

# Influence of CO<sub>2</sub> on upper airway muscles and chest wall/diaphragm corticomotor responses assessed by transcranial magnetic stimulation in awake healthy subjects

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**Borel J-C, Melo-Silva CA, Gakwaya S, Sériès F.** Influence of CO<sub>2</sub> on upper airway muscles and chest wall/diaphragm corticomotor responses assessed by transcranial magnetic stimulation in awake healthy subjects. *J Appl Physiol* 112: 798–805, 2012. First published December 8, 2011; doi:10.1152/jappphysiol.00713.2011.—*Rationale:* functional interaction between upper airway (UA) dilator muscles and the diaphragm is crucial in the maintenance of UA patency. This interaction could be altered by increasing respiratory drive. The aim of our study was to compare the effects of hypercapnic stimulation on diaphragm and genioglossus corticomotor responses to transcranial magnetic stimulation (TMS). *Methods:* 10 self-reported healthy men ( $32 \pm 9$  yr; body mass index =  $24 \pm 3$  kg/m<sup>2</sup>) breathed, in random order, room air or 5% and then 7% F<sub>I</sub>CO<sub>2</sub>, both balanced with pure O<sub>2</sub>. Assessments included ventilatory variables, isoflow UA resistance (at 300 ml/s), measurement of lower chest wall/diaphragm (LCW/diaphragm), and genioglossus motor threshold (MT) and motor-evoked potential (MEP) characteristics. TMS twitches were applied during early inspiration and end expiration at stimulation intensity 30% above LCW/diaphragm and genioglossus MT. *Results:* compared with room air, CO<sub>2</sub> inhalation significantly augmented minute ventilation, maximal inspiratory flow, tidal volume, and tidal volume/respiratory time ratio. UA resistance was unchanged with CO<sub>2</sub> inhalation. During 7% CO<sub>2</sub> breathing, LCW/diaphragm MT decreased by  $9.6 \pm 10.1\%$  whereas genioglossus MT increased by  $7.2 \pm 9\%$ . CO<sub>2</sub>-induced ventilatory stimulation led to elevation of LCW/diaphragm MEP amplitudes during inspiration but not during expiration. LCW/diaphragm MEP latencies remained unaltered both during inspiration and expiration. Genioglossus MEP latencies and amplitudes were unchanged with CO<sub>2</sub>. *Conclusion:* in awake, healthy subjects, CO<sub>2</sub>-induced hyperventilation is associated with heightened LCW/diaphragm corticomotor activation without modulating genioglossus MEP responses. This imbalance may promote UA instability during increased respiratory drive.

hypercapnia; respiratory muscle

DURING SPONTANEOUS BREATHING, diaphragm and upper airway (UA) dilator muscles are activated in a coordinated fashion, the latter being activated before the former (4, 39). This coordinate activation, resulting from respiratory output generated by the brain stem, is aimed at preventing closure of the UA's collapsible part during inspiration (14, 19). Furthermore, UA muscle activity is adjusted to the level of ventilation to maintain UA patency (i.e., the higher the ventilation level, the higher the UA muscle activity). Acute hypercapnia is known to increase phrenic nerve and hypoglossal nerve performance (44) and,

thus, both diaphragm and UA muscle actions (26). Nevertheless, several animal (18, 27) and human (29) studies have shown that the diaphragm could be activated at a lower CO<sub>2</sub> level than UA dilator muscles. Moreover, in animal experiments (17, 44), genioglossus (or hypoglossal nerve) and diaphragm (or phrenic nerve) activities have been demonstrated to be curvilinear, change in genioglossus activity being less than that of the diaphragm below a given chemical drive threshold, with an inverse situation above this threshold. These previous results indicate that uncoupling between diaphragm and UA dilator muscle activations may occur with increasing neural drive to breathe. Beyond automatic respiratory drive originating from the brainstem, the diaphragm and UA muscles can receive cortical commands that allow the respiratory system to perform volitional breathing (6) or non-respiratory tasks (16), meaning that corresponding peripheral motoneurons integrate brain stem and cortical commands.

Transcranial magnetic stimulation (TMS) represents a technique for investigating corticomotor activation of the peripheral muscles (9) as well as of the diaphragm and UA dilator muscles (8, 43). Several studies have revealed that volitional activation of the diaphragm (23, 25, 36), tongue, and pharyngeal muscles (12, 24, 33) increases TMS-related corticomotor responses, suggesting that TMS is a useful tool to assess the corticomotor facilitation phenomenon. Straus et al. (38), undertaking TMS during hypercapnic challenge, found that augmenting the automatic drive to breathe facilitated diaphragm corticomotor responses without influencing peripheral muscle responses (*abductor pollicis brevis*). These results illustrate that cortical magnetic stimulation is a reliable way of investigating the impact of brain stem automatic command on the diaphragm activation pattern, although fast-conducting corticospinal cells normally activate inspiratory motoneurons during voluntary breathing but are not required during involuntary contractions.

Given the notion that CO<sub>2</sub> has a differential impact on the diaphragm and UA dilator muscles (18, 27), our experiment tested the hypothesis that CO<sub>2</sub>-induced increases in respiratory drive will differentially alter the corticomotor responses of the diaphragm and the UA dilator muscles. Thus our aim was to compare the effects of hypercapnic stimulation on lower chest wall/diaphragm (LCW/diaphragm) and genioglossus corticomotor responses to TMS in awake, healthy subjects.

## MATERIALS AND METHODS

### Subjects

Ten self-reported healthy men were recruited by advertisement via a university e-mailing list. The ethics review board of our institution

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approved this protocol, and written informed consent was obtained from all study subjects.

