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

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Abstract

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

Methods: In 16 patients (age:65±13, 56% male, FEV_1:60±6%pred, Pimax:82±5%pred) assessments of respiratory muscle electromyography (EMG), esophageal (Pes) and gastric (Pga) pressures, breathing pattern, and noninvasive assessments of systemic (VO_2, cardiac output, oxygen delivery and extraction) and respiratory muscle hemodynamic and oxygenation responses (blood flow index [BFI], oxygen delivery index, deoxyhemoglobin concentration [HHb] and tissues oxygen saturation [$StiO_2$]), were performed under two different conditions of respiratory muscle loading (hyperpnea and loaded breathing).

Results: During hyperpnea, breathing frequency, minute ventilation, esophageal and diaphragm pressure-time product (PTP)/min, cardiac output and VO_2 were higher than during loaded breathing (P<0.05). Average inspiratory Pes and Pdi per breath scalene (SCA), sternocleidomastoid (SCM), and intercostal muscle activation was higher during loading breathing (P<0.05). Higher Pdi during loaded breathing compared to hyperpnea was due to higher Pes (P<0.05). Diaphragm activation, inspiratory and expiratory Pga and expiratory abdominal muscle activation did not differ between the two conditions (P>0.05). SCA-BFI and oxygen delivery index were lower and SCA-HHb was higher during loaded breathing. Furthermore, SCA and intercostal muscle $StiO_2$ were lower during loaded breathing compared to hyperpnea (P<0.05).

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

$StiO_2$ during loading breathing than during hyperpnea might indicates a mismatch between inspiratory muscle oxygen delivery and utilization.

Key Words: RESPIRATORY MUSCLE ACTIVATION, RESPIRATORY MUSCLE LOADING, RESPIRATORY MUSCLE METABOLISM, RESPIRATORY MUSCLE TRAINING.

INTRODUCTION

Improvements in both respiratory muscle endurance and strength can be observed in patients with COPD after either whole-body, or specific respiratory muscle endurance training.(1-3) The improvements in respiratory muscle function in response to endurance training are probably mainly due to the increased ventilatory demands induced by (exercise) hyperpnea. Hyperpneic training provides a high respiratory flow / low resistance stimulus to the respiratory muscles during a high number of consecutive repetitions.(2, 3) It has also been demonstrated that adding specific hyperpneic (i.e. endurance) respiratory muscle training can enhance the effects of whole body endurance training on respiratory muscle endurance. However, larger improvements in inspiratory muscle strength (i.e., pressure generating capacity) have been reported after specific inspiratory muscle strength training (IMT) in comparison with whole body endurance training (i.e. average increases of 16 vs 6 cmH_2O in maximal inspiratory mouth pressure [MIP] respectively).(4, 5) During inspiratory muscle strength training loading is induced by overcoming a "high external resistance" during a limited number of breathing cycles per session (e.g. 30-40 full vital capacity breaths against loads equaling about 30-50% of MIP).(4) Therefore, as much as limb muscles respond distinctively to endurance and strengthening stimuli,(6, 7) it can also be expected that different responses are induced when the respiratory muscles are exposed to "endurance" (i.e., hyperpnea) or "strengthening" (i.e., loaded breathing) stimuli. Differences in acute responses to either endurance or strengthening stimuli imposed on the respiratory muscle in terms of muscle recruitment and activation patterns as well as local and systemic oxygenation responses have however, to the best of our knowledge, never been comprehensively characterized. Therefore, we aimed to explore and compare the acute responses

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

METHODS

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

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

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

Hyperpnea. Patients were requested to maintain tidal volume, breathing frequency and minute ventilation reproducing their own breathing responses recorded during the CWRT for five minutes.(15) Thus, during the test investigators provided continuous verbal guidance aiming to maintain a maximum variation in minute ventilation of 5% throughout the test.(15) Visual feedback on breathing parameters was also provided on a screen displayed in front of the patient so as to adjust his/her breathing frequency and tidal volume to the level required by the investigator. Normocapnia was maintained by having subjects inspire from a Douglas bag containing 5% CO_2, 21% O_2 and 74% N_2 for balance, connected to a two-way non-rebreathing valve (model 2700, Hans Rudolph) by a piece of tubing.(15)

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

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

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

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

latter was calculated as the product of 1.39 x hemoglobin concentration [Hb] and %SpO_2.(25) Arterio-venous oxygen content (i.e., a-vO_2 diff) difference was calculated by dividing oxygen uptake by cardiac output. The systemic oxygen extraction ratio was calculated as the ratio of the a-vO2 diff to arterial oxygen content. In addition, systemic vascular conductance was calculated by dividing cardiac output by mean arterial blood pressure.

