forced expiratory capacity  
124 maneuvers (see online supplement) . Ribcage, i.e., scalene (SCA), sternocleidomastoid (SCM),  
125 parasternal intercostal and 7<sup>th</sup> intercostal (ICM and 7<sup>th</sup>ICM, respectively), and abdominal (ABD)  
126 muscle activation was measured by surface electromyography (sEMG) (Desktop Direct  
127 Transmission (DTS), NORAXON, Scottsdale, USA).(21) Electrodes were placed (1) on the  
128 posterior left triangle of neck at the level of cricoid process for scalene muscle measurements  
129 (EMGsca), (2) at the midpoint along the long axis of the right sternocleidomastoid muscle  
130 between the mastoid process and the medial clavicle for sternocleidomastoid muscle  
131 measurement (EMGscm), (3) at the right parasternal space of the 2<sup>nd</sup> and 3<sup>rd</sup> rib 3 cm lateral to  
132 the sternum for parasternal intercostal muscle measurements (EMGpicm), (4) at the line between  
133 the 7<sup>th</sup> and 8<sup>th</sup> intercostal space at mid axillary line for 7<sup>th</sup> intercostal muscle measurements  
134 (EMG 7<sup>th</sup> icm), (5) over upper 1/3 of rectus abdominis under costal cartilage level (EMGabd)  
135 (see online supplement)..

136 **Systemic hemodynamic and vascular responses during loaded breathing and**  
137 **hyperpnea.** Cardiac output, heart rate and stroke volume were continuously measured by a  
138 commercial impedance cardiography device (PhysioFlowPF50; Manatec Biomedical, Macheren,  
139 France) previously validated for COPD patients (see online supplement).(24) Estimated systemic  
140 oxygen delivery was calculated by the product of cardiac output and arterial oxygen content. The

141 latter was calculated as the product of  $1.39 \times$  hemoglobin concentration [Hb] and %SpO<sub>2</sub>.[\(25\)](#)  
142 Arterio-venous oxygen content (i.e., a-vO<sub>2</sub> diff) difference was calculated by dividing oxygen  
143 uptake by cardiac output. The systemic oxygen extraction ratio was calculated as the ratio of the  
144 a-vO<sub>2</sub> diff to arterial oxygen content. In addition, systemic vascular conductance was calculated  
145 by dividing cardiac output by mean arterial blood pressure.

146 **Respiratory muscles perfusion and oxygenation responses.** SCA, SCM and 7<sup>th</sup>ICM,  
147 and ABD blood flow indices (BFI) were calculated by using two commercial Near-Infrared  
148 Spectroscopy (NIRS; NIRO-200 and a NIRO-200NX; HAMAMATSU Photonics KK) devices in  
149 combination with light-absorbing indocyanine green dye (ICG) that was injected through a  
150 peripheral venous catheter as previously described and validated for patients with COPD (see  
151 online supplement). For the above-mentioned respiratory muscles oxygen delivery index was  
152 calculated by the product of BFI and arterial oxygen content. NIRS optodes were placed at the  
153 right posterior triangle of the neck, the left 7<sup>th</sup> intercostal space and over the upper 1/3 of rectus  
154 abdominis below costal cartilage level to respectively measure SCA, 7<sup>th</sup>ICM and rectus  
155 abdominis muscle perfusion. ICG injections for calculating BFI were performed during the last 5  
156 breaths during loaded breathing and during the last 30 seconds of the hyperpnea trial.

157 NIRS-derived changes in respiratory muscle deoxyhemoglobin concentration ([HHb])  
158 was used as an index of respiratory muscle oxygen extraction.[\(26\)](#) NIRS-derived tissue oxygen  
159 saturation index (i.e., St<sub>i</sub>O<sub>2</sub>) was considered as a measure of the dynamic balance between local  
160 tissue oxygen delivery and utilization [\(27\)](#) and, therefore, local muscle capacity to match oxygen  
161 supply relative to its metabolic demand (see online supplement) ).

162 **Statistical analysis.** A power >0.99 was found based on the difference between SCM  
163 muscle activation (EMG<sub>scm</sub>,%max) between the three tasks (i.e., rest, hyperpnea and loaded

164 breathing, see *Data analysis section* in the online supplement). Data are expressed as mean  $\pm$  SE  
165 or mean difference (95% confidence interval). Mean respiratory muscle activation, respiratory  
166 pressures and its derivatives, breathing pattern variables and central hemodynamic and metabolic  
167 variables during the last 60 seconds of rest, hyperpnea and loaded breathing were compared by  
168 one-way repeated measures ANOVA when normal distribution was not violated. Otherwise, the  
169 Friedman test was used. When statistical significance was met ( $P<0.05$ ) pairwise comparisons  
170 with Holm correction were performed as post-hoc analyses. Changes in respiratory muscle  
171 perfusion and oxygenation responses from rest to hyperpnea versus rest to loaded breathing were  
172 compared by paired t-tests when normally distributed, or by Mann-Whitney tests if normal  
173 distribution assumptions were not met (see online supplement).

## 174 **RESULTS**

175 **Subjects characteristics.** Subjects' characteristics are described in detail in Table 1. The  
176 sample was well balanced regarding sex and composed by patients classified as having mild to  
177 very severe COPD presenting resting lung hyperinflation (i.e., increased RV/TLC) (see *Subjects*  
178 *characteristics* in the supplemental material for more details). Six out of the sixteen included  
179 were not willing (n= 5) or able (n= 1) to undergo measurements of EMGdi, Pes and Pga with the  
180 esophageal catheter system. Three patients did not have respiratory muscle perfusion measured  
181 due to either technical reasons (n=1) or because of contraindications regarding ICG injections  
182 (n=2). Hence, nine out of the sixteen patients had concurrent measurements of EMGdi,  
183 respiratory pressures and respiratory muscle perfusion. There were no differences regarding  
184 pulmonary function, peak exercise and inspiratory muscle capacity between subjects with  
185 EMGdi and respiratory pressures measurements versus those subjects not able or not willing to  
186 undergo these specific experimental procedures.

187           **Respiratory symptoms during hyperpnea and loaded breathing tasks.** Neither  
188 breathlessness nor respiratory effort sensations were statistically different between hyperpnea  
189 and loaded breathing ( $5 \pm 1$  vs.  $4 \pm 1$ ,  $P=0.15$  and  $5 \pm 1$  vs.  $5 \pm 1$ ,  $P=0.93$ , respectively).

190           **Respiratory muscle activation.** We observed similar levels of diaphragm activation  
191 (EMGdi%max) (Figure 1a) between hyperpnea and loaded breathing ( $P= 0.35$ ). SCM, SCA and  
192 both intercostals muscle activation (i.e., parasternal and 7<sup>th</sup> intercostal) were significantly higher  
193 during loaded breathing as compared to hyperpnea (Figures 1b – 1e). There were no significant  
194 differences between expiratory activation of the abdominal muscle between hyperpnea and  
195 loaded breathing (EMGabd, %max:  $33 \pm 4$  vs.  $30 \pm 6$ , respectively;  $P=0.27$ ).

196           **Respiratory pressures and work of breathing.** Diaphragmatic and esophageal pressures  
197 per breath were significantly higher during loaded breathing in comparison to hyperpnea, gastric  
198 pressure, however, was similar between the two conditions ( $P= 0.64$ ; Table 2). Pes PTP and Pes  
199 WOB/b were significantly higher during loaded breathing in comparison to hyperpnea (Table 2).  
200 Inspiratory Pga and Pdi WOB/b were significantly greater during loaded breathing as compared  
201 to hyperpnea (Table 2). Pes WOB/min, and Pdi WOB/min tended to be higher during loaded  
202 breathing in comparison to hyperpnea ( $P=0.06$  and  $P=0.08$  respectively), but Pga WOB/min was  
203 similar ( $P= 0.96$ ) between the two conditions. Pes, Pga and Pdi PTP/min responses during  
204 hyperpnea were significantly higher as compared to loaded breathing (Table 2). There were no  
205 significant differences in expiratory Pga between hyperpnea and loaded breathing ( $P= 0.83$ ;  
206 Table 2).