### Measurements

**Ventilation and UA pressure.** Subjects were advised to avoid alcohol and caffeine for at least 4 h before the experiment. They were seated in a comfortable armchair. After local anesthesia (xylocaine 2% spray) of one nostril, a pressure-tipped catheter (model CT/S X1058, Gaeltec, Hackensack, NJ) was inserted through one nare and positioned 4 to 5 cm below the soft palate to record hypopharyngeal pressure. A nasal mask (Comfort Gel Nasal Mask, Philipps Respiroics, Murrysville, PA) was then placed over the nose with the catheter passing through a drilled hole. Occlusion of the mask opening during maximal inspiratory effort served to assess its air-tightness. Nasal pressure was measured continuously (MP45-18-871,  $\pm 5$  cmH<sub>2</sub>O, Validyne, Northridge, CA). The breathing circuit connected to the mask consisted of a pneumotachograph (model 112467-3850A, Hans Rudolf, Kansas City, MO) attached to an unidirectional three-way valve (model 1400, Hans Rudolf) whose inspiratory side could be switched from room air to 5% CO<sub>2</sub>-95% O<sub>2</sub> or 7% CO<sub>2</sub>-93% O<sub>2</sub> gas mixtures. A CO<sub>2</sub> analyzer (Ametek CD3A, Thermo Instruments Division, Pittsburgh, PA) was connected to the three-way valve expiratory port to measure end-tidal CO<sub>2</sub>. During the experiment, the study subjects were instructed to breathe exclusively through the nose. Tidal volume (Vt) was obtained by integration of the instantaneous airflow signal. The Vt/inspiratory time ratio (Vt/Ti) was then calculated. Isoflow pharyngeal resistance (0.3 l/s) was computed by referencing pharyngeal pressure to mask pressure. Respiratory parameters were acquired on a computer with Axoscope 10 and Digidata 1322 software at a 2-KHz sampling rate (Axon Instrument, Foster City, CA).

**Electromyography.** Surface recordings of LCW/diaphragm EMG were obtained with surface skin-taped Ag/AgCl disc electrodes (model FH-E5SCHC, Grass Technologies, West Warwick, RI) fixed with conductive paste. For the LCW/diaphragm, the active electrode was positioned over the 7th or 8th right intercostal space between the costochondral junction and the midclavicular line. The reference electrode was placed slightly laterally on the next higher rib (8). A ground Ag/AgCl disc electrode (model FH-E5SCHC, Grass Technologies) was positioned on the lower part of the sternum. The genioglossus EMG signal was recorded by intra-oral bipolar electrodes mounted on a mouthpiece. The electrodes were positioned on the bottom of the mouthpiece, lying in contact with the superior surface of the genioglossus, just behind the teeth, as described previously (11). Briefly, a plastic composite (TAK Systems, Wareham, MA), softened in boiling water, was molded on the lower teeth and bottom of the mouth. After achieving good fit and adequate rigidity, two Teflon-coated stainless steel wires (diameter: 0.0013 in. coated, 0.0010 in. bare) were sewn through the mouthpiece and placed 6–10 mm apart on the bottom surface of the mouthpiece, parallel to and on either side of the midline. The 10 mm of wires in contact with the mouth floor were bared and their tips were buried in the plastic material. Impedance of the transcutaneous electrodes was less than 5  $\Omega$ ; impedance of the intra-oral electrodes was not measured systematically.

EMG signals were amplified and filtered at 10–3,000 Hz (P122, Grass Technologies). EMG was recorded at a 10-kHz sampling rate with Axoscope 10 and Digidata 1320 software on another computer.

### TMS Procedure

TMS was delivered with an electromagnetic stimulator (Magstim 200, Whiteland, Dyfed, UK) equipped with a non-focal, 110-mm double cone coil (model P/N 9902-00) positioned at or close to the vertex. It was oriented in the anterior-posterior direction. Its optimal location was determined by identifying the stimulation site providing the highest amplitudes of genioglossus motor-evoked potentials

(MEP) at the lowest stimulator output intensity. The same stimulation site was targeted to evoke LCW/diaphragm MEP. The coil shape was drawn on the skull, and the same investigator held the coil in place to ascertain constant coil-positioning throughout the experiment. Genioglossus and LCW/diaphragm expiratory motor thresholds (MT) were defined as the lowest stimulation intensity level able to produce at least 3 MEP with 50–100  $\mu$ V amplitude (peak to peak) in six consecutive stimulations (3) applied at end-expiration according to instantaneous airflow tracing. We determined only expiratory motor threshold because this one is considered as the relaxed motor threshold according to the guidelines of the International Federation of Clinical Neurophysiology (30). This choice was justified by the fact that inspiratory twitches were applied very early during inspiration (magnetic twitches occurred when a 1 cmH<sub>2</sub>O subatmospheric pharyngeal pressure level was developed). Therefore, genioglossus and LWC/diaphragm activities at the time twitches were applied were dramatically lower than the 20% of maximal isometric voluntary strength that is the standard level of muscle activity used to determine active motor threshold (10, 36).

To ensure that stimulations occurred in the same mechanical conditions during inspiration and expiration, the stimulator was automatically triggered by the same driving pressure (33), with a custom-built electronic device evoked by changes in pharyngeal pressure direction. For each stimulation, we visually checked that its artifact occurred at the same pressure level.

Electromyographic responses to magnetic stimulations were quantified as follows: 1) MEP latency was considered as the time elapsed between the stimulation artifact and MEP onset and 2) MEP amplitude was measured peak to peak. The reported MEP characteristics are the average of 5 to 10 stimulations for a given subject and condition.

### Study Design

Figure 1 illustrates the study design. For any given subject, the experiment took 3 h to 3 h 30 min to be completed. TMS was performed while the subjects breathed three different gas mixtures during exclusive nose breathing: 1) room air, 2) 5% CO<sub>2</sub>-95% O<sub>2</sub>, and 3) 7% CO<sub>2</sub>-93% O<sub>2</sub>. The first two gas mixtures (1 and 2) were chosen in random order. Ten-minute acclimatization to the breathing circuit was allowed before beginning TMS in room air or 5% CO<sub>2</sub>-95% O<sub>2</sub> conditions. For each of these two respiratory conditions, genioglossus and diaphragm responses to TMS were assessed separately in random order. For each muscle, MT were measured first, and then 5 to 10 stimulations were delivered at 1.3 $\times$  MT intensity, both in early inspiration and end-expiration. A random inter-stimulus interval of several breathing cycles was maintained between twitches.

