Influence of continuous inspiratory resistive breathing trials on corticospinal excitability of lower limb muscles during isometric contraction

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BACKGROUND Increased work of breathing or fatigue of respiratory muscles has been suggested to reduce exercise performance.

PURPOSE The present study investigated the effects of continuous inspiratory resistive breathing (IRB) on the responsiveness of corticospinal pathway innervating the vastus lateralis (VL) to determine whether respiratory muscle fatigue affects the central motor output to exercising limb muscles.

METHODS Eight subjects underwent a 6-min IRB trial three times (IRB1–3) in two experiments. During each IRB trial, the subjects performed voluntary hyperventilation through a mask attached with an inspiratory resistive load (approximately 30% of maximal inspiratory pressure (PImax)). In Experiment 1, PImax was measured at baseline and after each IRB trial. In Experiment 2, VL responses to transcranial magnetic stimulation (motor evoked potentials (MEPs) and cortical silent periods (CSPs)) were assessed during 5 s isometric knee extension at an intensity of 15% of the maximal voluntary contraction over the same time course as in Experiment 1.

RESULTS PImax decreased from baseline after IRB2 and IRB3 ($P < 0.05$). MEPs increased from baseline after IRB1 and IRB2 ($P < 0.05$), whereas MEPs after IRB3 was not significantly different from baseline. CSPs did not change from baseline after IRB1 and IRB2, whereas CSPs after IRB3 increased from baseline ($P < 0.05$).

CONCLUSION These results suggest that as inspiratory muscle fatigue progresses, the corticospinal tract controlling the lower limb muscles can shift from excitatory dominance to inhibitory dominance as a whole, affecting the central motor output to working limb muscles.

KEYWORDS Respiratory muscle fatigue, lower limb muscles, corticospinal excitability, transcranial magnetic stimulation

Introduction

When the work of breathing becomes excessive, such as during sustained high-intensity leg exercise, respiratory muscle fatigue occurs. This respiratory muscle fatigue has been reported to induce leg muscle fatigue, resulting in a decrease in leg muscle force and power output [1–5]. As the mechanism, it has been suggested that metabolic receptor reflexes in respiratory muscles increase sympathetic nerve activity, causing vasoconstriction in leg muscles (decrease in leg muscle blood flow) [1, 6]. In other words, the model is that peripheral fatigue (accumulation of metabolites) generated in the respiratory muscles causes peripheral fatigue (e.g., decreased oxygen supply) in the leg muscles.

On the other hand, it has been reported that peripheral fatigue that occurs in one muscle induces central fatigue in another muscle [7–9]. For example, in a study using transcranial magnetic stimulation (TMS), Sidhu et al. (2014) showed that exhaustive endurance leg cycling exercise reduced corticospinal excitability of upper limb muscles not directly involved in the exercise [8]. However, such a phenomenon has only been examined between limb muscles. Therefore, in the present study, we attempted to investigate the effects of respiratory muscle fatigue on the corticospinal tracts controlling the lower limb muscles.

Continuous inspiratory resistive breathing (IRB) has been reported to result in a decrease in maximal inspiratory pressure (PImax) and respiratory muscle fatigue-induced vasoconstriction in limb muscles [10, 11]. Accordingly, we examined whether continuous IRB alters vastus lateralis muscle response to TMS during submaximal isometric knee extension without peripheral fatigue. We hypothesized that continuous IRB would decrease motor evoked potentials (MEPs, an indicator of corticospinal excitability) and increase cortical silent periods (CSPs, an indicator of intracortical inhibition) with the progression of respiratory muscle fatigue. To ascertain whether this

change in MEPs and CSPs is affected by the magnitude of respiratory muscle activity, two breathing conditions ("unloaded breathing" and "loaded breathing") were set in this study. Furthermore, since the MEPs and CSPs assessed in the vastus lateralis might be affected by peripheral fatigue of the leg muscle [12-14], the duration and intensity of isometric knee extension were set at 5 s and 15 % of maximal voluntary contraction (MVC), respectively. Thus, the experimental design of the present study did not fully simulate sustained high-intensity leg exercise.

Methods

Participants

Eight healthy males participated in the study as paid volunteers. Their mean ± standard deviation (SD) age, height, and weight were 23.1 ± 1.6 years, 170.3 ± 3.7 cm, and 64.1 ± 4.6 kg, respectively. Prior to participating in this study, each participant was fully informed of the experimental procedures and potential risks involved, and they provided informed consent and completed a health-screening questionnaire for participation in an experiment involving TMS. They were in good physical condition and had no cardiorespiratory or neurological disorders. The participants were requested to abstain from strenuous physical activity, consuming medicines or beverages containing alcohol or caffeine for 24 h prior to each test. The study was approved by the University Institutional Research Ethics Committee and conducted according to the Declaration of Helsinki.

Experimental design

Each participant participated in two experiments, Experiment 1 (PImax test) and Experiment 2 (TMS test) (Fig. 1A). Since it was technically difficult to perform the two

tests simultaneously, the two experiments were conducted at least one day apart, and the order was randomly assigned and counterbalanced among the participants. A familiarization session was provided on a different day prior to the two experiments and the participants were familiarized with the equipment and protocol that would be used in the two experiments. In addition, resting respiratory variables (breathing frequency, 20.2 ± 4.7 breaths/min; inspired tidal volume, 590.8 ± 96.5 ml; inspired minute ventilation, 11.6 ± 1.6 l/min; partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$), 36.0 ± 2.6 mmHg) were measured (means ± SD) in the familiarization session.

In each experiment, the participants performed a 6-min continuous IRB trial three times (IRB1, IRB2, and IRB3, separated by 7-min intervals) using an inspiratory muscle-training device (Threshold IMT, Phillips Respironics, USA) connected to a breathing mask. Inspiratory resistance was set to 40 cmH_2O (maximal resistance of the device), which has been adopted in previous studies [10, 11]. During the continuous IRB trials, breathing frequency was maintained at 60 breaths/min (target time for each inhalation and exhalation of one respiratory cycle was 0.5 s) via auditory feedback from a metronome, and inspired minute ventilation was maintained at 1.8 × the resting value with the aid of visual feedback from an oscilloscope showing the target inspired minute ventilation level. Since hyperventilation-induced hypocapnia ($P_{ET}CO_2$ level < 22 mmHg) may enhance brain excitability [15, 16], external dead space (500 mL) was added to the inspiratory resistance device to avoid hypocapnia due to hyperventilation during the continuous IRB trials. As a result, $P_{ET}CO_2$ was maintained at approximately 30 mmHg, and the decrease in $P_{ET}CO_2$ from the baseline value (approximately 36 mmHg) was comparable in the three continuous IRB trials.