207           **Breathing pattern.** In comparison to hyperpnea, absolute and relative inspiratory  
208 volumes were significantly higher during loaded breathing. Respiratory rate and minute  
209 ventilation however, was significantly lower during loading breathing compared to hyperpnea

210 ( $P < 0.05$ ; Table 2). Peak inspiratory flow was similar ( $P = 0.20$ ) and accompanied by longer  
211 inspiratory time and lower duty cycle during loaded breathing in comparison to hyperpnea  
212 ( $P < 0.05$ ; Table 2). During hyperpnea, end-inspiratory lung volume (EILV) achieved  $81 \pm 2\%$  of  
213 the vital capacity and during loaded breathing achieved  $59 \pm 4\%$  of the vital capacity.  
214 Representing an end-inspiratory reserve volume of  $1.76 \pm 0.12$  L during hyperpnea and  $2.90 \pm$   
215  $0.24$  during loaded breathing ( $P < 0.001$ ).

216 **Systemic hemodynamic, metabolic and cardiovascular responses.** Cardiac output,  
217  $VO_2$ , a- $vO_2$  diff and systemic vascular conductance responses were significantly greater during  
218 hyperpnea than during loaded breathing ( $P < 0.05$ ; Table 3). Mean arterial blood pressure did not  
219 significantly differ between the two conditions (Table 3).

220 **Respiratory muscle perfusion and oxygenation responses.** Increases from rest in  
221 SCABFI and oxygen delivery index were significantly less during loaded breathing as compared  
222 to hyperpnea ( $P < 0.05$ ; Table 4). The change from rest in SCA oxygen extraction (i.e., [HHb])  
223 was significantly higher during loading breathing as compared to hyperpnea ( $P < 0.05$ ; Table 4).  
224 During loading breathing SCA-StiO<sub>2</sub> decreased from rest whilst during hyperpnea SCA-StiO<sub>2</sub>  
225 increased leading to a significant difference in SCA-StiO<sub>2</sub> between the two conditions ( $P < 0.05$ ;  
226 Table 4). Increases from rest in 7<sup>th</sup>ICMBFI and oxygen delivery index were less (but not  
227 significant  $P = 0.27$  and  $P = 0.26$ , respectively) during loaded breathing as compared to hyperpnea.  
228 The change from rest in 7<sup>th</sup>ICM-HHB tended to be higher during loaded breathing as compared  
229 to hyperpnea ( $P = 0.06$ ). During loading breathing 7<sup>th</sup>ICM -StiO<sub>2</sub> decreased from rest whilst during  
230 hyperpnea 7<sup>th</sup>ICM -StiO<sub>2</sub> increased leading to a significant difference in 7<sup>th</sup>ICM -StiO<sub>2</sub> between  
231 the two conditions ( $P < 0.05$ ; Table 4). No significant changes in BFI ( $P = 0.09$ ), oxygen delivery

232 ( $P= 0.10$ ), [HHB] ( $P= 0.11$ ), and  $\text{StiO}_2$  ( $P= 0.50$ ) were observed for the ABDs between loaded  
233 breathing and hyperpnea.

## 234 **DISCUSSION**

235 *Main findings.* Our key findings are that by engaging either in hyperpnea (endurance  
236 stimulus) or loaded breathing (strength stimulus) differences in both local (i.e., respiratory  
237 muscle) and systemic responses are evoked in patients COPD. In both conditions the increase in  
238 systemic and respiratory muscle hemodynamics from rest seems to increase in association with  
239 the increase in  $\text{VO}_2$ , (Tables 3 and 4). Loaded breathing elicited greater activation of the SCA,  
240 SCM ICM and  $7^{\text{th}}$ ICM and inspiratory muscle and reduction in SCA and  $7^{\text{th}}$ ICM- $\text{StiO}_2$  (Figure 1  
241 and Table 4, respectively) compared to hyperpnea, thus reflecting the additional burden imposed  
242 on these muscles by a strengthening stimulus in comparison to an endurance loading stimulus  
243 (Table 2). In addition, increases in diaphragmatic activation during hyperpnea and loaded  
244 breathing relative to resting breathing were of similar magnitude in both conditions (Figure 1).

245 *Respiratory muscle activation during loaded breathing and hyperpnea.* The  
246 contribution of SCA, SCM and intercostal muscles to the act of breathing is known to be  
247 amplified with increased ventilatory demands.(28-30) Additionally, increased lung volumes are  
248 known to impact on the length-tension relationship of the diaphragm, by moving it away from its  
249 optimal length to generate pressure.(31-33).(31). Notably, as compared to diaphragm, increased  
250 lung volumes ensuing less length-tension impairment of SCA, SCM and intercostal muscles.  
251 These muscle undergo less severe length changes resulting in “less” sub-optimal length at higher  
252 volumes,(34-36) thereby relatively preserved pressure generating capacity.(36) Thus, SCA,  
253 SCM and intercostal muscles recruitment enables the respiratory system to compensate for the  
254 lost efficiency of the diaphragm by increasing lateral, dorsoventral (i.e., intercostals), and cranial

255 (i.e., SCA and SCM) displacement of the rib cage.(31), SCA, SCM and intercostal muscles  
256 recruitment serves as a reserve to overcome increasing demands imposed on the respiratory  
257 system under these conditions (i.e., performing faster and deeper inspiratory maneuvers as well  
258 as against higher loads).(34, 35) In our study, the recruitment of SCA, SCM and both intercostal  
259 muscles was further amplified during loaded breathing (Figure 1) when an additional external  
260 load was imposed on the respiratory system in addition to higher inspiratory volumes and flow  
261 rates. This resulted in further increases in respiratory demands (i.e., increased inspiratory  
262 pressures, WOB and PTP; Table 2). Furthermore, increases in inspiratory Pdi during loaded  
263 breathing (in comparison with hyperpnea) were mostly due to more negative inspiratory Pes but  
264 not more positive Pga (Table 2; see online supplement). These findings suggest that SCA, SCM  
265 and intercostal muscles were preferably recruited to perform the additional work imposed on the  
266 inspiratory muscles during loaded breathing.

267 ***Systemic and respiratory muscle metabolism during loaded breathing and hyperpnea.***

268 It is know that during exercise systemic responses such as cardiac output and  $VO_2$  increases  
269 proportionally to the work being performed by the working muscles per unit time.(37-39) Our  
270 study further supports these relations by demonstrating that increases in both  $VO_2$  and cardiac  
271 output during hyperpnea and loaded breathing appeared to have strong associations with PTP  
272 expressed per minute rather than per breath (Figure 2). Highlighting that increases in respiratory  
273 muscle oxygen requirements (i.e., cost of breathing) seems to be associated with the cumulative  
274 respiratory muscle effort that is developed during a given task rather than the respiratory muscle  
275 effort of each breath of a given task (figure 2).