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

NIRS-derived changes in respiratory muscle deoxyhemoglobin concentration ([HHb]) was used as an index of respiratory muscle oxygen extraction.(26) NIRS-derived tissue oxygen saturation index (i.e., $StiO_2$) was considered as a measure of the dynamic balance between local tissue oxygen delivery and utilization (27) and, therefore, local muscle capacity to match oxygen supply relative to its metabolic demand (see online supplement)).

Statistical analysis. A power >0.99 was found based on the difference between SCM muscle activation (EMGscm,%max) between the three tasks (i.e., rest, hyperpnea and loaded

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

RESULTS

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

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

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

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

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

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

Systemic hemodynamic, metabolic and cardiovascular responses. Cardiac output, VO_2, a-vO2 diff and systemic vascular conductance responses were significantly greater during hyperpnea than during loaded breathing (P<0.05; Table 3). Mean arterial blood pressure did not significantly differ between the two conditions (Table 3).

Respiratory muscle perfusion and oxygenation responses. Increases from rest in SCABFI and oxygen delivery index were significantly less during loaded breathing as compared to hyperpnea (P<0.05; Table 4). The change from rest in SCA oxygen extraction (i.e., [HHb]) was significantly higher during loading breathing as compared to hyperpnea (P<0.05; Table 4). During loading breathing SCA-$StiO_2$ decreased from rest whilst during hyperpnea SCA-$StiO_2$ increased leading to a significant difference in SCA-$StiO_2$ between the two conditions (P<0.05; Table 4). Increases from rest in 7^{th}ICMBFI and oxygen delivery index were less (but not significant *P*=0.27 and *P*=0.26, respectively) during loaded breathing as compared to hyperpnea. The change from rest in 7^{th}ICM-HHB tended to be higher during loaded breathing as compared to hyperpnea (*P*=0.06).During loading breathing 7^{th}ICM -$StiO_2$ decreased from rest whilst during hyperpnea 7^{th}ICM -$StiO_2$ increased leading to a significant difference in 7^{th}ICM -$StiO_2$ between the two conditions (P<0.05; Table 4). No significant changes in BFI (*P*= 0.09), oxygen delivery

(*P*= 0.10), [HHB] (*P*= 0.11), and $StiO_2$ (*P*= 0.50) were observed for the ABDs between loaded breathing and hyperpnea.

DISCUSSION

Main findings. Our key findings are that by engaging either in hyperpnea (endurance stimulus) or loaded breathing (strength stimulus) differences in both local (i.e., respiratory muscle) and systemic responses are evoked in patients COPD. In both conditions the increase in systemic and respiratory muscle hemodynamics from rest seems to increase in association with the increase in VO_2, (Tables 3 and 4). Loaded breathing elicited greater activation of the SCA, SCM ICM and ^{7th}ICM and inspiratory muscle and reduction in SCA and 7^{th}ICM-$StiO_2$ (Figure 1 and Table 4, respectively) compared to hyperpnea, thus reflecting the additional burden imposed on these muscles by a strengthening stimulus in comparison to an endurance loading stimulus (Table 2). In addition, increases in diaphragmatic activation during hyperpnea and loaded breathing relative to resting breathing were of similar magnitude in both conditions (Figure 1).

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

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

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

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

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

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

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

The observed acute increases in load and work being performed by the inspiratory muscles during both tasks (Table 2) are known to be important determinants of muscle improvements after exercise programs.(43) Furthermore, according to the specificity and overload principles of training,(43) in response to a low load (i.e., pressures), high repetition (i.e., breathing frequency and duration) and high exercise-volume (i.e., PTP cmH2O/s/min) (Table 2) stimulus as hyperpnea, an endurance benefit would be expected. While after loaded breathing, improvements in strength would be anticipated as consequence of the high load (i.e., pressures), low repetition (i.e., breathing frequency and duration) and high contraction-volume (PTP cmH2O/s/b) stimulus imposed by this regimen (Table 2). Noteworthy the additional recruitment of only SCA, SCM and both intercostal muscles (Figure 1) as the strategy to overcome the load imposed during loaded breathing in comparison to hyperpnea (Table 2) was accompanied by an increased metabolic burden (Table 3 It is therefore likely these inspiratory muscles will mostly benefit from this additional stimulus (i.e., increased load).(43) It has previously been observed that a period of high intensity inspiratory muscle strength training resulted in increases in specific hypertrophy of intercostal muscle fibers.(44)