Experiment 1 (PImax tests)

PImax measurement is a widely accepted non-invasive method to assess inspiratory muscle strength [10, 11, 17]. In Experiment 1, PImax was measured using a computerized spirometry system (AS-507, Minato Medical Science, Japan) at four stages: at baseline (before IRB1) and from 30 s after each IRB trial (Fig. 1A). In each stage, five measurements of PImax were carried out twice (total of 10 measurements) for 7 min with a 2-min interval, and eight measured values (excluding the maximum and minimum values) were averaged and defined as PImax (< 5% coefficient of variance).

Experiment 2 (TMS tests)

In Experiment 2, TMS tests were carried out to assess the responsiveness of the corticospinal pathway to the knee extensor during submaximal isometric contractions over the same time course as that in Experiment 1 (Fig. 1A). Prior to this test, to determine maximal voluntary contraction (MVC) in right knee extension, the subjects performed a 5-s unilateral maximal isometric knee extension three times interspersed with 1-min rest. The highest force was defined as the MVC for knee extension. Following this, each subject performed a single voluntary inhalation with maximal effort (as fast as possible) through a breathing mask equipped with inspiratory resistance (40 cmH_2O). This test was repeated three times with a short rest and the maximal flow value was defined as the maximal inspiration flow (MIF).

During the baseline period and after each IRB trial in Experiment 2, a 5-s isometric knee extension of the right leg was performed 14 times (Fig. 1). A 25-s rest was inserted between each knee extension trial. To prevent peripheral fatigue of the knee extensor muscles, a low contraction intensity (15% of MVC) was adopted as the intensity for the 5-s isometric knee extension. This isometric knee extension trial (=

IKE_{15}) was carried out in two breathing conditions (unloaded-breathing condition and loaded-breathing condition). In the loaded-breathing condition, each subject performed one quick inspiration from the resting expiratory level through the breathing mask equipped with inspiratory resistance (40 cmH_2O) approximately 2 s after the start of IKE_{15}. At that time, each subject was instructed to match the inspiration flow to the target line corresponding to 150% of the resting inspired tidal volume with the aid of visual feedback from an oscilloscope. Consequently, the peak value of inspiratory flow in the loaded-breathing condition was about 80% of MIF (baseline: 80.8 ± 6.7%, after IRB1: 82.0 ± 6.5%, after IRB2: 83.1 ± 6.2%, after IRB3: 84.9 ± 7.0%, means ± SD). The peak value of inspiratory flow after IRB3 tended to be higher than the other peak values, but the difference was not statistically significant ($\chi^2 = 2.70$, P = 0.44). In the unloaded-breathing condition, each subject performed spontaneous breathing through the same breathing mask without resistance. Each breathing condition was alternately repeated 7 times. TMS was applied to the cortical representation of the right vastus lateralis during IKE_{15} performed under the designated breathing condition. Throughout this experiment, participants maintained a seated position with a knee joint angle of 90° on a custom-built chair with a backrest equipped with two padded stoppers to prevent movement of the upper body and head.

Transcranial magnetic stimulation (TMS)

TMS was delivered by a magnetic stimulator (Magstim 200^2, Magstim Co., UK) as a monophasic current waveform. A double-cone coil (each 110 mm in diameter) was centered over the vertex. The TMS-induced current in the cortex was set to flow from posterior to anterior. The coil position was adjusted to elicit the largest MEP in the right vastus lateralis during IKE_{15} using the suprathreshold intensity (45%-60% of maximum

stimulator output, and this adjusted position was defined as the optimal position. The optimal position was marked on a tight-fitting swimming cap that covered the participant's head to ensure constant positioning of the coil throughout the experiment. Subsequently, the active motor threshold intensity (~ 43% of maximum stimulator output) was determined. Active motor threshold was defined as the minimal stimulus intensity required to elicit MEPs clearly distinguishable from the background electromyogram (EMG) in 5/10 pulses during IKE_{15}. Throughout Experiment 2 (TMS tests, Fig. 1A and B), the stimulus intensity was set at 120% of the active motor threshold [18]. During the loaded-breathing condition (Fig. 1), TMS was automatically triggered when inspiration flow reached 50% of the MIF in the latter part of the breath [18], while TMS during the unloaded-breathing condition was manually delivered in the inspiratory phase by monitoring a respiratory flow signal displayed on a display screen of the respiratory gas analyzer and change in thoracic circumference due to respiration, which were measured from a piezoelectric transducer (MLT1132, ADInstruments, Australia) strapped to the chest. In the present study, MEPs were measured during contraction rather than in the relaxed muscle, as we were interested in lower limb muscle corticospinal excitability during exercise [19].

Measurements and recordings

Respiratory flow and gas exchange variables were obtained by breath-by-breath measurements with a respiratory gas analyzer (AE-280s, Minato Medical Science, Japan). The flow meter and gas analyzer were calibrated prior to each test using a standard 2-l syringe and precision reference gas values (O_2: 15.16%, CO_2: 5.06%). The knee extension force was measured using a load cell (LC1205-K500, A&D, Japan) connected to a wire and belt fixed over the right ankle joint. The signals related to both

knee extension force and respiratory flow were converted into digital signals at a sampling rate of 1 kHz using an analog-digital converter (PowerLab 16/35, ADInstruments, Australia). The knee extension force signal was processed with a low-pass filter of 40 Hz and displayed on an oscilloscope so that the participants could produce the required knee extension force (15% of MVC).