276 The higher levels of both systemic and respiratory muscle oxygen extraction (i.e.,  $a-vO_2$   
277 difference and oxygen extraction and [HHb], respectively) during hyperpnea in comparison to

278 loaded breathing were accompanied by sufficient increase in both systemic and respiratory  
279 muscle oxygen delivery (Tables 3 and 4), thereby preserving the balance between respiratory  
280 muscle oxygen delivery and utilization (i.e.,  $StiO_2$ ; Table 4). During loaded breathing, however,  
281 despite higher respiratory pressure swings and PTP per breath (Table 2), PTP/min was lower  
282 than during hyperpnea (Table 3). Likewise, increases in  $VO_2$  and in cardiac output were less  
283 during loaded breathing in comparison to hyperpnea (Table 3). The lower “systemic” oxygen  
284 requirements (i.e.,  $VO_2$  and a- $vO_2$  diff, Table 3) during loaded breathing were accompanied by a  
285 smaller increase in respiratory muscle blood flow and oxygen delivery in comparison to  
286 hyperpnea (Table 4). These responses observed during loading breathing resulted in a mismatch  
287 between SCA and 7<sup>th</sup>ICM muscles oxygen delivery and utilization (Table 4), resulting in greater  
288 increases in muscle oxygen extraction (i.e., HHB) and lower  $StiO_2$  as compared to hyperpnea  
289 (Table 4). Higher intramuscular tensions imposed during loading breathing, might have  
290 contributed to limiting increased in muscle blood flow and oxygen delivery as compared to  
291 hyperpnea (Table 4).[\(40\)](#) The evidence of high intramuscular pressures during loading breathing  
292 is supported by the results demonstrating that mean arterial pressure did not statistically differ  
293 between the two conditions (Table 3) even that during loading breathing central hemodynamic  
294 responses were significantly lower compared to hyperpnea (Table 3). Indeed, studies have shown  
295 that increases in intramuscular pressure during dynamic exercise can reflexively increase mean  
296 arterial blood pressure (via the activation of the mechanoreceptor-mediated reflex within the  
297 skeletal muscle), the latter increases can be maintained throughout the exercise period.[\(41\)](#)

298 ***General considerations.*** Collectively, these results seem to support the notion that  
299 additional inspiratory pressures generated during loaded breathing are mainly a consequence of  
300 increased loading and activation of SCA, SCM and both intercostal muscles. The behavior of the



301 “respiratory effort-recruitment” ratio,(42) i.e., the “matching” between respiratory muscle effort  
302 (e.g., Pes, %max) and the recruitment of different inspiratory muscles (EMG, %max), is  
303 noteworthy. While during resting breathing a higher ratio indicates a “predominantly diaphragm  
304 contribution to breathing”, with increasing load (i.e., hyperpnea and loaded breathing), the ratio  
305 becomes similar between diaphragm and SCA, SCM and both intercostal muscles, thereby  
306 indicating that SCA, SCM and both intercostal muscles contribution to breathing becomes  
307 equally important as that of the diaphragm (supplemental material Figure E1).

308         The observed acute increases in load and work being performed by the inspiratory  
309 muscles during both tasks (Table 2) are known to be important determinants of muscle  
310 improvements after exercise programs.(43) Furthermore, according to the specificity and  
311 overload principles of training,(43) in response to a low load (i.e., pressures), high repetition  
312 (i.e., breathing frequency and duration) and high exercise-volume (i.e., PTP cmH<sub>2</sub>O/s/min)  
313 (Table 2) stimulus as hyperpnea, an endurance benefit would be expected. While after loaded  
314 breathing, improvements in strength would be anticipated as consequence of the high load (i.e.,  
315 pressures), low repetition (i.e., breathing frequency and duration) and high contraction-volume  
316 (PTP cmH<sub>2</sub>O/s/b) stimulus imposed by this regimen (Table 2). Noteworthy the additional  
317 recruitment of only SCA, SCM and both intercostal muscles (Figure 1) as the strategy to  
318 overcome the load imposed during loaded breathing in comparison to hyperpnea (Table 2) was  
319 accompanied by an increased metabolic burden (Table 3) It is therefore likely these inspiratory  
320 muscles will mostly benefit from this additional stimulus (i.e., increased load).(43) It has  
321 previously been observed that a period of high intensity inspiratory muscle strength training  
322 resulted in increases in specific hypertrophy of intercostal muscle fibers.(44)

323           *Implications.* The differences in physiological responses evoked by these different types  
324 (and intensities) of respiratory muscles loading support observations that had previously been  
325 done in clinical practice. It has long been assumed that while exercise hyperpnea constitutes a  
326 training load to the respiratory muscles a larger stimulus might be applied with specific  
327 respiratory muscle training.(45) This is supported by data from RCTs showing that adding  
328 specific inspiratory muscle strength training resulted in larger improvements in respiratory  
329 muscle function (strength and endurance), exercise capacity (cycling endurance time) and  
330 reduction in dyspnea(1) than standard endurance exercise training alone.(2, 4) The stimulus  
331 imposed during loaded breathing in this study (resembling a specific type of inspiratory muscle  
332 strength training) seems to be a good complimentary training stimulus for the respiratory  
333 muscles in addition to whole body exercise training.(46) Based on our data it provides a different  
334 additional load to the respiratory muscles in comparison to exercise hyperpnea. In contrast with  
335 earlier hypotheses this additional load did not result in stimulating the diaphragm in exceeding a  
336 plateau in motor unit recruitment that is typically observed early during exercise hyperpnea,(47)  
337 but by further stimulating SCA, SCM and intercostal muscle recruitment above levels observed  
338 during exercise breathing. Nevertheless, it is important to stress that the hyperpnea used herein  
339 resembles the load imposed to the respiratory system during exercise hyperpnea (i.e., 70% MVV  
340 for several minutes) and not necessarily loads imposed during specific respiratory muscle  
341 endurance training (i.e., 50 - 70% MVV for 15-30 minutes).(4) Whether higher volumes and  
342 longer durations of specific respiratory muscle endurance training might also lead to differential  
343 activation and recruitment patterns of respiratory muscles in comparison to the relatively short  
344 exercise hyperpnea stimulus provided in our study remains to be investigated.

345           *Strengths, limitations and technical considerations.* The multitude of variables  
346 simultaneously collected is a strength of the study. It allows the concurrent investigation of the  
347 behavior of respiratory muscles activation, pressure generation and metabolism under the same  
348 stimulus. Unfortunately, however, assessments of blood flow and oxygen requirements of the  
349 diaphragm could not be performed due to methodological and safety issues. A limitation of our  
350 study is the small sample size due to the complexity and the invasiveness of its methods and the  
351 fact that not all subjects were able or willing to undergo all experimental procedures. However,  
352 the sample was powered sufficiently (see *Data analysis* in the supplemental material for more  
353 details) to detect differences in a wide variety of physiological markers. Moreover, while  
354 reproducing the ventilatory pattern of exercise hyperpnea (i.e., breathing frequency, tidal volume  
355 and ventilation), there were also no statistically significant differences between the expiratory  
356 gastric pressures and expiratory ABD activation that were generated during cycling exercise in  
357 comparison to hyperpnea ( $P_{ga}$  cycling  $20 \pm 3$  vs  $P_{ga}$  hyperpnea  $25 \pm 5$ , cmH<sub>2</sub>O;  $P=0.3$ ; EMG<sub>abd</sub>  
358 cycling  $23 \pm 4$  vs EMG<sub>abd</sub> hyperpnea  $33 \pm 4$ , %max;  $P=0.10$ , respectively). Thus, providing an  
359 adequate reproducibility between exercise hyperpnea and the hyperpnea task used in our study.  
360 Arterial oxygen content,  $a\text{-VO}_2$  diff and systemic oxygen extraction were estimated using  
361 continuous SpO<sub>2</sub> measurements at the expense of acceptable reduced accuracy in the hypoxemic  
362 patients compared with invasive arterial blood sampling. In addition, it is known that the EMG  
363 signal from the costal diaphragm can generate noise on the activation of the 7<sup>th</sup>ICM we measured  
364 herein. However, the different pattern of diaphragm and 7<sup>th</sup>ICM activation between loaded  
365 breathing and hyperpnea suggested that this was not the case in our data. Nevertheless, it is  
366 possible that the EMG signal measured at these muscles as well as at SCA and SCM could be, at  
367 least in part, contaminated from nearby activity due to the use of superficial electrodes. In our

368 patients, the contribution of diaphragmatic blood flow to the overall NIRS signal on the 7th  
369 intercostal space is probably limited considering that adipose tissue thickness (fat + skin layer)  
370 (measurements were performed using a Harpenden skinfold caliper) indicated a mean value of  
371  $8.2 \pm 3.7$  mm. Therefore, the maximum penetration depth of NIRS light to the muscle tissue was  
372 reduced to approximately 12 mm. Taking into account the substantial distance between the  
373 sampling point of NIRS on the skin and the diaphragmatic appositional area compared with the  
374 shorter distance to the intercostals we believe that perfusion and oxygenation measures in our  
375 study at this site reflected mostly the external and internal intercostal muscles.