For subjects who were first allocated to the 5% CO<sub>2</sub>-95% O<sub>2</sub> gas mixture, a 40-min period of room air breathing was respected before deploying TMS with room air.

To prevent early experimentation failure attributable to intolerance, the third condition (7% CO<sub>2</sub>-93% O<sub>2</sub> gas mixture) was always implemented at the end of the protocol, and the acclimatization period was reduced to 5 min. In the latter condition, the following procedure was conducted: TMS was initially applied at the same intensity as in the 5% CO<sub>2</sub>-95% O<sub>2</sub> gas mixture condition. Then, if the study subjects were able to continue the experiment, LCW/diaphragm and genioglossus MT were measured, as described elsewhere. Finally, TMS was applied at 1.3 $\times$  MT while subjects continued to breathe the 7% CO<sub>2</sub>-93% O<sub>2</sub> gas mixture.

### Data and Statistical Analysis

Semi-automated software was designed for MEP averaging, rectification as well as visual estimation of MEP latencies, amplitudes and areas (JMP 9, SAS Institute, Cary, NC).

The ventilatory data reported in RESULTS are the averaged values of 1 min of recording before beginning TMS.

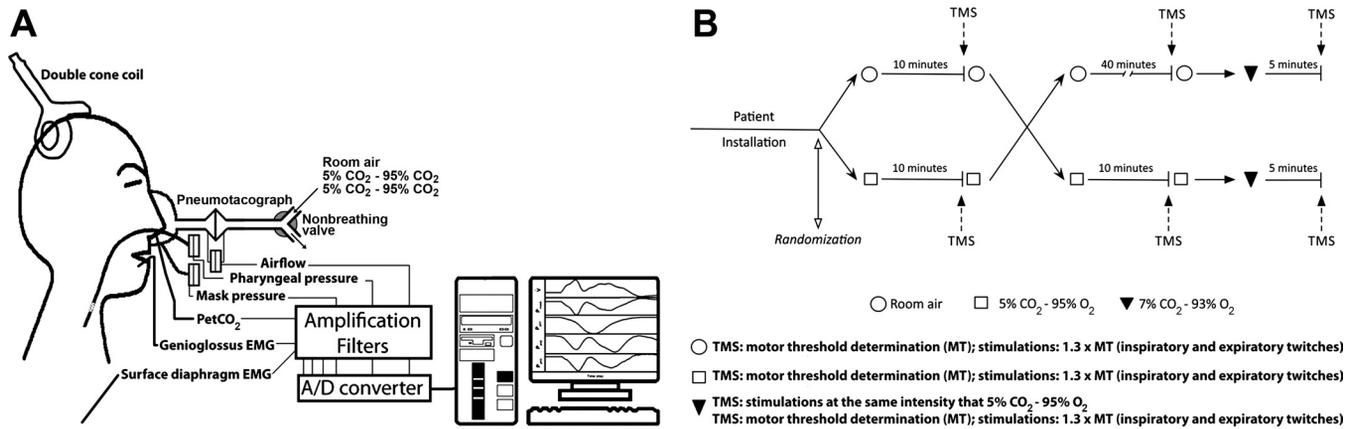


Fig. 1. Study design. TMS, transcranial magnetic stimulation; MT: motor thresholds.

The data are presented as means  $\pm$  SD in the tables. All analyses were performed with SigmaPlot 11.0 software (Systat Software, Chicago, IL). Changes in ventilatory data and electromyographic responses to TMS (MT, MEP latencies, and amplitudes) under different gas mixture breathing conditions were assessed by repeated-measures ANOVA. All pair-wise comparisons of the different conditions were made with the Tukey's test. Logarithmic transformation of variables was performed if distribution was skewed. The significance level was set to 5% for all comparisons.

The protocol time for the subjects exposed to room air initially was much shorter compared with the individuals initially exposed to 5% CO<sub>2</sub>. To verify whether such unbalance could alter the results under air or 5% CO<sub>2</sub> conditions, we performed a two-way repeated-measures ANOVA (one way = respiratory condition; one way = randomization group) on motor thresholds and MEP amplitudes. The results of the 7% CO<sub>2</sub> trial were not entered into these analyses because this condition was not completed in random order but systematically at the end of the protocol.

## RESULTS

Mean age of the study subjects was  $32 \pm 9$  yr, and their mean body mass index was  $24 \pm 3$  kg/m<sup>2</sup>. None of them was taking any medication; they did not report snoring and did not complain of any symptoms suggestive of sleep-related breathing disorders or respiratory diseases.

### Tidal Ventilation Features

As expected, CO<sub>2</sub>-induced hyperventilation augmented minute ventilation, tidal volume, maximal inspiratory flow,

and Vt/Ti ratio. Peak pharyngeal pressure decreased with increasing CO<sub>2</sub> level. In contrast, respiratory rate, inspiratory time, and pharyngeal resistance did not change significantly (Table 1).

### Responses to TMS

As presented in Table 2, inspiratory and expiratory twitches were triggered at the same pharyngeal pressure values regardless of respiratory condition (room air, 5% CO<sub>2</sub>-95% O<sub>2</sub> and 7% CO<sub>2</sub>-93% O<sub>2</sub> gas mixtures). Table 3 reports the point within a given phase at which stimulation was administered (% of inspiration or % of expiration). When F<sub>I</sub>CO<sub>2</sub> increased, stimulation occurred earlier in the inspiration phase and later in the expiration phase.