A surface EMG was recorded from the right vastus lateralis with a bipolar EMG sensor with an interelectrode distance of 20 mm (SX230, Biometrics Ltd., UK). Before attachment of the EMG sensor, the skin was shaved, abraded, and cleaned with alcohol to reduce skin impedance. The sensor was placed longitudinally over the belly of the muscle. The ground electrode was placed over the styloid process of the left wrist. The raw EMG signals were amplified using an amplifier embedded in the EMG sensor (bandwidth = 20-450 Hz; common mode rejection ratio > 96 dB; input impedance > $10^{13}\Omega$; gain = 1,000) and converted into digital signals at a sampling rate of 2 kHz.

Respiratory effort sensation (RES) was evaluated using the modified Borg scale (12 points, 0-10) [20]. Participants were asked to rate RES by pointing to the Borg scale at baseline (before IRB1) and immediately after each IRB trial in the two experiments.

Data processing

Data were analyzed using off-line analysis software (LabChart 8, ADInstruments, Australia). The peak-to-peak amplitudes of 7 MEPs were calculated in each breathing condition. The MEP that was farthest from the median value was excluded from further data analysis [18]. The background EMG signal was full-wave-rectified, and the mean EMG corresponding to the MEP after outliers were excluded was calculated from a 100-ms window prior to TMS delivery. The background force was defined as the mean knee extension force averaged over the same period and number as those of the

background EMG. Since the evoked potentials induced during muscle contraction could be influenced by background muscle activity, MEPs were normalized to background EMG for further analysis [13]. Normalized MEPs were calculated as the ratio of the MEP amplitude to the background EMG and presented as arbitrary units (AUs). The duration of the CSPs was visually determined and calculated as the interval from the time of TMS onset to the reappearance of continuous voluntary EMG during IKE_{15} [21–23].

Statistical analysis

The SPSS (v20) statistical package was used for data analysis. Normality of the data was checked using the Shapiro-Wilk test. One-way repeated-measures analysis of variance (ANOVA) with a Dunnett post-hoc comparison to the baseline value was performed to examine the effect of the IRB trial on PImax. Two-way repeated-measures ANOVA was used to compare changes in RES, inspired tidal volume and inspired minute ventilation during the IRB trials between the two experiments and among the three IRB trials. In the case of significant results, a Bonferroni post-hoc test was performed for multiple comparisons. For breathing frequency during the IRB trials, a Friedman test was used to examine the effect of the IRB trial, and a Wilcoxon signed-rank test was used to evaluate the difference between the two experiments. Background EMG, background force, MEPs and CSPs were examined for the effects of the IRB trial and the breathing condition using two-way repeated-measures ANOVA. If a main effect was observed in each factor, a paired *t*-test was performed to compare between the two breathing conditions and a Dunnett post-hoc test was performed to compare between the baseline value and after IRB trial values. The magnitudes of the changes in variables were expressed as the standardized effect size (ES, Cohen's d for a paired *t*-test and a

Dunnett post-hoc test, r for a Wilcoxon signed-rank test). The effect size can be classified as small ($0.2 < d < 0.5$, $0.1 < r < 0.3$), medium ($0.5 < d < 0.8$, $0.3 < r < 0.5$), and large ($d > 0.8$, $r > 0.5$) [24]. All data are reported as means ± SD. Statistical significance was set at $P < 0.05$.

Results

The respiratory variables during IRB trials in Experiments 1 and 2 are shown in Table 1. The Friedman test and the Wilcoxon signed-rank test did not show a main effect of the IRB trial ($\chi^2 = 4.69$, $P = 0.10$ in Experiments 1, $\chi^2 = 0.74$, $P = 0.69$ in Experiments 2) or a significant difference between the two experiments ($P = 0.40$, $r = 0.34$ in IRB1, $P = 0.44$, $r = 0.30$ in IRB2, $P = 0.40$, $r = 0.34$ in IRB3) in breathing frequency. For inspired tidal volume and inspired minute ventilation, two-way repeated-measures ANOVA showed no significant interaction between the factor of the breathing condition and the factor of the IRB trial (inspired tidal volume: $F = 3.21$, $P = 0.07$, inspired minute ventilation: $F = 0.26$, $P = 0.78$). There were no significant differences in inspired tidal volume ($F = 0.08$, $P = 0.79$) and inspired minute ventilation ($F < 0.001$, $P = 0.98$) between the two experiments, and no main effect of the IRB trial was found for these variables (inspired tidal volume: $F = 0.68$, $P = 0.52$, inspired minute ventilation: $F = 1.61$, $P = 0.23$). In Experiments 1 and 2, the values of inspired tidal volume during IRB trials were 196 ± 9.2% and 196 ± 10.0% of the resting value, respectively. There was no significant difference in RES between the two experiments ($F = 0.01$, $P = 0.92$, Table 2). RES immediately after each IRB trial was significantly higher than the baseline value ($P < 0.001$, $d > 4.00$ in both experiments). RES in IRB3 was significantly higher than that in IRB1 ($P = 0.048$, $d = 0.98$ in Experiment 1 and $d = 0.40$ in Experiment 2).

In Experiment 1, there was a significant main effect of the IRB trial on PImax ($F = 8.21$, $P < 0.001$). As shown in Fig. 2, PImax was significantly decreased after IRB2 (110.1 ± 10.4 cmH_2O, $P = 0.002$, $d = 1.31$) and after IRB3 (108.4 ± 17.4 cmH_2O, $P < 0.001$, $d = 1.10$) from the baseline value (125.3 ± 12.7 cmH_2O). PImax for all but one of the eight participants showed a maximum value at baseline (n = 5) or after IRB1 (n = 2) and a minimum value after IRB2 (n = 2) or IRB3 (n = 5). For reasons unknown, the other participant had the lowest PImax at baseline. When data for that exceptional participant were excluded, the decrease in PImax from baseline to after IRB3 was significantly correlated with the increase in RES from baseline to after IRB3 ($P = 0.03$, Pearson's $r = 0.80$; Fig. 3).