### 376 **CONCLUSION**

377 During loaded breathing there was greater respiratory muscle effort compare to  
378 hyperpnea which ensued larger ribcage and neck muscle activation during inspirations. This  
379 response reflects the additional burden imposed on these muscles by a strengthening stimulus in  
380 comparison to an endurance loading stimulus. In addition, the decrease in ribcage and neck  
381 muscle tissue oxygen saturation during loading breathing compared to hyperpnea might indicates  
382 a mismatch between inspiratory muscle oxygen delivery and utilization .

383

384

385

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392

### 393 **Conflict of interest**

394 The authors have no conflict of interest to disclose. The results presented herein do not  
395 constitute endorsement by ACSM and are presented clearly, honestly, and without fabrication,  
396 falsification, or inappropriate data manipulation.

### 397 **REFERENCES**

- 398 1. Charususin N, Gosselink R, Decramer M, et al. Randomised controlled trial of adjunctive  
399 inspiratory muscle training for patients with COPD. *Thorax*. 2018;73(10):942-50. doi:  
400 10.1136/thoraxjnl-2017-211417. PubMed PMID: 29914940.
- 401 2. Mador MJ, Deniz O, Aggarwal A, Shaffer M, Kufel TJ, Spengler CM. Effect of  
402 respiratory muscle endurance training in patients with COPD undergoing pulmonary  
403 rehabilitation. *Chest*. 2005;128(3):1216-24. doi: 10.1378/chest.128.3.1216. PubMed PMID:  
404 16162709.
- 405 3. Illi SK, Held U, Frank I, Spengler CM. Effect of respiratory muscle training on exercise  
406 performance in healthy individuals: a systematic review and meta-analysis. *Sports Med*.  
407 2012;42(8):707-24. Epub 2012/07/07. doi: 10.2165/11631670-000000000-00000. PubMed  
408 PMID: 22765281.
- 409 4. Gosselink R, De Vos J, van den Heuvel SP, Segers J, Decramer M, Kwakkel G. Impact  
410 of inspiratory muscle training in patients with COPD: what is the evidence? *The European*  
411 *respiratory journal*. 2011;37(2):416-25. doi: 10.1183/09031936.00031810. PubMed PMID:  
412 21282809.

- 413 5. Beaumont M, Forget P, Couturaud F, Reyckler G. Effects of inspiratory muscle training  
414 in COPD patients: A systematic review and meta-analysis. *The clinical respiratory journal*.  
415 2018;12(7):2178-88. doi: 10.1111/crj.12905. PubMed PMID: 29665262.
- 416 6. Richardson RS, Sheldon J, Poole DC, Hopkins SR, Ries AL, Wagner PD. Evidence of  
417 skeletal muscle metabolic reserve during whole body exercise in patients with chronic  
418 obstructive pulmonary disease. *American journal of respiratory and critical care medicine*.  
419 1999;159(3):881-5. doi: 10.1164/ajrccm.159.3.9803049. PubMed PMID: 10051266.
- 420 7. Shoemaker JK, Hodge L, Hughson RL. Cardiorespiratory kinetics and femoral artery  
421 blood velocity during dynamic knee extension exercise. *Journal of applied physiology*.  
422 1994;77(6):2625-32. doi: 10.1152/jappl.1994.77.6.2625. PubMed PMID: 7896601.
- 423 8. Mahler DA, Weinberg DH, Wells CK, Feinstein AR. The measurement of dyspnea.  
424 Contents, interobserver agreement, and physiologic correlates of two new clinical indexes.  
425 *Chest*. 1984;85(6):751-8. PubMed PMID: 6723384.
- 426 9. Singh D, Agusti A, Anzueto A, et al. Global Strategy for the Diagnosis, Management,  
427 and Prevention of Chronic Obstructive Lung Disease: The GOLD Science Committee Report  
428 2019. *The European respiratory journal*. 2019. doi: 10.1183/13993003.00164-2019. PubMed  
429 PMID: 30846476.
- 430 10. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *The European*  
431 *respiratory journal*. 2005;26(2):319-38. doi: 10.1183/09031936.05.00034805. PubMed PMID:  
432 16055882.
- 433 11. Wanger J, Clausen JL, Coates A, et al. Standardisation of the measurement of lung  
434 volumes. *The European respiratory journal*. 2005;26(3):511-22. doi:  
435 10.1183/09031936.05.00035005. PubMed PMID: 16135736.

- 436 12. Black LF, Hyatt RE. Maximal respiratory pressures: normal values and relationship to  
437 age and sex. *The American review of respiratory disease*. 1969;99(5):696-702. doi:  
438 10.1164/arrd.1969.99.5.696. PubMed PMID: 5772056.
- 439 13. Laveneziana P, Albuquerque A, Aliverti A, et al. ERS Statement on Respiratory Muscle  
440 Testing at Rest and during Exercise. *The European respiratory journal*. 2019. doi:  
441 10.1183/13993003.01214-2018. PubMed PMID: 30956204.
- 442 14. Puente-Maestu L, Palange P, Casaburi R, et al. Use of exercise testing in the evaluation  
443 of interventional efficacy: an official ERS statement. *The European respiratory journal*.  
444 2016;47(2):429-60. doi: 10.1183/13993003.00745-2015. PubMed PMID: 26797036.
- 445 15. Vogiatzis I, Athanasopoulos D, Habazetl H, et al. Intercostal muscle blood flow  
446 limitation during exercise in chronic obstructive pulmonary disease. *American journal of*  
447 *respiratory and critical care medicine*. 2010;182(9):1105-13. doi: 10.1164/rccm.201002-0172OC.  
448 PubMed PMID: 20622032.
- 449 16. Langer D, Charususin N, Jacome C, et al. Efficacy of a Novel Method for Inspiratory  
450 Muscle Training in People With Chronic Obstructive Pulmonary Disease. *Physical therapy*.  
451 2015;95(9):1264-73. doi: 10.2522/ptj.20140245. PubMed PMID: 25858974.
- 452 17. Borg GA. Psychophysical bases of perceived exertion. *Medicine and science in sports*  
453 *and exercise*. 1982;14(5):377-81. Epub 1982/01/01. PubMed PMID: 7154893.
- 454 18. Louvaris Z, Habazetl H, Wagner H, Zakyntinos S, Wagner P, Vogiatzis I. Near-infrared  
455 spectroscopy using indocyanine green dye for minimally invasive measurement of respiratory  
456 and leg muscle blood flow in patients with COPD. *Journal of applied physiology*.  
457 2018;125(3):947-59. doi: 10.1152/jappphysiol.00959.2017. PubMed PMID: 29927736.