LCW/diaphragm and genioglossus MT were assessed in every subject (no missing data) under room air and 5% F<sub>I</sub>CO<sub>2</sub>, with stimulations being delivered 30% above these respective thresholds. During the 7% F<sub>I</sub>CO<sub>2</sub> trial, TMS of the genioglossus and LCW/diaphragm was undertaken at the same stimulator output intensities as subjects breathing 5% F<sub>I</sub>CO<sub>2</sub> for each specific muscle (i.e., stimulation intensity = 1.3  $\times$  MT of these muscles, as assessed during the 5% F<sub>I</sub>CO<sub>2</sub> trial). In the 7% CO<sub>2</sub> condition, LCW/diaphragm MT were measured in every subject, and genioglossus MT were quantified in all but one subject. However, only five subjects tolerated a longer hypercapnic hyperventilation trial at this F<sub>I</sub>CO<sub>2</sub> level; thus only five complete data sets were obtained at the intensity of 1.3  $\times$  MT

Table 1. Ventilatory features

	AIR	5% CO <sub>2</sub> -95% O <sub>2</sub>	7% CO <sub>2</sub> -93% O <sub>2</sub>	P Value of rm-ANOVA
Ve, l/min	6.8 $\pm$ 1.2 $\ddagger$	14.8 $\pm$ 3.5	22.6 $\pm$ 6.9*	<0.001
Vt, liter	0.5 $\pm$ 0.1 $\ddagger$	1.1 $\pm$ 0.3* $\ddagger$	1.6 $\pm$ 0.6* $\ddagger$	<0.001
Vi-max, l/min	0.4 $\pm$ 0.07 $\ddagger$	0.7 $\pm$ 0.2* $\ddagger$	1.1 $\pm$ 0.3* $\ddagger$	<0.001
Vt/Ti, l/s	0.28 $\pm$ 0.06 $\ddagger$	0.54 $\pm$ 0.16* $\ddagger$	0.80 $\pm$ 0.28* $\ddagger$	<0.001
Ti, s	2.0 $\pm$ 0.5	2.0 $\pm$ 0.5	2.1 $\pm$ 0.6	ns
Respiratory rate, breaths/min	13 $\pm$ 3	15 $\pm$ 3	15 $\pm$ 5	ns
Pphar-peak, cmH <sub>2</sub> O	-2.1 $\pm$ 0.6 $\ddagger$	-4.8 $\pm$ 1.1* $\ddagger$	-7.6 $\pm$ 2.8* $\ddagger$	<0.001
UA-R, cmH <sub>2</sub> O $\cdot$ s <sup>-1</sup>	2.0 $\pm$ 1.5	1.8 $\pm$ 1.5	1.7 $\pm$ 1.5	ns
PetCO <sub>2</sub> , kPa	5.6 $\pm$ 0.3 $\ddagger$	6.2 $\pm$ 0.2* $\ddagger$	7.0 $\pm$ 0.3* $\ddagger$	<0.001

Values are means  $\pm$  SD. Ve, minute ventilation; Vt, tidal volume; Vi-max, maximal inspiratory flow; Ti, inspiratory time; Pphar-peak, value of pharyngeal pressure at peak inspiration; UA-R, upper airway resistance measured at 300 ml/s of inspiratory flow. PetCO<sub>2</sub>, end-tidal CO<sub>2</sub> pressure. P value: results of comparisons in changes between the 3 conditions (repeated-measures ANOVA): \*, $\ddagger$ , $\ddagger$ Significant difference between pairwise comparisons (Tukey's test); \*significantly different from Air;  $\ddagger$ significantly different from 5% CO<sub>2</sub>;  $\ddagger$ significantly different from 7% CO<sub>2</sub>. Example: Ve in air condition is significantly different from Ve at 7% CO<sub>2</sub>.

Table 2. *Inspiratory and expiratory pharyngeal pressures triggering the TMS twitches in each condition (room air, 5% FiCO<sub>2</sub>, 7% FiCO<sub>2</sub>)*

Pharyngeal Pressure, cmH <sub>2</sub> O	Inspiration				Expiration			
	Room Air	5% CO <sub>2</sub> -95% O <sub>2</sub>	7% CO <sub>2</sub> -93% O <sub>2</sub>	P Value	Room Air	5% CO <sub>2</sub> -95% O <sub>2</sub>	7% CO <sub>2</sub> -93% O <sub>2</sub>	P Value
LCW/diaphragm	-0.88 ± 0.08	-0.92 ± 0.07	-0.88 ± 0.07	0.12	0.81 ± 0.07	0.79 ± 0.13	0.83 ± 0.26	ns
Genioglossus	-0.84 ± 0.08	-0.99 ± 0.07	-0.92 ± 0.33	0.36	0.83 ± 0.09	0.84 ± 0.14	0.85 ± 0.24	ns

Data are reported as means ± SD. LCW, lower chest wall.

at 7% FiCO<sub>2</sub>. Consequently, the results of MEP amplitudes and latencies reported below are those recorded at the same intensity while subjects breathed 5% FiCO<sub>2</sub>. Neither motor thresholds nor MEP amplitudes were significantly affected by the randomization order (i.e., respiratory condition × randomization group interaction nonsignificant).

### MT

The average value of expiratory MT at room air was 65 ± 8% (95% CI: 59.5 to 70.5%) of maximal stimulator output for the LCW/diaphragm and 38 ± 6% (95% CI: 34.2 to 42.4%) for the genioglossus. Regardless of respiratory condition (room air, 5% CO<sub>2</sub> or 7% CO<sub>2</sub>), expiratory LCW/diaphragm MT were always significantly higher than expiratory genioglossus MT. Compared with room air, expiratory LCW/diaphragm MT decreased with increasing FiCO<sub>2</sub> (Fig. 2A). The mean decline in expiratory LCW/diaphragm MT was -9.6 ± 10.1% (95% CI: -16.9; -2.3) at 7% FiCO<sub>2</sub>. In contrast, expiratory genioglossus MT grew with a mean of +7.2 ± 9% (95% CI: 0.2; 14.1) between room air and 7% FiCO<sub>2</sub> (Fig. 2B).