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

Strengths, limitations and technical considerations. The multitude of variables simultaneously collected is a strength of the study. It allows the concurrent investigation of the behavior of respiratory muscles activation, pressure generation and metabolism under the same stimulus. Unfortunately, however, assessments of blood flow and oxygen requirements of the diaphragm could not be performed due to methodological and safety issues. A limitation of our study is the small sample size due to the complexity and the invasiveness of its methods and the fact that not all subjects were able or willing to undergo all experimental procedures. However, the sample was powered sufficiently (see *Data analysis* in the supplemental material for more details) to detect differences in a wide variety of physiological markers. Moreover, while reproducing the ventilatory pattern of exercise hyperpnea (i.e., breathing frequency, tidal volume and ventilation), there were also no statistically significant differences between the expiratory gastric pressures and expiratory ABD activation that were generated during cycling exercise in comparison to hyperpnea (Pga cycling 20 ± 3 vs Pga hyperpnea 25 ± 5, cmH_2O; P=0.3; EMGabd cycling 23 ± 4 vs EMGabd hyperpnea 33 ± 4, %max; P=0.10, respectively). Thus, providing an adequate reproducibility between exercise hyperpnea and the hyperpnea task used in our study. Arterial oxygen content, a-VO_2 diff and systemic oxygen extraction were estimated using continuous SpO_2 measurements at the expense of acceptable reduced accuracy in the hypoxemic patients compared with invasive arterial blood sampling. In addition, it is known that the EMG signal from the costal diaphragm can generate noise on the activation of the 7^{th}ICM we measured herein. However, the different pattern of diaphragm and 7^{th}ICM activation between loaded breathing and hyperpnea suggested that this was not the case in our data. Nevertheless, it is possible that the EMG signal measured at these muscles as well as at SCA and SCM could be, at least in part, contaminated from nearby activity due to the use of superficial electrodes. In our

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

CONCLUSION

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

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Conflict of interest

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

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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^{th} icm, %max: relative 7^{th} 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 og 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

	n: 16
Demographics / Anthropometrics	
Sex, male/female	9 / 7
Age, yrs	65 ± 13
BMI, kg/m^2	27 ± 1.6
Pulmonary function	
FEV_1, L	1.44 ± 0.15
FEV_1, %pred	60 ± 6
FVC, L	3.23 ± 0.22
FVC, %pred	99 ± 8
FEV_1/FVC, %	44 ± 3
MVV, L/min	52 ± 5
MVV, %pred	65 ± 8
TLC, L	6.4 ± 0.46
TLC, %pred	118 ± 5
RV, L	3.45 ± 0.33
RV, %pred	155 ± 12
RV/TLC, %	54 ± 2
VC, L	2.9 ± 0.2
TLCO, mmol/min/kpa	4.3 ± 0.4
TLCO, %pred	56 ± 4

Peak exercise data and inspiratory muscle capacity	
Wpeak, W	81 ± 7
Wpeak, %max	71 ± 5
VO_2, peak, L/min	1.371 ± 0.116
VO_2, peak, %max	87 ± 8
COpeak, L/min	12.0 ± 0.5
MIP, cmH_2O	74 ± 4
MIP, %pred	82 ± 5
MIP <LLN, n(%)	9 (56)
Hb, g/dl	14.5 ± 0.3

Data are mean ± SE or n (%). FEV_1: forced expiratory volume in the first second; FVC: forced - vital capacity; MVV: maximum voluntary ventilation; TLC: total lung capacity; RV: residual volume; TLCO: transfer factor for carbon monoxide; MIP: maximal inspiratory pressure; Insp. mm. weakness: maximum inspiratory pressure bellow the lower limit of normality; Wpeak; peak exercise capacity; VO2peak: peak oxygen consumption; COpeak; peak cardiac output; LLN: lower limit of normality.