- 458 19. Piiper J, Haab P. Oxygen supply and uptake in tissue models with unequal distribution of  
459 blood flow and shunt. *Respiration physiology*. 1991;84(2):261-71. PubMed PMID: 1876763.
- 460 20. Langer D, Ciavaglia C, Faisal A, et al. Inspiratory muscle training reduces diaphragm  
461 activation and dyspnea during exercise in COPD. *Journal of applied physiology*.  
462 2018;125(2):381-92. doi: 10.1152/jappphysiol.01078.2017. PubMed PMID: 29543134.
- 463 21. Hawkes EZ, Nowicky AV, McConnell AK. Diaphragm and intercostal surface EMG and  
464 muscle performance after acute inspiratory muscle loading. *Respiratory physiology &*  
465 *neurobiology*. 2007;155(3):213-9. doi: 10.1016/j.resp.2006.06.002. PubMed PMID: 16846758.
- 466 22. American Thoracic Society/European Respiratory S. ATS/ERS Statement on respiratory  
467 muscle testing. *American journal of respiratory and critical care medicine*. 2002;166(4):518-624.  
468 doi: 10.1164/rccm.166.4.518. PubMed PMID: 12186831.
- 469 23. Faisal A, Alghamdi BJ, Ciavaglia CE, et al. Common Mechanisms of Dyspnea in  
470 Chronic Interstitial and Obstructive Lung Disorders. *American journal of respiratory and critical*  
471 *care medicine*. 2016;193(3):299-309. Epub 2015/09/26. doi: 10.1164/rccm.201504-0841OC.  
472 PubMed PMID: 26407036.
- 473 24. Louvaris Z, Spetsioti S, Andrianopoulos V, et al. Cardiac output measurement during  
474 exercise in COPD: A comparison of dye dilution and impedance cardiography. *The clinical*  
475 *respiratory journal*. 2019;13(4):222-31. doi: 10.1111/crj.13002. PubMed PMID: 30724023.
- 476 25. Borghi-Silva A, Oliveira CC, Carrascosa C, et al. Respiratory muscle unloading improves  
477 leg muscle oxygenation during exercise in patients with COPD. *Thorax*. 2008;63(10):910-5. doi:  
478 10.1136/thx.2007.090167. PubMed PMID: 18492743.
- 479 26. Grassi B, Quaresima V. Near-infrared spectroscopy and skeletal muscle oxidative  
480 function in vivo in health and disease: a review from an exercise physiology perspective. *Journal*



481 of biomedical optics. 2016;21(9):091313. doi: 10.1117/1.JBO.21.9.091313. PubMed PMID:  
482 27443955.

483 27. Boushel R, Langberg H, Olesen J, Gonzales-Alonzo J, Bulow J, Kjaer M. Monitoring  
484 tissue oxygen availability with near infrared spectroscopy (NIRS) in health and disease.  
485 Scandinavian journal of medicine & science in sports. 2001;11(4):213-22. PubMed PMID:  
486 11476426.

487 28. Washino S, Kanehisa H, Yoshitake Y. Neck inspiratory muscle activation patterns during  
488 well-controlled inspiration. European journal of applied physiology. 2017;117(10):2085-97. doi:  
489 10.1007/s00421-017-3699-5. PubMed PMID: 28823081.

490 29. Campbell EJ. The role of the scalene and sternomastoid muscles in breathing in normal  
491 subjects; an electromyographic study. Journal of anatomy. 1955;89(3):378-86. PubMed PMID:  
492 13251968; PubMed Central PMCID: PMC1244766.

493 30. Raper AJ, Thompson WT, Jr., Shapiro W, Patterson JL, Jr. Scalene and sternomastoid  
494 muscle function. Journal of applied physiology. 1966;21(2):497-502. doi:  
495 10.1152/jappl.1966.21.2.497. PubMed PMID: 5934453.

496 31. De Troyer A, Boriek AM. Mechanics of the respiratory muscles. Comprehensive  
497 Physiology. 2011;1(3):1273-300. doi: 10.1002/cphy.c100009. PubMed PMID: 23733642.

498 32. De Troyer A, Wilson TA. Effect of acute inflation on the mechanics of the inspiratory  
499 muscles. Journal of applied physiology. 2009;107(1):315-23. doi:  
500 10.1152/japplphysiol.91472.2008. PubMed PMID: 19265064.

501 33. De Troyer A, Leduc D, Cappello M, Mine B, Gevenois PA, Wilson TA. Mechanisms of  
502 the inspiratory action of the diaphragm during isolated contraction. Journal of applied

503 physiology. 2009;107(6):1736-42. doi: 10.1152/jappphysiol.00753.2009. PubMed PMID:  
504 19797686.

505 34. Decramer M, De Troyer A. Respiratory changes in parasternal intercostal length. Journal  
506 of applied physiology: respiratory, environmental and exercise physiology. 1984;57(4):1254-60.  
507 doi: 10.1152/jappl.1984.57.4.1254. PubMed PMID: 6501034.

508 35. Farkas GA, Rochester DF. Contractile characteristics and operating lengths of canine  
509 neck inspiratory muscles. Journal of applied physiology. 1986;61(1):220-6. doi:  
510 10.1152/jappl.1986.61.1.220. PubMed PMID: 3733607.

511 36. Farkas GA. Mechanical properties of respiratory muscles in primates. Respiration  
512 physiology. 1991;86(1):41-50. PubMed PMID: 1759052.

513 37. Roussos C, Macklem PT. The respiratory muscles. The New England journal of  
514 medicine. 1982;307(13):786-97. doi: 10.1056/NEJM198209233071304. PubMed PMID:  
515 7050712.

516 38. Rochester DF, Pradel-Guena M. Measurement of diaphragmatic blood flow in dogs from  
517 xenon 133 clearance. Journal of applied physiology. 1973;34(1):68-74. doi:  
518 10.1152/jappl.1973.34.1.68. PubMed PMID: 4572509.

519 39. Andersen P, Saltin B. Maximal perfusion of skeletal muscle in man. The Journal of  
520 physiology. 1985;366:233-49. PubMed PMID: 4057091; PubMed Central PMCID:  
521 PMC1193029.

522 40. Bellemare F, Wight D, Lavigne CM, Grassino A. Effect of tension and timing of  
523 contraction on the blood flow of the diaphragm. Journal of applied physiology: respiratory,  
524 environmental and exercise physiology. 1983;54(6):1597-606. doi:  
525 10.1152/jappl.1983.54.6.1597. PubMed PMID: 6874482.

- 526 41. Gallagher KM, Fadel PJ, Smith SA, et al. Increases in intramuscular pressure raise  
527 arterial blood pressure during dynamic exercise. *Journal of applied physiology*. 2001;91(5):2351-  
528 8. doi: 10.1152/jappl.2001.91.5.2351. PubMed PMID: 11641380.
- 529 42. Ertl P, Kruse A, Tilp M. Detecting fatigue thresholds from electromyographic signals: A  
530 systematic review on approaches and methodologies. *J Electromyogr Kinesiol*. 2016;30:216-30.  
531 Epub 2016/08/17. doi: 10.1016/j.jelekin.2016.08.002. PubMed PMID: 27529668.
- 532 43. Kraemer WJ, Ratamess NA. Fundamentals of resistance training: progression and  
533 exercise prescription. *Medicine and science in sports and exercise*. 2004;36(4):674-88. PubMed  
534 PMID: 15064596.
- 535 44. Ramirez-Sarmiento A, Orozco-Levi M, Guell R, et al. Inspiratory muscle training in  
536 patients with chronic obstructive pulmonary disease: structural adaptation and physiologic  
537 outcomes. *American journal of respiratory and critical care medicine*. 2002;166(11):1491-7. doi:  
538 10.1164/rccm.200202-075OC. PubMed PMID: 12406842.
- 539 45. Decramer M. Response of the respiratory muscles to rehabilitation in COPD. *Journal of*  
540 *applied physiology*. 2009;107(3):971-6. Epub 2009/04/04. doi:  
541 10.1152/japplphysiol.91459.2008. PubMed PMID: 19342436.
- 542 46. Polkey MI, Ambrosino N. Inspiratory muscle training in COPD: can data finally beat  
543 emotion? *Thorax*. 2018;73(10):900-1. doi: 10.1136/thoraxjnl-2018-212070. PubMed PMID:  
544 29945956.
- 545 47. Sinderby C, Spahija J, Beck J, et al. Diaphragm activation during exercise in chronic  
546 obstructive pulmonary disease. *American journal of respiratory and critical care medicine*.  
547 2001;163(7):1637-41. Epub 2001/06/13. doi: 10.1164/ajrccm.163.7.2007033. PubMed PMID:  
548 11401887.