In Experiment 2, there was no significant interaction between the factor of the breathing condition and the factor of the IRB trial in any variables (background EMG: $F = 0.74$, $P = 0.54$, force: $F = 1.19$, $P = 0.34$, MEPs: $F = 0.44$, $P = 0.72$, CSPs: $F = 0.08$, $P = 0.97$). There was no significant main effect of the IRB trial or the breathing condition on background EMG ($F = 0.27$, $P = 0.62$) and force ($F = 0.49$, $P = 0.51$). As shown in Fig. 4, there was no significant difference between the unloaded-breathing condition and loaded-breathing condition in MEPs ($F = 0.01$, $P = 0.94$) and CSPs ($F = 3.80$, $P = 0.09$). However, there were significant main effects of the IRB trial on MEPs ($F = 3.76$, $P = 0.03$) and CSPs ($F = 6.13$, $P = 0.004$). MEPs were significantly increased after IRB1 (19.7 ± 5.5 in the unloaded-breathing condition, 18.1 ± 6.4 in the loaded-breathing condition, $P = 0.03$, $d = 0.58$) and IRB2 (18.8 ± 4.7 in the unloaded-breathing condition, 19.7 ± 9.2 in the loaded-breathing condition, $P = 0.02$, $d = 0.61$) from baseline value (15.6 ± 3.8 in the unloaded breathing condition, 16.6 ± 6.0 in the loaded-breathing condition) but not after IRB3 (18.0 ± 3.5 in the unloaded-breathing condition, 18.3 ± 8.6 in the loaded-breathing condition, $P = 0.14$, $d = 0.42$). The duration of CSPs was

significantly increased after IRB3 (107.6 ± 14.7 ms in the unloaded-breathing condition, 111.5 ± 21.2 ms in the loaded-breathing condition, P = 0.001, d = 0.72) compared to the baseline duration (94.8 ± 14.5 ms in the unloaded-breathing condition, 99.5 ± 20.5 ms in the loaded-breathing condition) but not after IRB1 (98.9 ± 20.0 ms in the unloaded-breathing condition, 103.6 ± 25.8 ms in the loaded-breathing condition, P = 0.38, d = 0.21) and IRB2 (101.0 ± 15.9 ms in the unloaded-breathing condition, 106.9 ± 19.9 ms in the loaded-breathing condition, P = 0.08, d = 0.40).

Discussion

The main results of the present study are as follows: (1) PImax decreased significantly from baseline after IRB2 and IRB3, (2) MEPs increased significantly from baseline after IRB1 and IRB2, whereas MEP after IRB3 was not significantly different from baseline, (3) CSPs did not change significantly from baseline after IRB1 and IRB2, whereas CSP after IRB3 was increased significantly from baseline, and (4) there was no significant difference in MEPs and CSPs between unloaded-breathing and loaded-breathing conditions. These results appear to indicate that as inspiratory muscle fatigue progresses, the corticospinal tracts controlling the vastus lateralis might have shifted from excitatory dominance to inhibitory dominance as a whole.

There were no significant differences in respiratory variables between the three IRB trials or between the two experiments (Table 1). In addition, RES values during the IRB trials did not differ between the two experiments (Table 2). These results indicate that the work done by the respiratory muscles during each IRB trial was equivalent in the two experiments. It can therefore be assumed that the decrease in PImax (Fig. 2) observed in Experiment 1 (PImax tests) was similarly induced in Experiment 2 (TMS tests). The decrease in PImax observed in Experiment 1 was significantly correlated

with the increase in RES observed in both Experiment 1 (Fig. 3) and Experiment 2 (not shown in the figure). While respiratory sensation originates from multiple neurophysiological mechanisms, RES is thought to increase depending on the intensity of the central motor drive to the respiratory muscles [25, 26]. The central motor drive to working muscles usually increases when the muscle is required to sustain a constant output to overcome fatigue. Therefore, the observed decrease in PImax associated with an increase in RES suggests that continuous IRB trials induced inspiratory muscle fatigue.

In the present study, although the lack of data on phrenic nerve stimulation-induced respiratory pressures (e.g., transdiaphragmatic pressure) makes interpretation of inspiratory muscle fatigue difficult [27], the observed correlation between PImax and RES does not rule out the possibility of changes at the muscular level and perceptual process. An inspiratory loading protocol can activate respiratory afferents, including C-fibers and group III/IV muscle afferents, and intensify dyspnea (i.e., sense of breathing effort) without a decrease in transdiaphragmatic pressure [28]. As such, even though it is unclear whether transdiaphragmatic pressure was reduced, continuous IRB trials might have activated respiratory afferents along with an increase in RES. The fact that RES in IRB3 was significantly higher than that in IRB1 is considered to indicate that the degree of respiratory muscle fatigue was greater in IRB3 than in IRB1 and IRB2.

MEPs after IRB1 and IRB2 were significantly higher than MEP at baseline, but MEP in IRB3 showed no significant difference from the baseline value. This implies that while corticospinal excitability of lower limb muscles increased with the progression of inspiratory muscle fatigue, the excitability might have begun to be suppressed after respiratory muscle fatigue reached a certain level. In parallel with the change in MEPs, CSP was increased significantly from baseline after IRB3. Therefore,

it is considered that the effect of continuous IRB on MEPs and CSPs depends on the degree of respiratory muscle fatigue.

The reduction in PImax in the present study was comparable to the reduction found in previous studies [10, 11] in which arm muscle sympathetic nerve activity during leg-cycling exercise increased with progression of respiratory muscle fatigue. This respiratory muscle fatigue-induced sympathetic reflex, which is termed 'respiratory muscle metaboreflex', is thought to arise from an increased group III/IV phrenic afferent discharge [6, 29]. Although not measured in the present study, human studies have indicated that inspiratory resistive loading causes accumulation of lactate in respiratory muscles [30, 31]. Therefore, it is possible that the decrease in PImax observed in the present study was accompanied by an increase in group III/IV phrenic afferent activity. It has been demonstrated that fatiguing lower limb exercise decreased MEPs in an upper limb muscle performing submaximal contraction at a low intensity (20% of MVC) and that this decrease in MEPs was mediated by group III/IV lower limb muscle afferent feedback [8]. Therefore, it is likely that the phenomenon of MEPs that had increased after IRB1 and IRB2 returning to baseline after IRB3 might have been due to the activation of respiratory muscle afferents associated with the progression of inspiratory muscle fatigue. This suggests that like in the case of limb muscle fatigue, respiratory muscle fatigue can decrease excitability of the corticospinal pathway innervating other remote limb muscles. Furthermore, it has been demonstrated that CSPs evaluated in the exercising muscle increased during fatiguing exercise with the same muscle [12, 32] and that such an increase in CSPs was prevented by blocking the central projection of the limb muscle group III/IV afferents [12, 33]. An increase in CSPs is considered to reflect an increase in intracortical inhibition mediated by activation of the GABA system [34, 35]. Therefore, the increase in CSPs observed after