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

				Mean diff (95% CI)		
	Rest	**Hyperpnea**	**Loaded breathing**	**Hyperpnea - Rest**	**Loaded breathing - Rest**	**Loaded breathing - Hyperpnea**
Respiratory pressures and work of breathing (n= 10)						
Pes, cmH_2O	-9 ± 1	-15 ± 1	-35 ± 2	-6 (-11 - -2)*	-26 (-30 - -21)*	-19 (-24 - -15)*
Pes, %max	14 ± 2	23 ± 2	54 ± 5	10 (-2 – 21)*	40 (27 – 51)*	30 (18 – 41)*
inspPga, cmH2O	10 ± 2	12 ± 2	15 ± 4	1 (-9 – 12)	5 (-5 – 15)	3 (-7 – 13)
expPga, cmH2O	10 ± 1	21 ± 4	21 ± 4	10 (-1 – 21)	11 (0 – 22)	1 (-10 – 12)
inspPga, %max	21 ±	22 ± 4	26 ± 6	1 (-15 – 17)	5 (-11 – 21)	4 (-12 – 20)
Pdi, cmH_2O	19 ± 1	27 ± 2	50 ± 4	7 (17 - -2)*	30 (40 – 20)*	22 (32 – 12)*
Pdi, %max	21 ± 2	28 ± 1	53 ± 4	7 (-2 – 16)*	32 (22 – 41)*	24 (15 – 34)*
Pes WOB, L/cmH_2O	6± 1	16 ± 2	113 ± 16	10 (-22 – 42)*	108 (75 – 140)*	97 (65 – 130)*
inspPga WOB,, L/cmH_2O	3 ± 1	9 ± 2	33 ±5	6 (-6 – 17)	30 (18 – 41)*	24 (13 – 36)*
Pdi WOB, L/cmH_2O	7 ± 2	14 ± 4	104 ± 15	7 (-25 – 39)*	97 (65 – 128)*	90 (58 – 122)*
PTP Pes, cmH2O/s/b	4 ± 0	6 ± 0	8 ± 1	2 (0 – 4)*	4 (-2 – 4)*	2 (0 – 4)*
inspPTP Pga, cmH2O/s/b	4 ± 1	4 ± 1	3 ± 1	0 (-3 – 3)	-1 (4 – 2)	-1 (-4 – 2)

PTP Pdi, cmH2O/s/b	8 ± 1	10 ± 1	11 ± 1	2 (0 – 6)	3 (0 – 6)	1 (-2 – 4)
Pes WOB, L/cmH_2O/min	95 ± 11	495 ± 62	624 ± 71	400 (209 – 591)*	529 (337 – 720)*	129 (-62 – 320)
inspPga WOB, L/cmH2O/min	52 ± 7	276 ± 58	198 ± 38	224 (83 - 365)*	147 (6 – 288)*	-77 (-218 – 64)
Pdi WOB, L/cmH_2O/min	109 ± 16	430 ± 107	567 ± 66	321 (64 – 578)*	458 (200 – 715)*	136 (-120 - 394)
PTP Pes, cmH_2O/s/min	71 ± 12	184 ± 16	49 ± 9	112 (69 – 157)*	-21 (-66 – 22)	-135 (-179 – 91)*
inspPTP Pga, cmH2O/s/min	84 ± 18	142 ± 28	21 ± 7	58 (-12 – 127)*	-62 (-132 – 7)*	-120 (-190 - -51)*
PTPPdi, cmH_2O/s/min	154 ± 26	325 ± 35	68 ± 13	171 (79 – 262)*	-85(-177 – 6)*	-256 (-348 - -1654.73)*
Breathing pattern (n= 16)						
	Rest	**hyperpnea**	**loaded breathing**	**hyperpnea - Rest**	**loaded breathing - Rest**	**loaded breathing - hyperpnea**
VE, L	13 ± 1	38 ± 3	12 ± 1	25 (18 – 32)*	-1 (-8 - 5)	-26 (-33 - -19)*
Insp. vol., L	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)*
Bf, b/min	20 ± 1	34 ± 1	7 ± 1	14 (10 – 18)*	-13 (-17 - -8)*	-27 (-31 - -22)*
Insp. peak flow, L/sec	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)
Insp. time, s	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)*
Ti/Ttot, %	38 ± 1	37 ± 1	24 ± 2	-2 (-6 - 4)	-14 (-19 - -8)*	-12 (-18 - -7)*