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551 Supplemental digital content 2. tif

552

553 **Figure 1.** Comparisons between the EMG activation among the different tasks. EMGdi, %max:  
554 relative diaphragmatic activation; EMGsca, %max: relative scalenes activation; EMGscm,  
555 %max: relative sternocleidomastoid activation; EMGicm, %max: relative parasternal intercostal  
556 activation; EMG 7<sup>th</sup> icm, %max: relative 7<sup>th</sup> intercostal activation. Boxplots shows median at  
557 central line, first and third quantiles for lower and upper box's limits, respectively, and minimum  
558 and maximum values for lower and upper limits. Dots are single patients' values. Dots outside  
559 the limits are outliers' values. \* $P < 0.05$ ; NS;  $P > 0.05$ . EMGdi:  $n = 10$ ; sEMG  $n = 16$ .

560

561 **Figure 2.** Relationship between work of breathing (WOB) and pressure-time product (PTP) with  
562 oxygen consumption ( $VO_2$ ; a and d, respectively) and cardiac output (CO; b and e, respectively);  
563 and between systemic oxygen delivery ( $O_2$  del) and oxygen consumption ( $VO_2$ ) and vascular  
564 conductance (Vasc. cond.; c and f, respectively). r: Pearson coefficient correlations;  $R^2$ : Adjusted  
565 R squared (univariate linear regression); NA: not applicable; NS:  $P > 0.05$  (non-significant). Lines  
566 are the best-fitting line and shadow areas are 95% confidence interval. Circles: rest; triangles:  
567 normocapnic hyperpnea; cross: tapered flow resistive loading.

568 **Table 1.** Subjects characteristics, pulmonary function and peak exercise and inspiratory muscle  
 569 capacity data

	<b>n: 16</b>
<b>Demographics / Anthropometrics</b>	
<b>Sex, male/female</b>	9 / 7
<b>Age, yrs</b>	65 ± 13
<b>BMI, kg/m<sup>2</sup></b>	27 ± 1.6
<b>Pulmonary function</b>	
<b>FEV<sub>1</sub>, L</b>	1.44 ± 0.15
<b>FEV<sub>1</sub>, %pred</b>	60 ± 6
<b>FVC, L</b>	3.23 ± 0.22
<b>FVC, %pred</b>	99 ± 8
<b>FEV<sub>1</sub>/FVC, %</b>	44 ± 3
<b>MVV, L/min</b>	52 ± 5
<b>MVV, %pred</b>	65 ± 8
<b>TLC, L</b>	6.4 ± 0.46
<b>TLC, %pred</b>	118 ± 5
<b>RV, L</b>	3.45 ± 0.33
<b>RV, %pred</b>	155 ± 12
<b>RV/TLC, %</b>	54 ± 2
<b>VC, L</b>	2.9 ± 0.2
<b>TLCO, mmol/min/kpa</b>	4.3 ± 0.4
<b>TLCO, %pred</b>	56 ± 4

### Peak exercise data and inspiratory muscle capacity

<b>W<sub>peak</sub>, W</b>	81±7
<b>W<sub>peak</sub>, %max</b>	71±5
<b>VO<sub>2</sub>, peak, L/min</b>	1.371±0.116
<b>VO<sub>2</sub>, peak, %max</b>	87±8
<b>CO<sub>peak</sub>, L/min</b>	120±0.5
<b>MIP, cmH<sub>2</sub>O</b>	74±4
<b>MIP, %pred</b>	82±5
<b>MIP &lt;LLN, n(%)</b>	9(56)
<b>Hb, g/dl</b>	14.5±0.3

570 Data are mean ± SE or n (%). FEV<sub>1</sub>: forced expiratory volume in the first second; FVC: forced -  
571 vital capacity; MVV: maximum voluntary ventilation; TLC: total lung capacity; RV: residual  
572 volume; TLCO: transfer factor for carbon monoxide; MIP: maximal inspiratory pressure; Insp.  
573 mm. weakness: maximum inspiratory pressure bellow the lower limit of normality; W<sub>peak</sub>; peak  
574 exercise capacity; VO<sub>2peak</sub>: peak oxygen consumption; CO<sub>peak</sub>; peak cardiac output; LLN:  
575 lower limit of normality.  
576

577 **Table 2.** Respiratory pressures and work of breathing and breathing pattern data during hyperpnea and loaded breathing

	<b>Mean diff (95% CI)</b>					
	<b>Rest</b>	<b>Hyperpnea</b>	<b>Loaded breathing</b>	<b>Hyperpnea - Rest</b>	<b>Loaded breathing - Rest</b>	<b>Loaded breathing - Hyperpnea</b>
<b>Respiratory pressures and work of breathing (n= 10)</b>						
<b>Pes, cmH<sub>2</sub>O</b>	-9±1	-15±1	-35±2	-6(-11 - -2)*	-26(-30 - -21)*	-19(-24 - -15)*
<b>Pes, %max</b>	14±2	23±2	54±5	10(-2 - 21)*	40(27 - 51)*	30(18 - 41)*
<b>inspPga, cmH<sub>2</sub>O</b>	10±2	12±2	15±4	1(-9 - 12)	5(-5 - 15)	3(-7 - 13)
<b>expPga, cmH<sub>2</sub>O</b>	10±1	21±4	21±4	10(-1 - 21)	11(0 - 22)	1(-10 - 12)
<b>inspPga, %max</b>	21±	22±4	26±6	1(-15 - 17)	5(-11 - 21)	4(-12 - 20)
<b>Pdi, cmH<sub>2</sub>O</b>	19±1	27±2	50±4	7(17 - -2)*	30(40 - 20)*	22(32 - 12)*
<b>Pdi, %max</b>	21±2	28±1	53±4	7(-2 - 16)*	32(22 - 41)*	24(15 - 34)*
<b>Pes WOB, L/cmH<sub>2</sub>O</b>	6±1	16±2	113±16	10(-22 - 42)*	108(75 - 140)*	97(65 - 130)*
<b>inspPga WOB, L/cmH<sub>2</sub>O</b>	3±1	9±2	33±5	6(-6 - 17)	30(18 - 41)*	24(13 - 36)*
<b>Pdi WOB, L/cmH<sub>2</sub>O</b>	7±2	14±4	104±15	7(-25 - 39)*	97(65 - 128)*	90(58 - 122)*
<b>PTPPes, cmH<sub>2</sub>O/s/b</b>	4±0	6±0	8±1	2(0 - 4)*	4(-2 - 4)*	2(0 - 4)*
<b>inspPTPPga, cmH<sub>2</sub>O/s/b</b>	4±1	4±1	3±1	0(-3 - 3)	-1(4 - 2)	-1(-4 - 2)

<b>PTPPdi, cmH<sub>2</sub>O/s/b</b>	8±1	10±1	11±1	2(0–6)	3(0–6)	1(-2–4)
<b>Pes WOB, L/cmH<sub>2</sub>O/min</b>	95±11	495±62	624±71	400(209–591)*	529(337–720)*	129(-62–320)
<b>inspPga WOB, L/cmH<sub>2</sub>O/min</b>	52±7	276±58	198±38	224(83–365)*	147(6–288)*	-77(-218–64)
<b>Pdi WOB, L/cmH<sub>2</sub>O/min</b>	109±16	430±107	567±66	321(64–578)*	458(200–715)*	136(-120–394)
<b>PTPPes, cmH<sub>2</sub>O/s/min</b>	71±12	184±16	49±9	112(69–157)*	-21(-66–22)	-135(-179–91)*
<b>inspPTPPga, cmH<sub>2</sub>O/s/min</b>	84±18	142±28	21±7	58(-12–127)*	-62(-132–7)*	-120(-190–51)*
<b>PTPPdi, cmH<sub>2</sub>O/s/min</b>	154±26	325±35	68±13	171(79–262)*	-85(-177–6)*	-256(-348–-1654.73)*