### LCW/Diaphragm MEP

FiCO<sub>2</sub> elevation increased inspiratory MEP LCW/diaphragm amplitudes (Fig. 3A) and MEP areas (Table 3). In contrast, expiratory MEP amplitudes remained unaltered (Fig. 3A). Inspiratory and expiratory LCW/diaphragm MEP latencies did not change significantly between the three conditions (room air, 5% FiCO<sub>2</sub>, 7% FiCO<sub>2</sub>; Fig. 3B). For the five subjects in whom TMS of LCW/diaphragm was also undertaken at an intensity of 1.3 × 7% FiCO<sub>2</sub> MT, the mean inspiratory LCW/diaphragm MEP amplitude was 1.3 ± 0.5 mV. This result was not significantly different from the one obtained with 7% FiCO<sub>2</sub> and using a stimulation intensity of 1.3 × 5% FiCO<sub>2</sub> MT (1.5 ± 0.8 mV).

### Genioglossus MEP

Neither genioglossus amplitudes nor latencies were significantly modified with increasing FiCO<sub>2</sub> (Fig. 3, C and D). The raw records in Fig. 4 illustrate the changes in MEP amplitude

for the LCW/diaphragm and genioglossus during early inspiration.

For the subjects in whom TMS of genioglossus was also undertaken at the intensity of 1.3 × 7% FiCO<sub>2</sub> MT, the mean inspiratory genioglossus MEP amplitude was 0.24 ± 0.12 mV and did not differ from that obtained at 7% FiCO<sub>2</sub> using the intensity that was employed at 5% FiCO<sub>2</sub> (0.20 ± 0.14 mV).

### DISCUSSION

This study assessed the influence of hypercapnia-induced increases in respiratory drive on UA dilator muscle and LCW/diaphragm corticomotor responses to TMS in awake, healthy subjects. We demonstrated that the augmented respiratory drive facilitated LCW/diaphragm corticomotor responses by decreasing MT and elevating inspiratory MEP amplitudes. On the other hand, under the same conditions, genioglossus MT increased and MEP latencies and amplitudes were unchanged.

### Methodological Considerations

Twitch-related LCW/diaphragm EMG was recorded by surface electrodes, making it impossible to exclude cross-talk signals from adjacent muscles. LCW/diaphragm MEP are likely to be contaminated by signals from intercostal muscles, serratus anterior, and/or abdominal muscles, particularly when the cerebral cortex is stimulated with a non-focal coil. We acknowledge this risk of contamination. Nevertheless, electrode placement respected the positions described by Demoule et al. (8) and was the same as in earlier experiments investigating diaphragm responses to non-focal TMS (5, 23, 36, 38).

Another issue relates to study design. As opposed to room air and 5% CO<sub>2</sub>-95% O<sub>2</sub> conditions, the 7% CO<sub>2</sub>-93% O<sub>2</sub> trial was not randomized and was always performed at the end. This decision was justified by the fact that, for a given condition (room air or 5% CO<sub>2</sub>-95% O<sub>2</sub>), measuring specific LCW/diaphragm and genioglossus MT lasted ~15 min or more, and TMS at each 1.3× MT required an equivalent amount of time. Taking this total duration into account and considering the impact of muscle fatigue on TMS responses (41, 42), we chose not to introduce such bias in the 7% CO<sub>2</sub> experiment and to

Table 3. *Percentage of respiratory time at which stimulation was administered (for genioglossus or LCW/diaphragm) according to the breathing condition*

	Inspiratory phase				Expiratory phase			
	Room air	5% CO <sub>2</sub> -95% O <sub>2</sub>	7% CO <sub>2</sub> -93% O <sub>2</sub>	P Value	Room Air	5% CO <sub>2</sub> -95% O <sub>2</sub>	7% CO <sub>2</sub> -93% O <sub>2</sub>	P Value
Genioglossus	10.6 ± 6.0†‡	6.3 ± 2.0*‡	2.0 ± 0.9*†	P < 0.01	56 ± 21†‡	80 ± 17*	98 ± 3‡	P < 0.01
LCW/diaphragm	12.0 ± 6.0†‡	6.1 ± 2.0*‡	2.3 ± 1.6*†	P < 0.01	53 ± 17†‡	85 ± 16*	94 ± 7‡	P < 0.01

Data are reported as means ± SD in % of cycle. P value: results of comparisons in changes between the 3 conditions (repeated-measures ANOVA); \*, †, ‡ significant difference between pairwise comparisons (Tukey's test); \*air; †5% CO<sub>2</sub>; ‡7% CO<sub>2</sub>. Inspiratory and expiratory phase duration were determined according to the flow recording.

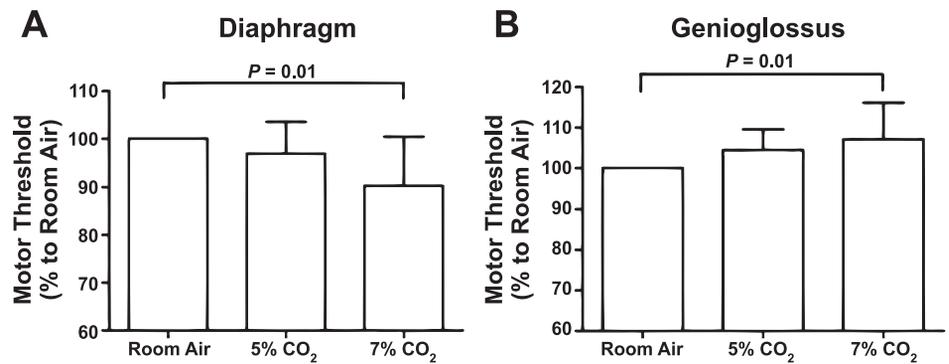


Fig. 2. Lower chest wall (LCW)/diaphragm and genioglossus expiratory MT measured in different breathing conditions (room air, 5% CO<sub>2</sub>, 7% CO<sub>2</sub>). Genioglossus MT at 7% F<sub>I</sub>CO<sub>2</sub> were recorded in only 9 subjects.

complete the last step of the experiment in an uncontrolled condition.