IRB3 in the present study indicates the possibility that the activation of respiratory muscle group III/IV afferents by respiratory muscle fatigue activates the GABA system within the motor cortex neurons innervating the limb muscle. A recent study has shown that activation of the GABA system is involved in an increase in muscle sympathetic activity and pressor responses to skeletal muscle metaboreflex activation in humans [36]. Therefore, the increase in CSPs observed in the present study indicates that activation of respiratory muscle group III/IV afferents with respiratory muscle fatigue may have an inhibitory effect not only on the circulation control systems (e.g., a decrease in blood flow to limb muscles [2, 3]) but also on the motor control systems (i.e., motor cortex neurons innervating limb muscles).

In the present study, MEPs increased significantly from baseline after IRB1 and IRB2. Sidhu et al. (2014) have showed group III/IV-mediated leg afferent feedback inhibits the responsiveness of the corticospinal projection (i.e., MEP) to a remote muscle (unexercised arm muscle) in the presence of leg fatigue but that the afferent feedback to the remote muscle might act as an excitatory signal in the absence of leg fatigue [8]. Therefore, in IRB1 and IRB2, respiratory muscle fatigue may not have been sufficient to stimulate the respiratory muscle group III/IV afferents. Another possible reason for the observed increase in MEPs may be related to activation of breathing-associated cortical motor areas. This activation during voluntary breathing has been suggested to facilitate neighboring cortical areas controlling the non-respiratory muscles such as muscles in the fingers [15, 37] and legs [18]. Breathing under respiratory muscle fatigue is expected to increase central respiratory drive to maintain a required respiratory muscle output like that during voluntary breathing. In the present study, cortical motor areas associated with voluntary breathing must have been activated during each continuous IRB trial, which would have acted to increase MEPs after each

trial including IRB3. This action would facilitate limb exercise, but after IRB3, it might have been offset by the effect of respiratory afferent feedback described above.

Although we had predicted that MEPs would be higher in the loaded-breathing condition than in the unloaded-breathing condition because breathing during TMS was performed voluntarily in the former condition, there was no difference in MEPs between the two conditions. In the loaded-breathing condition, since TMS was delivered in the latter part of voluntary inspiration (80% of MIF, see Fig. 1), lung volume at which TMS was delivered during the loaded-breathing condition must have been greater than that during the unloaded-breathing condition. Pulmonary respiratory afferents associated with increases in lung volume have been shown to attenuate the monosynaptic reflex evoked from limb muscles [38] and abolish locomotion [39] in anesthetized animals. Therefore, it is likely that in the loaded-breathing condition, increase in respiratory muscle activity enhanced inhibitory feedback from the above-mentioned afferents, resulting in an offset of the increase in vastus lateralis MEPs that would be induced via the activation of voluntary breathing-associated cortical motor areas.

Limitations

We recognize small sample size (n = 8) as one of the study's limitations. Although significant differences were found in the main analyses (PImax, MEPs, CSPs), the effect sizes were not necessarily large (medium). As such, the results of the present study would need to be validated in a larger study.

In the present study, MEPs could not be normalized by maximal M-wave (Mmax). This must be acknowledged as a major weakness of the present study. As the exercise task in this study was a brief (5 s) low-intensity (15% MVC) extension,

peripheral fatigue is unlikely to have occurred and it is assumed that there was no significant change in Mmax of VL, but this would need to be confirmed directly.

TMS-induced MEPs consist of both spinal and cortical processes. Thus, the results of the present study reflect changes in the overall excitability of the corticospinal tracts innervating the knee extensor muscles. Likewise, CSPs include processes in the spinal cord as well as in the cortex. Takahashi et al. (2011) reported that short interval intracortical inhibition was reduced in the non-fatigue upper limb muscles during and after fatiguing lower limb exercise [9]. Therefore, we cannot rule out the possibility that the increase in CSPs observed in the present study is due to an increase in inhibition at the spinal cord level rather than intracortical inhibition. Indeed, CSPs of up to 150 ms have been suggested to be spinal origin [40]. In the present study, CSPs after IRB3 were significantly greater than that before IRB1, while these CSPs were within 150 ms (Fig. 4). Although it is outside the scope of the purpose of the present study, it will be necessary to measure indices such as short interval intracortical inhibition or cervicomedullary motor evoked potential to distinguish cortex involvement and spinal cord involvement.

Application to real-world exercise

In the present study, vastus lateralis responses to TMS were assessed at 15% MVC. It has been suggested that corticospinal excitability of the upper limb muscles recorded during voluntary contractions is modulated by lower limb fatiguing exercise and that this modulation depends on the contraction intensity of the upper limb muscle [23]. It is also known that there are task-dependent differences in corticospinal excitability with respect to locomotion and tonic motor output [19, 41]. Furthermore, muscle activity of vastus lateralis during cycling exercise at a power output equivalent to maximal oxygen

uptake has been reported to be 20-40% of MVC [42, 43]. These findings mean that the results of the present study should not be applied to high-intensity leg muscle exercise. Nevertheless, in populations prone to respiratory muscle fatigue (i.e., COPD patients and those with low physical fitness), even low- to moderate-intensity exercise is still likely to induce an inhibitory effect of inspiratory muscle fatigue on corticospinal pathway innervating leg muscles.

