Title page

Relationship between disturbances of CO_2 homeostasis and force output characteristics during isometric knee extension

Takahiro Yunoki[1*], Kejun Zang[1], Kei Hatano[2], Ryouta Matsuura[3], Yoshinori Ohtsuka[4]

[1] Department of Health and Physical Education, Graduate School of Education, Hokkaido University, Sapporo, Japan

[2] Japan Institute of Sports Sciences, Japan

[3] Graduate School of Education, Joetsu University of Education, Japan

[4] Department of Sports and Human Studies, Sapporo International University, Japan

[*]Corresponding author: Takahiro Yunoki

Department of Health and Physical Education, Graduate School of Education, Hokkaido University, Kita-11, Nishi-7, Kita-ku, Sapporo 060-0811, Japan

Email: yunoki@edu.hokudai.ac.jp

Abstract

To determine whether disturbances of CO_2 homeostasis alter force output characteristics of lower limb muscles, participants performed four isometric knee extension trials ($MVC_{30\%}$, 10 s each with 20-s rest intervals) in three CO_2 conditions (normocapnia [NORM], hypercapnia [HYPER], and hypocapnia [HYPO]). Respiratory frequency and tidal volume were matched between CO_2 conditions. In each $MVC_{30\%}$, the participants exerted a constant force (30% of maximum voluntary contraction [MVC]). The force coefficient of variation (Fcv) during each $MVC_{30\%}$ and MVC before and after the four $MVC_{30\%}$ trials were measured. For the means of the four trials, Fcv was significantly lower in HYPER than in HYPO. However, within HYPER, a significant positive correlation was found between the increase in end-tidal CO_2 partial pressure and the increase in Fcv. MVCs in NORM and HYPO decreased significantly over the four trials, while no such reduction was observed in HYPER. These results suggest that perturbed CO_2 homeostasis influences the force output characteristics independently of breathing pattern variables.

Keywords: Hypercapnia, Force fluctuations, Breathing interventions, Isometric contraction, Persistent inward currents.

Introduction

The ability to stabilize or maximize the force exerted by limb muscles during voluntary actions is an important motor control function that underlies the foundation of activities of daily living. Motor output from the limb muscles is generated by spinal motoneurons innervating the muscle fibers and is influenced by a variety of factors (e.g., fatigue, aging, neuromodulatory input) that modulate motor unit recruitment and rate coding (Enoka and Duchateau 2017). In addition to such factors intrinsic to limb muscles, external factors associated with respiratory muscle activity, such as voluntary breathing (David et al., 2012; Li and Yasuda, 2007; Shirakawa et al. 2015; Smith et al., 2008), resistive loaded breathing (Balzamo et al., 1997; Turner, 2003), and respiratory muscle fatigue (Dempsey, 2006), have also been suggested to influence the force generated by limb muscles.

In some studies (David et al., 2012; Smith et al., 2008), voluntary hyperpnea was compared with involuntary hyperpnea driven by CO_2 rebreathing ("CO_2-driven hyperpnea") to examine the effects of cortical motor control of breathing on motor control of limb muscles. For example, voluntary hyperpnea has been reported to result in greater displacements of the center of mass during quiet standing compared to CO_2-driven hyperpnea (David et al., 2012). This suggests that motor control of lower limb muscles

involved in postural stability may be impaired in situations in which breathing relies more on cortical motor control by the “cortical respiratory center” than on autonomic control by the brainstem respiratory centers (David et al., 2012). However, in this study (David et al., 2012), since ventilation level in voluntary hyperpnea was greater than that in CO_2-driven hyperpnea, mechanical factors such as body movement may have affected the results.