Finally, MT at 7% CO<sub>2</sub>-93% O<sub>2</sub> were quantified after the completion of stimulation at the same intensity as with the 5% CO<sub>2</sub>-95% O<sub>2</sub> gas mixture. As the time required to measure MT was largely variable, it was impossible to know a priori whether the subjects could or could not complete the 7% CO<sub>2</sub> experiment without experiencing respiratory muscle fatigue, especially in the context of exclusive nose breathing. LCW/diaphragm MT decreased and genioglossus MT increased with 7% F<sub>I</sub>CO<sub>2</sub>. Considering that the MEP amplitudes and latencies reported here with 7% F<sub>I</sub>CO<sub>2</sub> were obtained with the same stimulator output intensities as those with 5% F<sub>I</sub>CO<sub>2</sub>, stimulation intensities at 7% F<sub>I</sub>CO<sub>2</sub> could actually be above 1.3× MT for the LCW/diaphragm and below 1.3× MT for the genioglossus.

#### LCW/Diaphragm Corticomotor Pathway Facilitation Under Hypercapnic Stimulation

An important factor that could flaw interpretation of the present study is the ability of TMS to depict the relative

contribution of voluntary and automatic control in the LCW/diaphragm and UA muscle activation pathway. Twitch-related MEP reflects the effective neural output delivered by the motoneurons to the muscles. These motoneurons could be depolarized through different pathways. One is the volitional drive that acts mainly via a direct corticospinal pathway, which is not required during hypercapnic-induced hyperventilation. Therefore, such volitional stimulus could not account for the difference between genioglossus and LCW/diaphragm responses to cortical magnetic stimulation under hypercapnic stimulation. On the other hand, the drive coming from the medullary respiratory centers is increased during hypercapnia. The difference between genioglossus and LCW/diaphragm responses to cortical magnetic stimulation observed in the present study may be related to a different impact of hypercapnia on medullary respiratory centers and hypoglossus nucleus that modulate the non-volitional activity of the LCW/diaphragm and the genioglossus.

One could also question the possible impact of type II error in interpretation of the present results. The percent increase in LCW/diaphragm MEP amplitude between room air and 7%

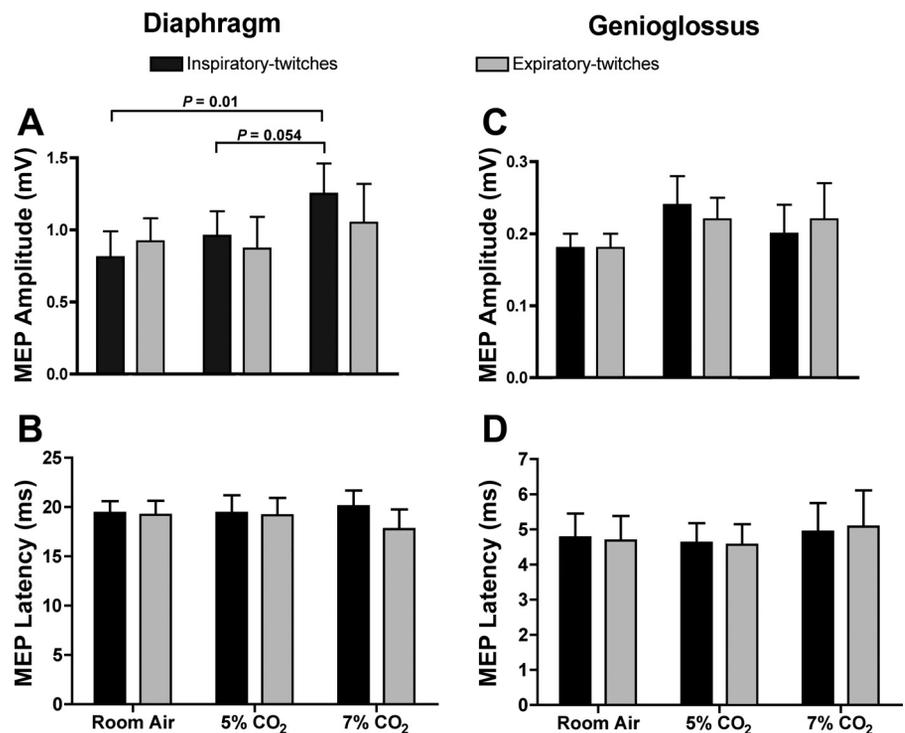


Fig. 3. LCW/diaphragm, genioglossus motor evoked potential (MEP) recorded in different breathing conditions (room air, 5% CO<sub>2</sub>, 7% CO<sub>2</sub>). For room air and 5% F<sub>I</sub>CO<sub>2</sub> conditions, LCW/diaphragm and genioglossus MEP were analyzed in response to twitch intensity set at 1.3× MT of each specific muscle. With 7% F<sub>I</sub>CO<sub>2</sub>, transcranial magnetic stimulation (TMS) was delivered at the same stimulator output intensities for each specific muscle as subjects breathing 5% F<sub>I</sub>CO<sub>2</sub> (see RESULTS). MEP amplitude values were log-transformed for statistical analysis.