#### Breathing pattern (n= 16)

	<b>Rest</b>	<b>hyperpnea</b>	<b>loaded breathing</b>	<b>hyperpnea - Rest</b>	<b>loaded breathing - Rest</b>	<b>loaded breathing - hyperpnea</b>
<b>VE, L</b>	13±1	38±3	12±1	25(18–32)*	-1(-8–5)	-26(-33–-19)*
<b>Insp. vol., L</b>	0.74±0.06	1.17±0.11	1.9±0.21	0.43(-0.05–0.91)*	1.16(0.68–1.64)*	0.73(0.25–1.21)*
<b>Bf, b/min</b>	20±1	34±1	7±1	14(10–18)*	-13(-17–-8)*	-27(-31–-22)*
<b>Insp. peak flow, L/sec</b>	0.91±0.05	2.47±0.18	2.23±0.2	1.56(1.03–2.09)*	1.32(0.80–1.85)*	-0.24(-0.77–0.28)
<b>Insp. time, s</b>	1.27±0.1	0.67±0.04	2.26±0.22	-0.60(-1.09–0.11)*	0.99(0.50–1.47)*	1.58(1.10–2.07)*
<b>Ti/Ttot, %</b>	38±1	37±1	24±2	-2(-6–4)	-14(-19–-8)*	-12(-18–-7)*



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578 Data are mean  $\pm$  SE or mean difference (95% confidence interval). Ti/Ttot: duty cycle of respiration; Bf: breathing frequency; Pes:  
579 Esophageal pressure; Pdi: Transdiaphragmatic pressure; WOB: work of breathing; PTP: Pressure Time Product. \*  $P < 0.05$ .  
580

581 **Table 3.** Central hemodynamic and metabolic responses

	Mean diff (95% CI)					
	Rest	hyperpnea	loaded breathing	hyperpnea - Rest	loaded breathing - Rest	loaded breathing - hyperpnea
<b>HR, bpm</b>	76±3	90±4	89±4	14(2–26)*	13(1–25)*	-1(-13–11)
<b>SV, ml</b>	70±4	84±6	73±4	15(-1–31)*	4(-13–20)	-11(-27–5)*
<b>CO, L/min</b>	5.2±0.3	7.5±0.5	6.5±0.4	2.3(0.9–3.7)*	1.1(0.2–2.6)*	-1.1(-4–0.3)*
<b>CO, %max</b>	44±3	62±4	54±4	19(6–32)	10(-3–23)	-8(-21–5)
<b>VO<sub>2</sub>, ml/min</b>	283±20	625±42	443±34	342(229–454)*	161(46–275)*	-181(-296–-67)*
<b>VO<sub>2</sub>, %max</b>	25±4	54±7	39±5	29(10–48)*	13(-6–32)*	-16(-34–3)*
<b>VCO<sub>2</sub>, ml/min</b>	224±14	412±69	409±32	188(35–341)*	185(29–340)*	-4(-159–151)
<b>CaO<sub>2</sub>, mlO<sub>2</sub>/100ml</b>	189±0.5	192±0.5	192±0.4	0.3(-1.2–1.9)	0.3(-1.2–1.9)	0(-1.5–1.6)
<b>O<sub>2</sub> delivery, LO<sub>2</sub>/min</b>	0.98±0.05	1.42±0.1	1.23±0.07	0.44(0.17–0.71)*	0.25(-0.01–0.52)*	-0.18(-0.45–0.08)*
<b>O<sub>2</sub> extraction, %</b>	29±2	46±4	38±3	16(6–26)*	8(-2–19)*	-8(-18–3)*
<b>a-vO<sub>2</sub> difference, mlO<sub>2</sub>/100ml</b>	5.6±0.3	8.73±0.75	7.3±0.7	3.2(1.1–5.3)*	1.7(-0.4–3.9)*	-1.4(-3.5–0.7)*
<b>SVC, ml/min/mmHg</b>	56±3	74±5	63±4	18(5–32)*	7(-7–21)*	-11(-26–3)*
<b>SpO<sub>2</sub>, %</b>	94±1	95±1	94±1	2(-1–4)	0(-2–3)	-1(-4–1)

<b>SBP,mmHg</b>	120±3	139±6	133±5	19(4–33)*	13(-3–28)*	-5.9(-22–10)
<b>DBP,mmHg</b>	80±2	88±2	90±4	8(-1–17)	10(1–20)*	2(-8–12)
<b>MAP</b>	93±2	105±3	104±4	12(2–22)*	11(1–21)*	-1(-11–10)

582 Data are mean ± SE or mean difference (95% confidence interval). HR: heart rate; SV: stroke volume; CO: cardiac output; VO<sub>2</sub>:  
583 oxygen consumption; VCO<sub>2</sub>: carbon dioxide production; CaO<sub>2</sub>: arterial oxygen content; a-vO<sub>2</sub> difference: arterio-venous oxygen  
584 difference; SVC: systemic vascular conductance SpO<sub>2</sub>: peripheral oxygen saturation; SBP: systolic blood pressure; DBP: diastolic  
585 blood pressure; Vasc. Cond.: systemic vascular conductance. \**P* <0.05.

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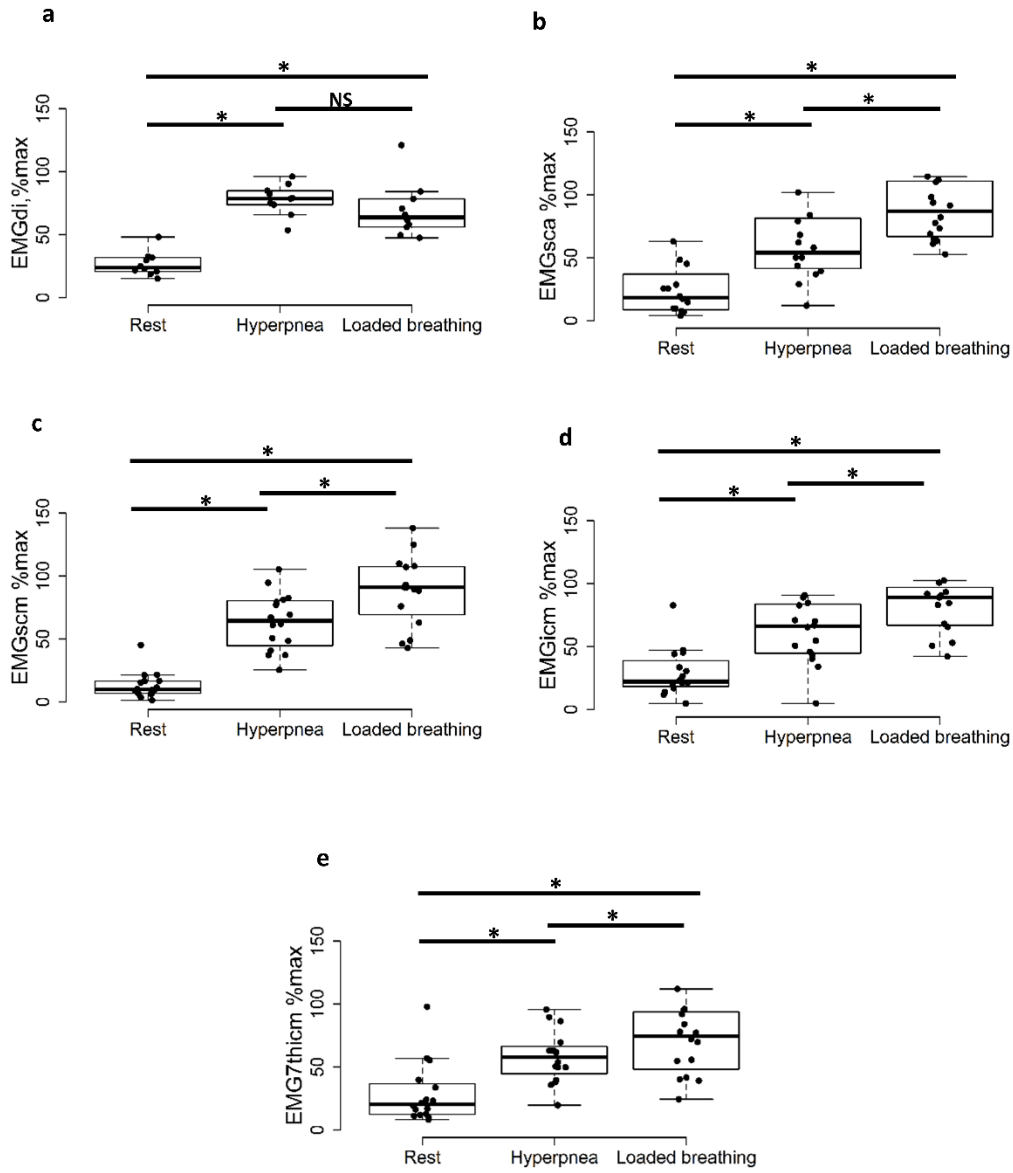
588 **Table 4.** Respiratory muscles perfusion and oxygenation responses during hyperpnea and loaded  
 589 breathing