Conclusion

PImax and RES gradually decreased and increased, respectively, with repetition of the IRB trial. MEPs increased until after IRB2 and then showed a tendency to return to the baseline level after IRB3. CSPs increased after IRB3 compared with the baseline. These results suggest that as inspiratory muscle fatigue progresses, the corticospinal tract controlling the lower limb muscles may shift from excitatory dominance to inhibitory dominance as a whole, directly affecting the central motor output to working limb muscles. Thus, it is possible that the changes in MEPs and CSPs in the limb muscles depending on the degree of respiratory muscle fatigue exert a potentially positive or negative effect on exercise performance. A larger study is needed to validate these results.

Notes on contributors

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Figure Legends

Figure 1. A: Overview of the experimental protocol. The inspiratory resistive breathing (IRB) trial was repeated three times in both experiments. In Experiment 1, maximal inspiratory pressure (PImax) was measured at baseline (before IRB1) and after each IRB trial (vertical white arrows). Five measurements of PImax were carried out twice (total of 10 measurements) for 7 min with a 2-min interval. In Experiment 2, transcranial magnetic stimulation (TMS) was delivered at baseline and after each IRB trial under the unloaded-breathing condition (vertical gray arrows) and the loaded-breathing condition (vertical black arrows). Each breathing condition was alternately repeated 7 times. Motor evoked potentials (MEPs) and cortical silent periods (CSPs) in the right vastus lateralis were measured during approximately 5-s isometric knee extension at an intensity of 15% of maximal voluntary contraction. B: Respiratory flow, knee extension force, and vastus lateralis electromyogram (EMG) from a representative participant during a TMS test under the loaded-breathing condition in Experiment 2. The vertical dotted line indicates the stimulation point. C: raw traces of EMG evoked responses to TMS from a single representative subject under the unloaded-breathing condition and the loaded-breathing condition in Experiment 2.

Figure 2. Maximal inspiratory pressure (PImax) at baseline (before IRB1) and after each IRB trial. Data are presented as means ± SD (n =8). * $P < 0.05$, significantly different from the baseline value

Figure 3. Relationship between maximal inspiratory pressure (PImax) and respiratory effort sensation (RES). ΔPImax denotes the decrease in PImax from baseline to after IRB3. ΔRES denotes the increase in RES from baseline to after IRB3. The obtained

relationship suggests that the decrease in PImax was significantly ($r = 0.80$, $P = 0.03$) correlated with the increase in RES. Data for one participant were not included in this relationship (see text)

Figure 4. Normalized motor evoked potentials (MEPs, left panel) and cortical silent periods (CSPs, right panel) in the unloaded-breathing condition (open circles) and the loaded-breathing condition (closed circles) at baseline (before IRB1) and after each IRB trial. Data are presented as means ± SD ($n = 8$). * $P < 0.05$, significantly different from the baseline value

Table 1. Respiratory variables during inspiratory resistance breathing (IRB) trials

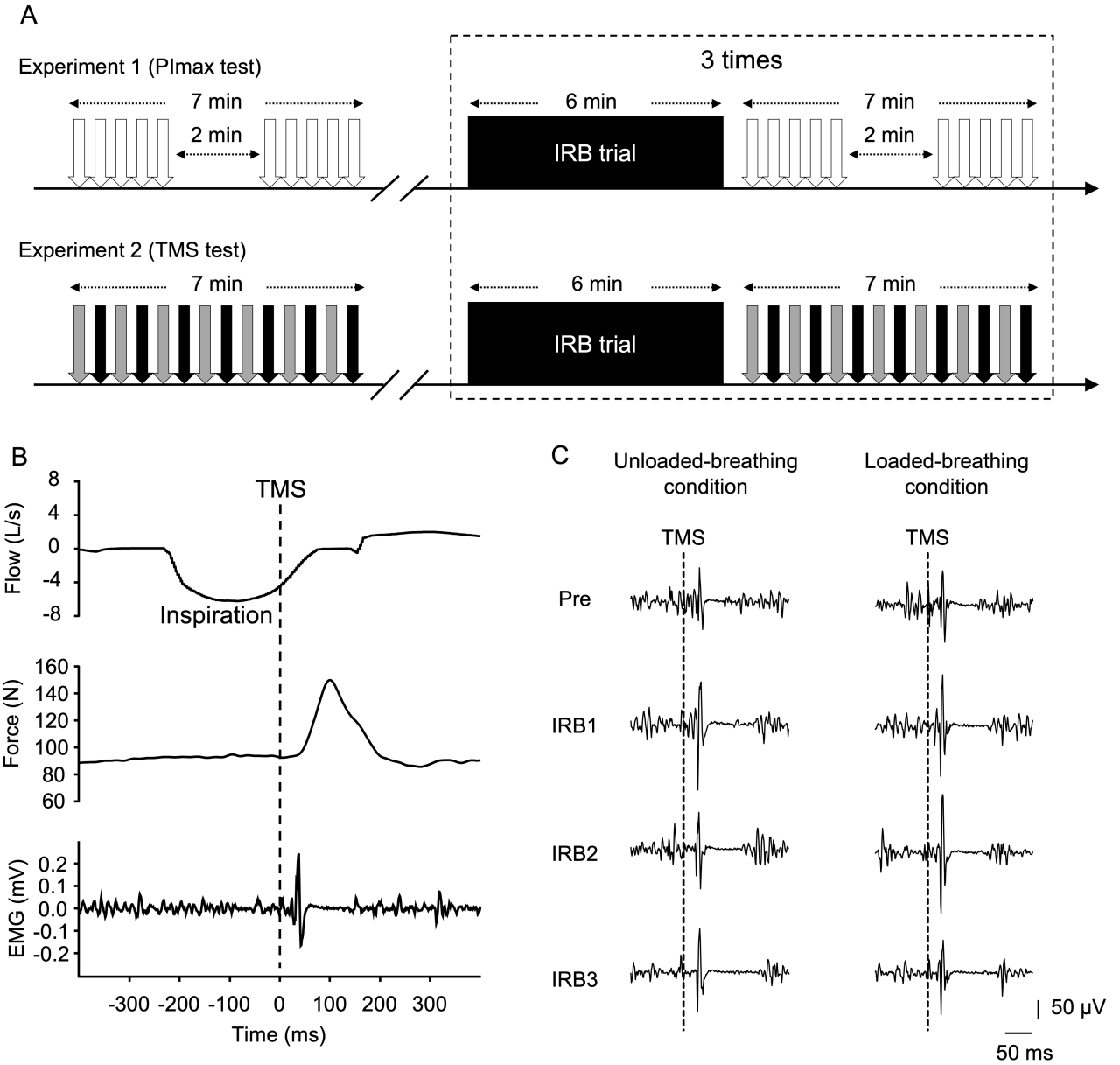
	IRB1	IRB2	IRB3
BF (breaths/min)			
Experiment 1	60.0 ± 0.7	60.0 ± 1.1	59.9 ± 0.4
Experiment 2	59.7 ± 0.7	59.9 ± 0.1	60.1 ± 0.3
TVI (ml)			
Experiment 1	1140.9 ± 146.1	1152.5 ± 134.8	1158.9 ± 138.5
Experiment 2	1155.0 ± 138.5	1168.4 ± 124.8	1140.8 ± 142.4
$\dot{V}$I (l/min)			
Experiment 1	68.4 ± 8.9	69.6 ± 8.9	68.8 ± 8.5
Experiment 2	68.7 ± 8.6	69.8 ± 7.8	68.3 ± 8.9