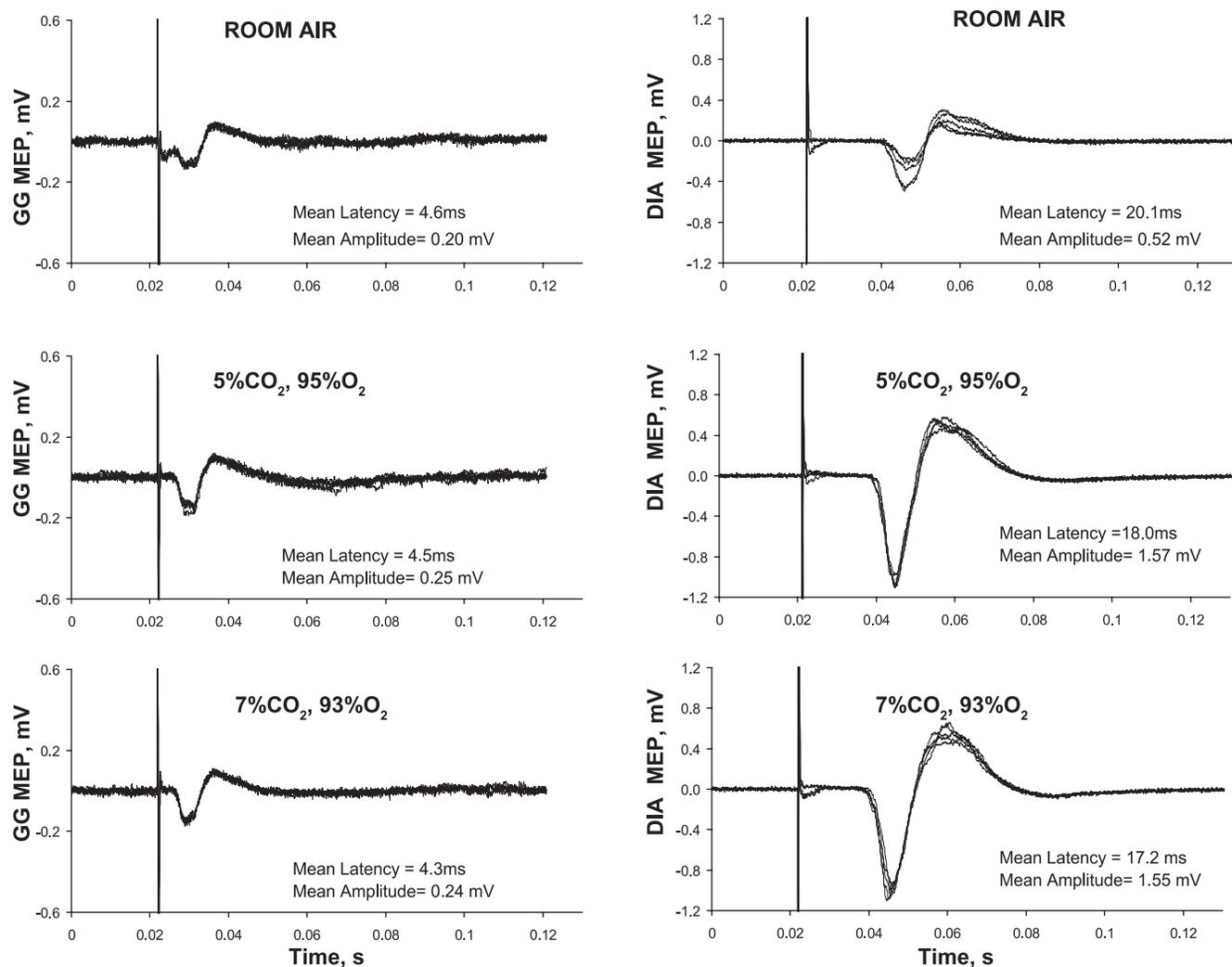


Fig. 4. Raw records illustrating changes in MEP amplitude for the LCW/diaphragm (DIA) and genioglossus (GG) during early inspiration.

$F_{iCO_2}$  was  $157 \pm 233\%$ ; in contrast, the mean increment in genioglossus MEP amplitude was  $8 \pm 51\%$ . Considering this huge difference, a sample size of 300 subjects would be necessary to show a significant rise in genioglossus MEP amplitude from room air to 7%  $F_{iCO_2}$ . Sample size was more than 10 times larger than that required to detect a difference in LCW/diaphragm responses. Even if it is virtually impossible to definitively rule out type II error, the dramatic difference between genioglossus and LCW/diaphragm corticomotor responses to hypercapnic challenge remains remarkable.

In the present study, CO<sub>2</sub>-induced LCW/diaphragm corticomotor facilitation was demonstrated by a decrease in MT and an increase in MEP amplitude. The range of LCW/diaphragm MT values was in perfect accordance with that published by Sharshar et al. (36) deploying the same coil. MT are influenced by many factors, such as sleep/awake status (2, 3) and degree of muscular activation (33). In the present work, any confounding effect, represented by the amount of prestimulation muscle activation, was controlled by performing twitches at a fixed hypo-pharyngeal pressure regardless of experimental condition (room air, 5% CO<sub>2</sub>-95% O<sub>2</sub> and 7% CO<sub>2</sub>-93% O<sub>2</sub>). Compared with our results, two studies have determined that hypocapnia increases the corticomotor excitability of peripheral muscles

(35, 37). Sparing et al. (37) showed that hypocapnia (15 Torr) was associated with a decrease in first dorsal interosseous MT and an increase in MEP amplitudes. Furthermore, compared with voluntary isocapnic hyperpnea (22), non-voluntary hypercapnic hyperpnea is not linked with an increment in primary motor cortex activation (7). Finally, employing low-intensity TMS as a means of assessing the contribution of the cortex to different respiratory tasks, Petersen et al. (28) demonstrated that corticospinal output has little influence on respiratory muscle activation during hypercapnic hyperpnea. Altogether, these results suggest that the likely origin of CO<sub>2</sub>-related LCW/diaphragm facilitation is infra-cortical.

Such a LCW/diaphragm corticomotor facilitation phenomenon is in accordance with data published by Straus et al. (38), although we did not observe a decrease in diaphragm MEP latency with the same hypercapnic paradigm. Such disparities could be linked to differences in TMS intensities between the two experiments. Straus et al. delivered cortical stimulation at maximal stimulator output intensity, which was largely higher than in the present investigation. Several studies have disclosed that TMS twitch-related MEP amplitudes and latencies are linked to stimulus intensity (24, 36): the higher the intensity, the higher the MEP amplitudes and the lower the latencies.