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

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

				Mean diff (95% CI)		
	Rest	**hyperpnea**	**loaded breathing**	**hyperpnea - Rest**	**loaded breathing - Rest**	**loaded breathing - hyperpnea**
HR, bpm	76 ± 3	90 ± 4	89 ± 4	14 (2 – 26)*	13 (1 – 25)*	-1 (-13 – 11)
SV, ml	70 ± 4	84 ± 6	73 ± 4	15 (-1 – 31)*	4 (-13 – 20)	-11 (-27 – 5)*
CO, L/min	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)*
CO, %max	44 ± 3	62 ± 4	54 ± 4	19 (6 – 32)	10 (-3 – 23)	-8 (-21 – 5)
VO_2, ml/min	283 ± 20	625 ± 42	443 ± 34	342 (229 – 454)*	161 (46 – 275)*	-181 (-296 - -67)*
VO_2, %max	25 ± 4	54 ± 7	39 ± 5	29 (10 – 48)*	13 (-6 – 32)*	-16 (-34 – 3)*
VCO_2, ml/min	224 ± 14	412 ± 69	409 ± 32	188 (35 – 341)*	185 (29 – 340)*	-4 (-159 – 151)
CaO_2mlO_2/100ml	18.9 ± 0.5	19.2 ± 0.5	19.2 ± 0.4	0.3 (-1.2 – 1.9)	0.3 (-1.2 – 1.9)	0 (-1.5 – 1.6)
O_2 delivery, LO_2/min	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)*
O_2 extraction, %	29 ± 2	46 ± 4	38 ± 3	16 (6 - 26*	8 (-2 – 19)*	-8 (-18 – 3)*
a-vO_2 difference, mlO_2/100ml	5.6 ± 03	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)*
SVC, ml/min/mmHg	56 ± 3	74 ± 5	63 ± 4	18 (5 – 32)*	7 (-7 – 21)*	-11 (-26 – 3)*
SpO_2, %	94 ± 1	95 ± 1	94 ± 1	2 (-1 – 4)	0 (-2 – 3)	-1 (-4 – 1)

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

Data are mean ± SE or mean difference (95% confidence interval). HR: heart rate; SV: stroke volume; CO: cardiac output; VO_2: oxygen consumption; VCO_2: carbon dioxide production; CaO_2; arterial oxygen content; $a\text{-}vO_2$ difference: arterio-venous oxygen difference; SVC: systemic vascular conductance SpO_2: peripheral oxygen saturation; SBP: systolic blood pressure; DBP: diastolic blood pressure; Vasc. Cond.: systemic vascular conductance. *P <0.05.

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

			Mean diff (95% CI)
	hyperpnea	**loaded breathing**	**loaded breathing - hyperpnea**
Respiratory muscle perfusion, n= 13			
ΔSCA BFI, nmol/L	4.67 ± 1.3	2.81 ± 1.17	-1.86 (-3.2 - -0.5)*
Δ7th IC BFI, nmol/L	0.76 ± 0.2	0.5 ± 0.2	0.27 (-0.78 - 0.2)
ΔABD BFI, nmol/L	1.2 ± 0.5	0.4 ± 0.3	-0.8 (-1.7 – 0.2)
Respiratory muscle O2delivery			
ΔSCA O2del, au	90 ± 24	54 ± 22	-36 (-11 - -62)*
Δ7th IC O2del, au	14 ± 4	10 ± 5	-5 (4 - -14)
ΔABD O2del, au	23 ± 10	8 ± 6	-14 (3 – 33)
Respiratory muscle oxygen saturation, n= 15			
ΔSCA StiO2, %	1.25 ± 0.9	-2.84 ± 1.27	-4.1 (-6 - -2.1)*
Δ7th IC StiO2, %	1.5 ± 0.71	-1.52 ± 0.86	-3 (-4.9 - -1.3)*
ΔABD StiO2, %	1.00 ± 1.00	-0.40 ± 1.52	-1.38 (-3.6 - 0.9)
Respiratory muscle oxygen extraction, n= 15			
ΔSCA [HHb], µmol/L	2.94 ± 1.33	7.68 ± 2.08	4.73 (1.88 -7.58)*
Δ7th IC [HHb], µmol/L	0.42 ± 0.61	1.9 ± 0.87	1.48 (-0.05 - 3)
ΔABD [HHb], µmol/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). Δ: changes from rest; SCA:
591 Scalenes; 7th IC: 7th Intercostal; ABD: Rectus Abdominis; [HHb]: deoxyhemoglobin
592 concentration; $StiO_2$: Tissue oxygen saturation index; BFI: blood flow index. *P <0.05.