	Mean diff (95% CI)		
	hyperpnea	loaded breathing	loaded breathing - hyperpnea
<b>Respiratory muscle perfusion, n= 13</b>			
$\Delta$ SCA BFI, nmol/L	4.67 ± 1.3	2.81 ± 1.17	-1.86 (-3.2 - -0.5)*
$\Delta$ 7 <sup>th</sup> IC BFI, nmol/L	0.76 ± 0.2	0.5 ± 0.2	0.27 (-0.78 - 0.2)
$\Delta$ ABD BFI, nmol/L	1.2 ± 0.5	0.4 ± 0.3	-0.8 (-1.7 - 0.2)
<b>Respiratory muscle O<sub>2</sub> delivery</b>			
$\Delta$ SCA O <sub>2</sub> del, au	90 ± 24	54 ± 22	-36 (-11 - -62)*
$\Delta$ 7 <sup>th</sup> IC O <sub>2</sub> del, au	14 ± 4	10 ± 5	-5 (4 - -14)
$\Delta$ ABD O <sub>2</sub> del, au	23 ± 10	8 ± 6	-14 (3 - -33)
<b>Respiratory muscle oxygen saturation, n= 15</b>			
$\Delta$ SCA St <i>i</i> O <sub>2</sub> , %	1.25 ± 0.9	-2.84 ± 1.27	-4.1 (-6 - -2.1)*
$\Delta$ 7 <sup>th</sup> IC St <i>i</i> O <sub>2</sub> , %	1.5 ± 0.71	-1.52 ± 0.86	-3 (-4.9 - -1.3)*
$\Delta$ ABD St <i>i</i> O <sub>2</sub> , %	1.00 ± 1.00	-0.40 ± 1.52	-1.38 (-3.6 - 0.9)
<b>Respiratory muscle oxygen extraction, n= 15</b>			
$\Delta$ SCA [HHb], $\mu$ mol/L	2.94 ± 1.33	7.68 ± 2.08	4.73 (1.88 - 7.58)*
$\Delta$ 7 <sup>th</sup> IC [HHb], $\mu$ mol/L	0.42 ± 0.61	1.9 ± 0.87	1.48 (-0.05 - 3)
$\Delta$ ABD [HHb], $\mu$ mol/L	-1.67 ± 0.86	0.03 ± 1.1	1.70 (-0.82 - 3.48)

590 Data are mean ± SE or mean difference (95% confidence interval).  $\Delta$ : changes from rest; SCA:  
 591 Scalenes; 7<sup>th</sup> IC: 7<sup>th</sup> Intercostal; ABD: Rectus Abdominis; [HHb]: deoxyhemoglobin  
 592 concentration; St*i*O<sub>2</sub>: Tissue oxygen saturation index; BFI: blood flow index. \**P* <0.05.

593 Figure 1

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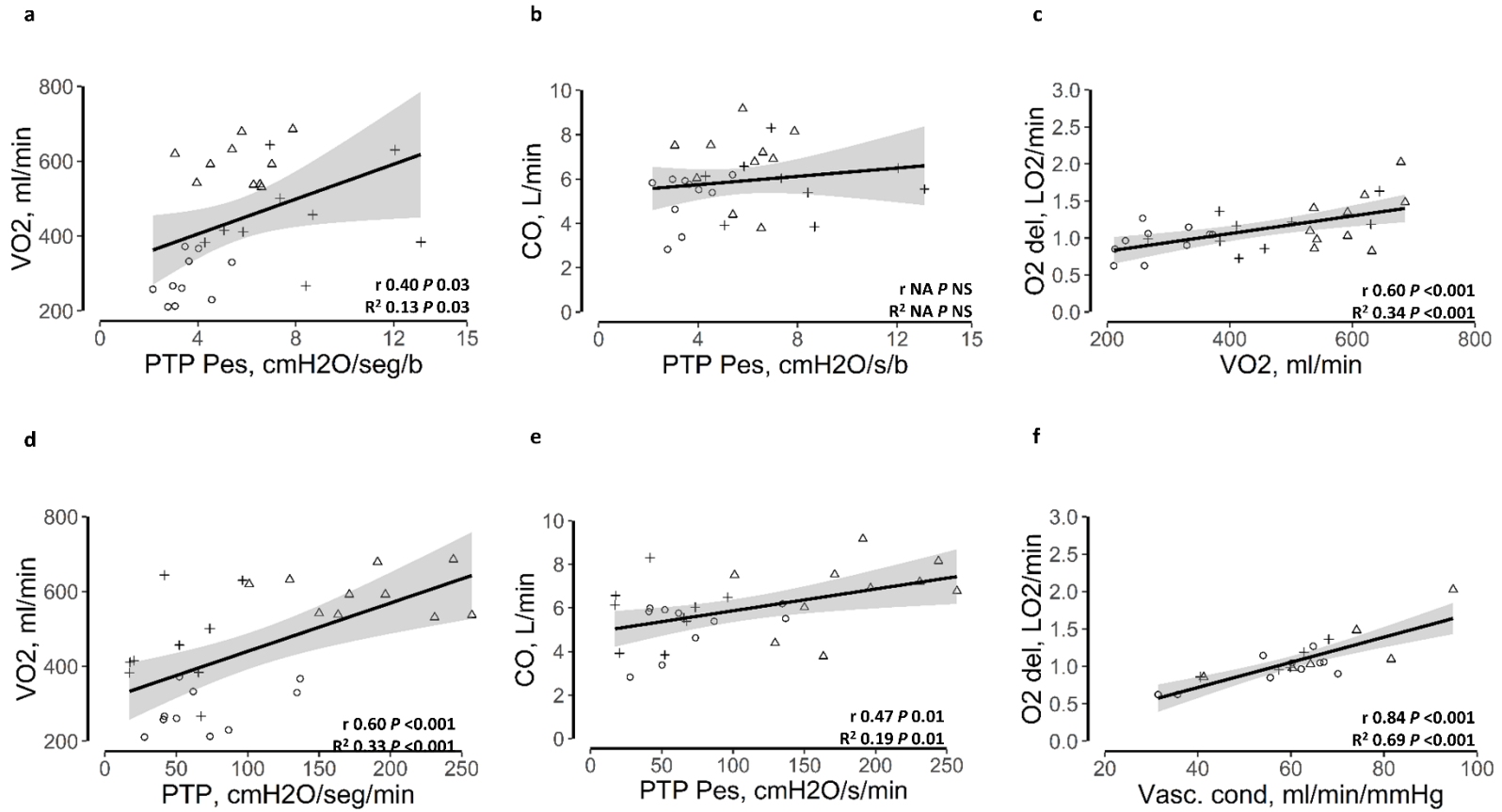


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597 Figure 2.

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