Table 4. Diaphragm and genioglossus baseline activity (100 ms before stimulation) and MEP areas according to the breathing condition (room air, 5% CO<sub>2</sub>, 7% CO<sub>2</sub>)

	Inspiration				Expiration			
	Room air	5% CO <sub>2</sub> -95%O <sub>2</sub>	7%CO <sub>2</sub> -93%O <sub>2</sub>	P Value	Room Air	5% CO <sub>2</sub> -95% O <sub>2</sub>	7% CO <sub>2</sub> -93% O <sub>2</sub>	P Value
LCW/diaphragm baseline activity	0.46 ± 0.23	0.43 ± 0.12	0.43 ± 0.12	ns	0.38 ± 0.12	0.39 ± 0.11	0.37 ± 0.03	ns
LCW/diaphragm MEP area	6.2 ± 5.0‡	7.7 ± 4.6	11.7 ± 7.8*	0.04	6.9 ± 3.4	6.7 ± 5.1	9.7 ± 7.8	ns
Genioglossus baseline activity	0.42 ± 0.04‡	0.44 ± 0.06	0.52 ± 0.19*	0.03	0.41 ± 0.05†‡	0.43 ± 0.05*‡	0.60 ± 0.22*†	0.02
Genioglossus MEP area	1.0 ± 0.4	1.3 ± 0.9	1.0 ± 0.8	ns	1.0 ± 0.5	1.2 ± 0.8	1.2 ± 0.8	ns

Data are reported as means ± SD in mV/ms. P value: results of comparisons in changes between the three conditions (repeated-measures ANOVA); \*, †, ‡ significant difference between pairwise comparisons (Tukey's test); \*air; †5% CO<sub>2</sub>; ‡7% CO<sub>2</sub>.

Moreover, the MEP amplitude/stimulation intensity relationship has been shown to best fit the Boltzmann sigmoid model (36), suggesting that increasing stimulation intensity above MT is associated with a huge rise in MEP amplitude.

#### Lack of Genioglossus Corticomotor Facilitation Under Hypercapnic Stimulation

Although numerous authors have established that CO<sub>2</sub> is consistently coupled with heightened genioglossus muscular activity (21, 26, 29, 31), we did not observe an increase in genioglossus corticomotor excitability under hypercapnic stimulation. While hypocapnia decreases peripheral muscle MT (37), the effects of hypercapnia on MT are less clear. Krnjevic et al. (20) demonstrated that neuronal excitability in the isolated cat cortex is depressed by 7% F<sub>I</sub>CO<sub>2</sub>. Grippo et al. (15) recently investigated corticomotor excitability of the first dorsal interosseous muscle via the TMS paradigm. They reported that the central silent period, reflecting a motor inhibitory phenomenon, is related to PaCO<sub>2</sub> in apneic patients. Their results suggest that corticomotor excitability could be altered by hypercapnia and could thus explain the increase in genioglossus MT.

#### Discrepancy Between Genioglossus and LCW/Diaphragm Corticomotor Activity Under Hypercapnic Stimulation

If we consider that hypercapnia has an homogeneous impact on the entire primary motor cortex, the mechanisms involved in the discrepancy between genioglossus and LCW/diaphragm corticomotor responses should occur at the infra-cortical level. Several studies have established that, during hypercapnic challenge, the CO<sub>2</sub>-related increase in diaphragm activity takes place earlier (i.e., at a lower CO<sub>2</sub> level) than CO<sub>2</sub>-related increments of genioglossus activity (18, 27, 29). It is noteworthy, however, that non-chemical stimuli play an important role in the quantification of genioglossus muscle activity. During hypercapnic stimulation, it is difficult to distinguish between the relative contributions of chemical (increased PaCO<sub>2</sub>) and mechanical stimuli (decreased pharyngeal pressure) to the rise in genioglossus activity (31). Akahoshi et al. (1) have shown that the mechanical component could be more potent than the chemical stimulus in heightening genioglossus activity. Their results are in accordance with earlier data from our laboratory (32), demonstrating that vigorous deep breathing, reflecting high negative pharyngeal pressure, is associated with increased genioglossus corticomotor responses. In contrast, in the present study, when genioglossus corticomotor responses were assessed at the same pharyngeal pressure regardless of hypercapnia-induced hyperventilation level, they remained unchanged.

This supports the importance of negative airway pressure feedback as a determinant of UA muscle activity and the distinct impact of changes in respiratory drive on respiratory and UA dilator muscle activity.

Two other hypotheses can be drawn to account for the difference in LCW/diaphragm and genioglossus corticomotor responses related to hypercapnia. The first one concerns central fatigue. We do not believe that this phenomenon could be involved in such difference because the study was conducted in normal subjects who do not have high baseline electromyographic activity during both wakefulness and sleep and because genioglossus histochemical characteristics make this muscle physiologically highly resistant to fatigue (34). Another hypothesis could be that vagal feedback could differentially affect these two muscles groups as it highly impacts on genioglossus activity in animals (40). However, TMS were applied during late expiration and early inspiration, making it unlikely that variation in the input of vagal afferences originating from lung mechanoreceptor interacted with genioglossus responsiveness. In fact our results can be put in parallel with those of Strauss et al. (38) who found that corticomotor responsiveness of peripheral muscles decreases during CO<sub>2</sub>-induced hyperventilation, whereas that of the diaphragm is enhanced. In the absence of data published in humans, one can speculate that hypercapnia decreases excitability in hypoglossal nerves as it does for peripheral nerves (13).

#### Conclusion

LCW/diaphragm and genioglossus corticomotor responses are affected differently by hypercapnia in awake, healthy men. This imbalance indicates that CO<sub>2</sub> may differentially influence the infra-cortical structures that govern diaphragm and UA dilator muscle activities. Such a situation may potentially promote UA instability during sleep, particularly in subjects with altered UA muscle responses to decreased airway pressure. Further studies are needed to compare the effects of CO<sub>2</sub> on UA muscle corticomotor responses in apneic patients and non-apneic subjects.

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#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

#### AUTHOR CONTRIBUTIONS

Author contributions: J.-C.B. and F.S. conception and design of research; J.-C.B. and S.G. performed experiments; J.-C.B. and S.G. analyzed data;

J.-C.B., C.A.M.-S., S.G., and F.S. interpreted results of experiments; J.-C.B. and C.A.M.-S. prepared figures; J.-C.B. drafted manuscript; J.-C.B., C.A.M.-S., S.G., and F.S. edited and revised manuscript; J.-C.B., C.A.M.-S., S.G., and F.S. approved final version of manuscript.

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