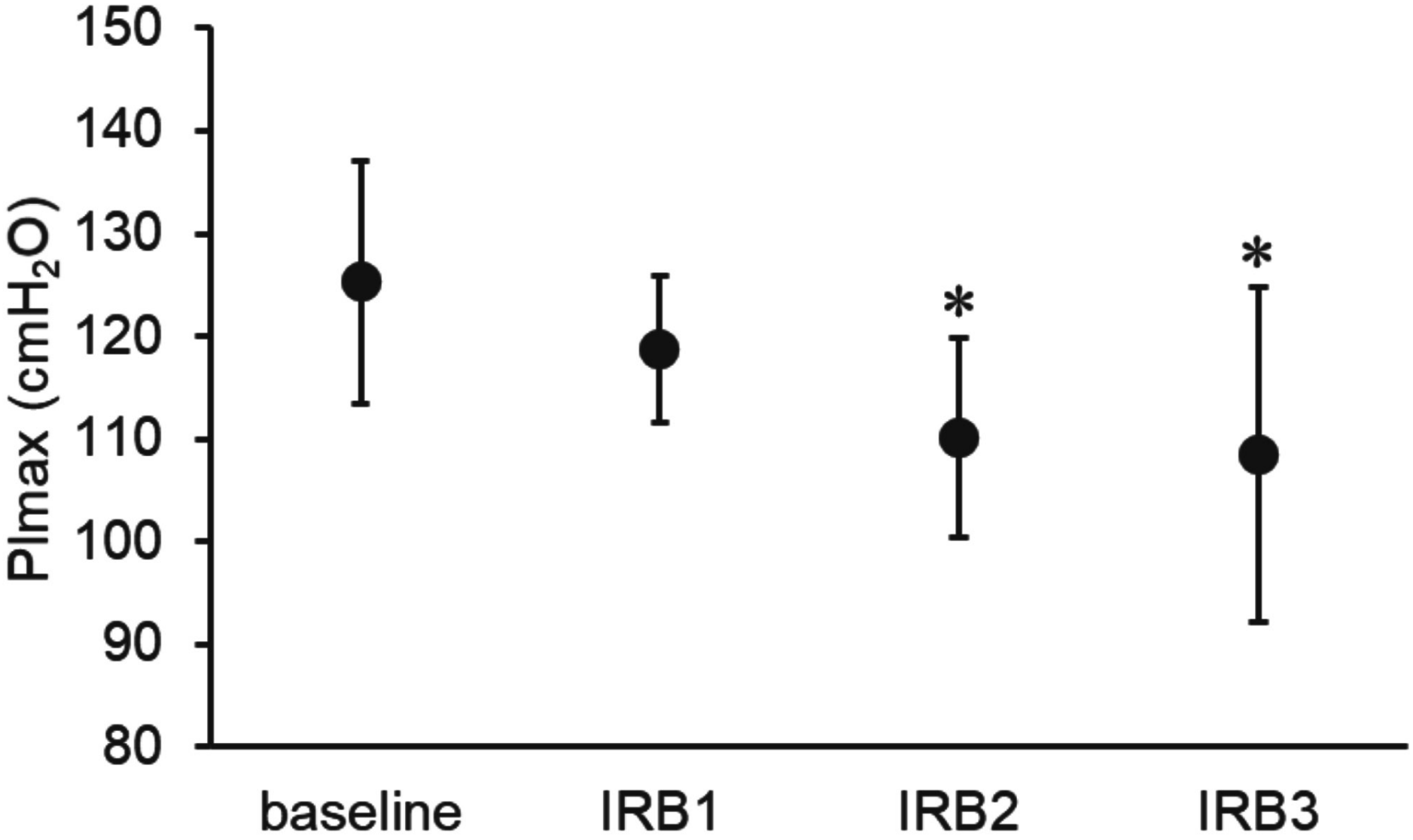
Values are mean ± SD (n = 8). BF, breathing frequency; TVI, inspired tidal volume; $\dot{V}$I, inspired minute ventilation