593 Figure 1

594

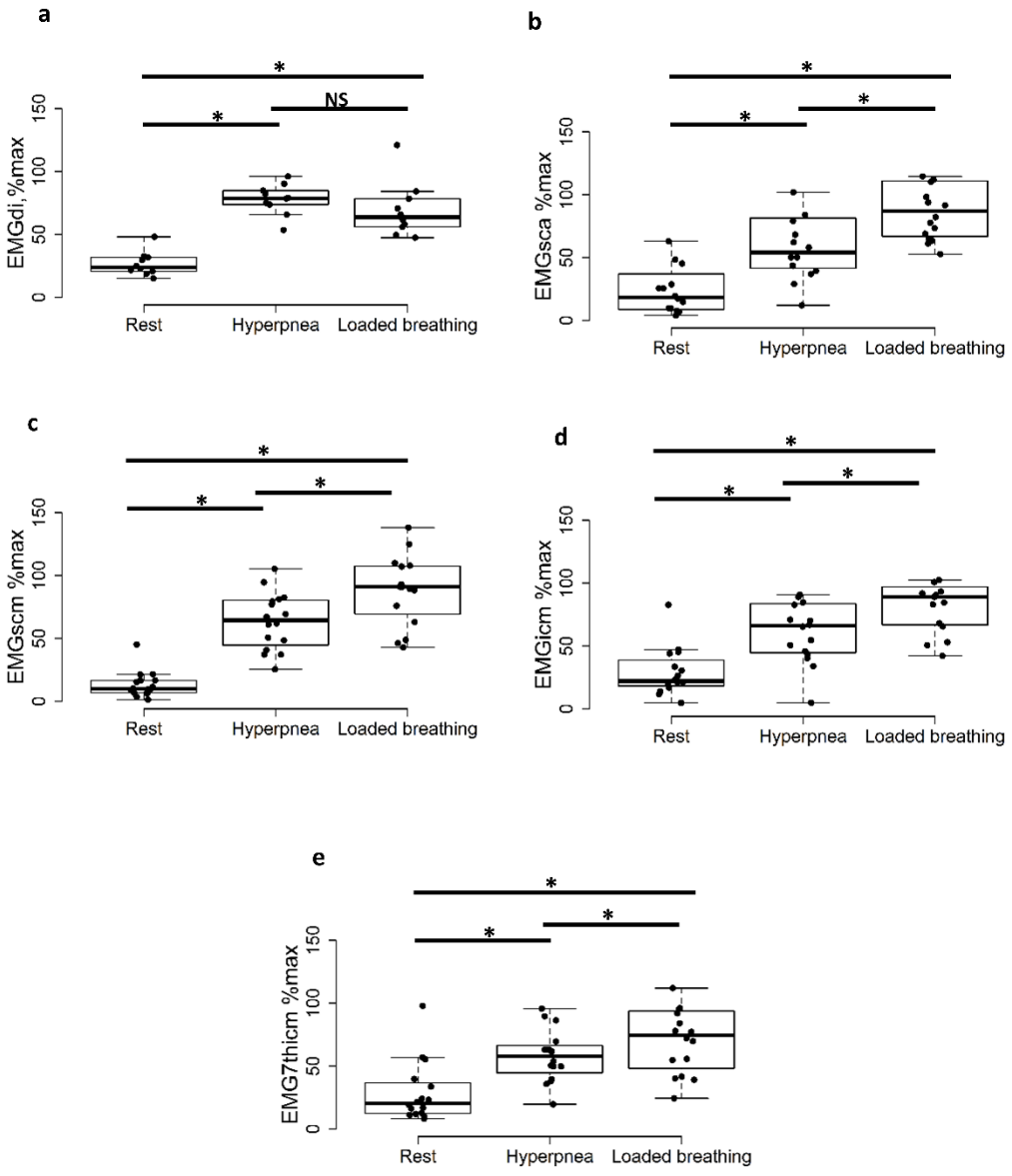

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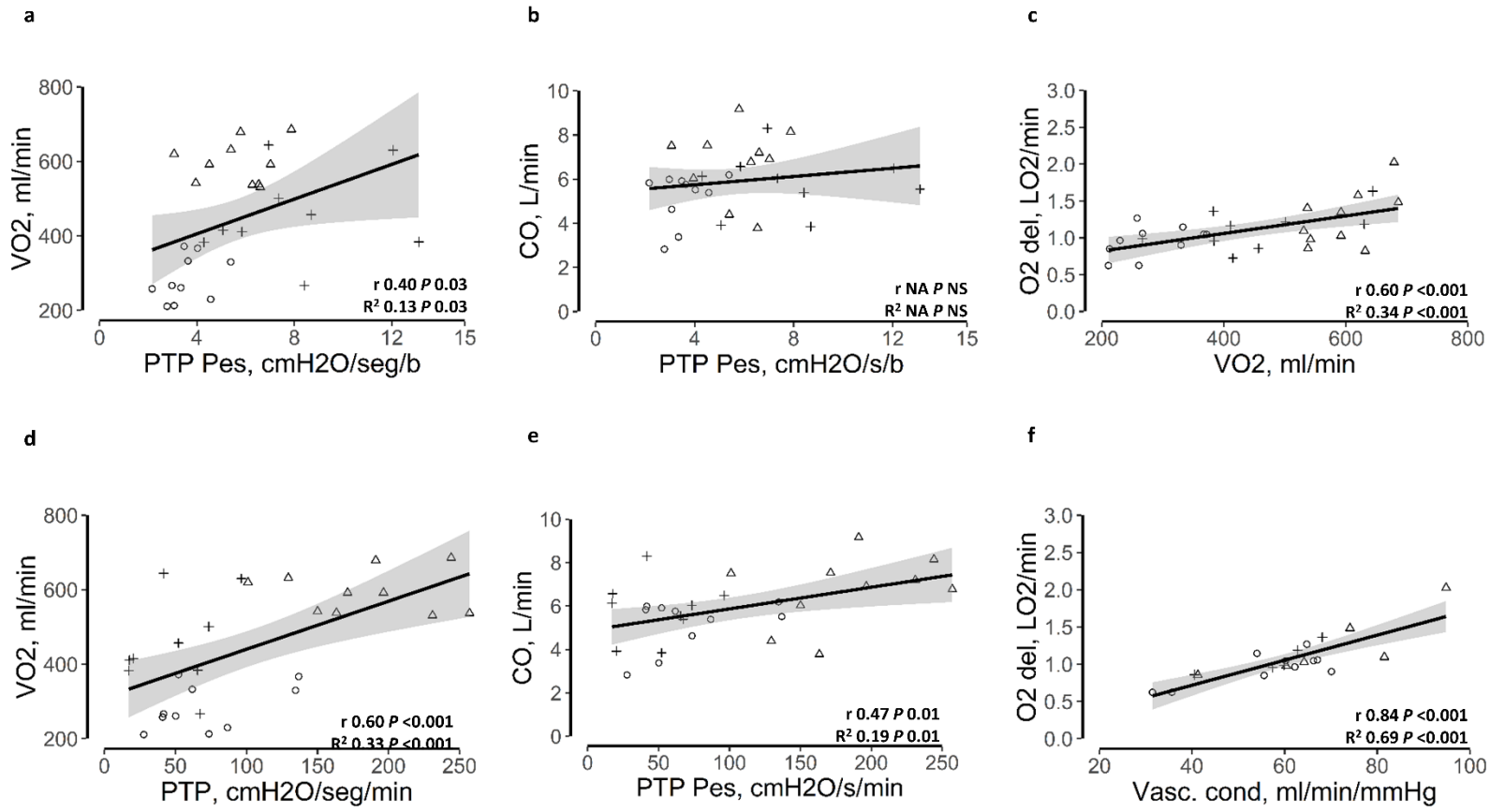
Figure 2.


a
VO2, ml/min
PTP Pes, cmH2O/seg/b
r 0.40 P 0.03
R² 0.13 P 0.03
b
CO, L/min
PTP Pes, cmH2O/s/b
r NA P NS
R² NA P NS
c
O2 del, LO2/min
VO2, ml/min
r 0.60 P <0.001
R² 0.34 P <0.001
d
VO2, ml/min
PTP, cmH2O/seg/min
r 0.60 P <0.001
R² 0.33 P <0.001
e
CO, L/min
PTP Pes, cmH2O/s/min
r 0.47 P 0.01
R² 0.19 P 0.01
f
O2 del, LO2/min
Vasc. cond, ml/min/mmHg
r 0.84 P <0.001
R² 0.69 P <0.001

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