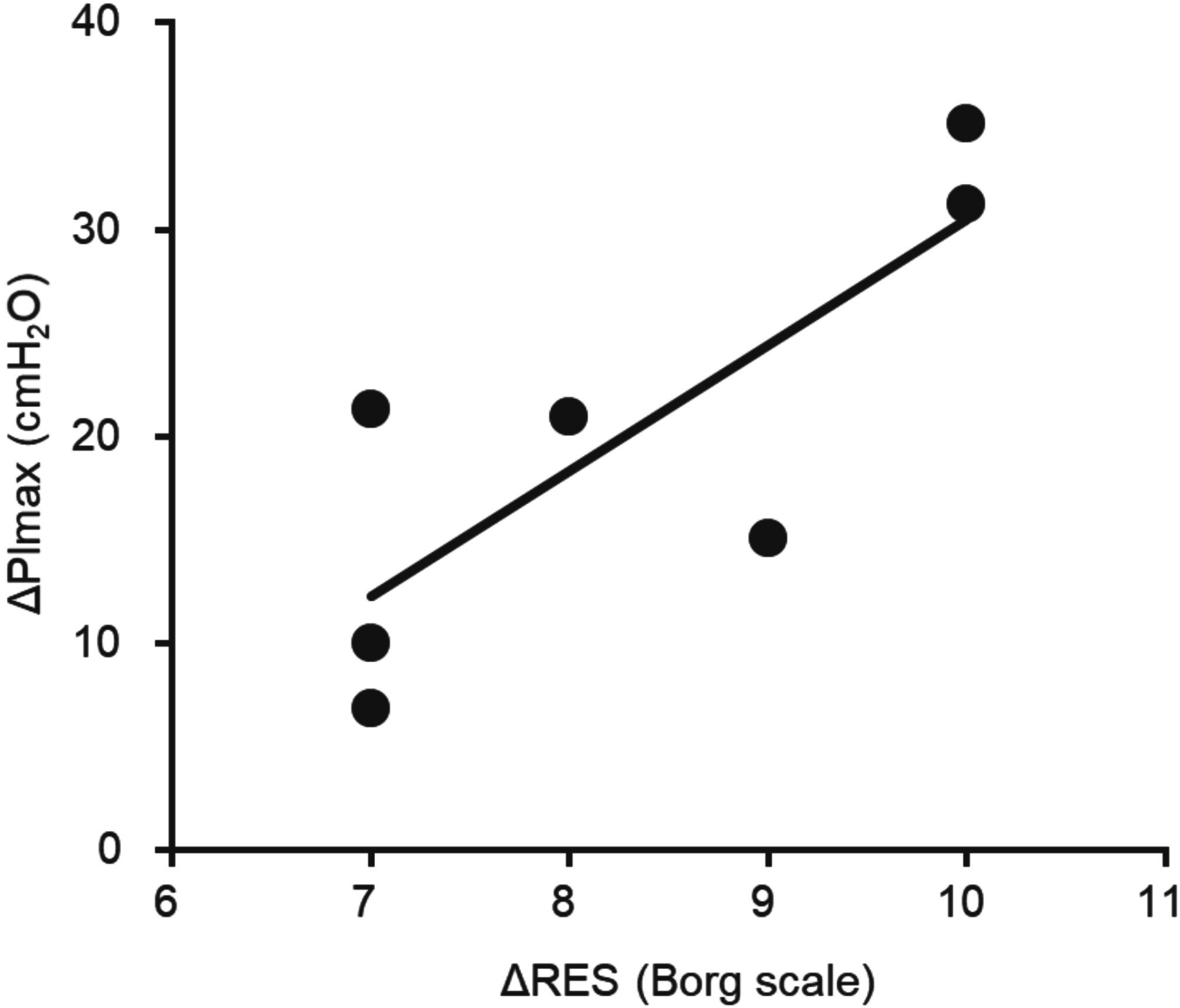
Table 2. Respiratory effort sensation (RES) before and immediately after inspiratory resistive breathing (IRB) trials

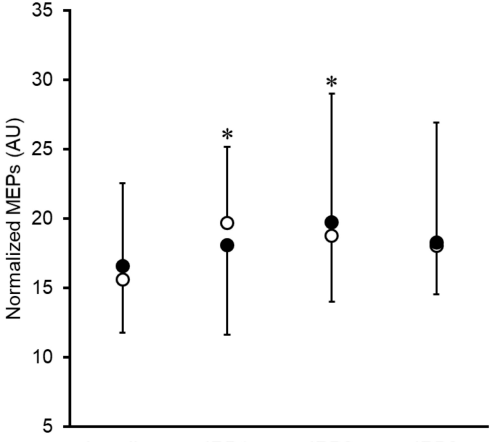
RES	Baseline	IRB1	IRB2	IRB3
Experiment 1	0.0 ± 0.0	6.9 ± 1.7 *	8.0 ± 1.5 *	8.4 ± 1.3 *$^{\#}$
Experiment 2	0.0 ± 0.0	7.4 ± 1.6 *	8.0 ± 1.5 *	8.0 ± 1.5 *$^{\#}$

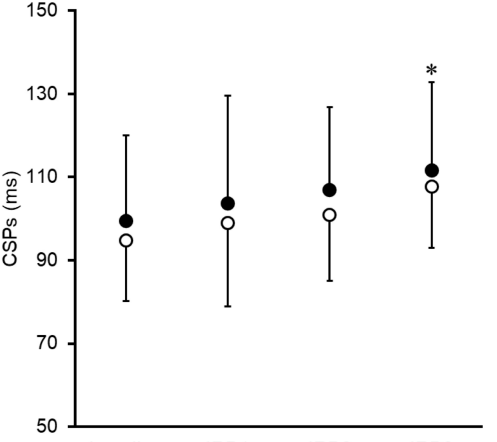
Values are mean ± SD (n = 8). * $P < 0.05$, significantly different from the baseline value. $^{\#}$ $P < 0.05$, significantly different from the IRB1


A
Experiment 1 (PImax test)
7 min
2 min
3 times
6 min
IRB trial
7 min
2 min
Experiment 2 (TMS test)
7 min
6 min
IRB trial
7 min
B
TMS
Flow (L/s)
8
4
0
-4
-8
Inspiration
Force (N)
160
140
120
100
80
60
EMG (mV)
0.2
0.1
0.0
-0.1
-0.2
-300 -200 -100 0 100 200 300
Time (ms)
C
Unloaded-breathing condition
Loaded-breathing condition
TMS
TMS
Pre
IRB1
IRB2
IRB3
50 μV
50 ms


150
140
130
120
110
100
90
80
Plmax (cmH2O)
*
*
baseline
IRB1
IRB2
IRB3


40
30
20
10
0
ΔPImax (cmH2O)
6
7
8
9
10
11
ΔRES (Borg scale)


35
30
25
20
15
10
5
Normalized MEPs (AU)
*
*
baseline
IRB1
IRB2
IRB3


150
130
110
90
70
50
CSPs (ms)
*
baseline
IRB1
IRB2
IRB3