We previously compared conditions of CO_2-driven hyperpnea and voluntary hyperpnea at equivalent ventilation levels and reported that self-sustained muscle activity (SSMA) of the resting soleus muscle was higher in the former condition than in the latter condition (Hatano et al., 2022). SSMA is EMG activity that persists after continuous electrical stimulation of peripheral nerves and is one of the indirect indicators of the behavior of persistent inward currents (PICs) in spinal motoneurons (Collins et al., 2001; Mesquita et al., 2021; Nozaki et al., 2003; Trajano et al., 2014; Walton et al., 2002). PICs are activated by brainstem-derived serotonin (5-HT) input and are known to amplify the gain of motoneuron output (Heckman et al., 2003; Hultborn et al., 2004). Since increases in arterial CO_2 partial pressure ($PaCO_2$) stimulate brainstem raphe serotonergic neurons (Nattie et al., 2004; Richerson, 2004; Wang et al., 2001), it has been speculated that hypercapnia caused by CO_2 rebreathing etc. may

activate 5-HT-induced PICs in spinal motoneurons innervating lower limb muscles (Hatano et al., 2018; Hatano et al., 2022). On the other hand, hypercapnia has been shown to have no effect on maximal voluntary force and voluntary activation (VA) of the elbow flexor muscles during and after a 2-min maximal voluntary contraction (MVC) (Smith et al., 2008). This lack of hypercapnia effects may be because the contraction task was MVC and the expected breathing intervention effect was limited by the upper limit of muscle contractile capacity. Even if this is the case, the effects of hypercapnia on force generation may become apparent in a submaximal contraction task.

Therefore, the aim of the present study was to determine the effects of an increase or decrease in end-tidal CO_2 partial pressure ($P_{ET}CO_2$), a surrogate measurement for $PaCO_2$, on force output characteristics during isometric knee extension between conditions with matched ventilation levels. We hypothesized that the force fluctuations (coefficient of variation for force) during submaximal isometric contraction, a measure of force steadiness, would be greater under a hypercapnia condition than under a hypocapnia condition.

Methods

Participants

Ten healthy male participants (age, 23.9 ± 3.2 years; body mass, 79.0 ± 13.8 kg; height, 173.6 ± 6.1 cm) with no cardiorespiratory or neurological disorders participated in this study as paid volunteers. The sample size was calculated using G-power software (version 3.1.9.5). Based on a previous study (Wei et al., 2014; sample size: 7-9) showing a significant increase in the coefficient of variation (CV) of force fluctuations with paroxetine, which enhances 5-HT, and a significant decrease in the CV with cyproheptadine, which inhibits the effects of 5-HT, an effect size (f) of 0.89, an alpha level of 0.05 and, a power of 0.8 were set. Consequently, a sample size of 10 was required. All participants gave written informed consent prior to entering the study. This study was approved by the Ethical Committee of Hokkaido University Graduate School of Education (no. 19-27) and conducted according to the Declaration of Helsinki excluding registration in a database.

Experimental design

The study consisted of a preliminary experiment and a main experiment. The participants were instructed to refrain from strenuous physical activity, medication, and alcohol/caffeinated beverages for 24 hours prior to each experiment. Each experiment for

each participant was conducted at the same time of day on different days within a week. As described below, the preliminary, main, and additional experiments were conducted on different days, but the three intervention protocols (NORM, HYPER, and HYPO) in the main experiment were conducted on the same day.

Preliminary experiment

In the preliminary experiment, each participant performed familiarization with the tasks and measurements in the main experiment. Resting minute ventilation ($\dot{V}E$) and $P_{ET}CO_2$ were then measured to determine the external dead space (tube) volume and target tidal volume (V_T) for the main experiment.

Main experiment

In the main experiment, each participant sat in a custom-built chair with a backrest board with two padded stoppers to maintain the position of the upper body with the knee and hip angles positioned at 90° and 110°, respectively. First, maximum voluntary contraction (MVC) with isometric knee extension (5 s) of the right leg was measured. For each participant, MVC was measured three times at 1-min intervals, and the maximum value was used as MVCpre. The participants then participated in three

experimental protocols with different levels of $P_{ET}CO_2$ after approximately 15 min of rest (Fig. 1). Each protocol consisted of resting breathing (0 – 180 s) and voluntary breathing (180 – 490 s) periods. The voluntary breathing was performed under one of three CO_2 conditions: normocapnia (NORM), hypercapnia (HYPER), and hypocapnia (HYPO). During the voluntary breathing, participants performed isometric knee extension tasks ($MVC_{30\%}$, intensity: 30% of MVC, duration: 10 s) four times at 20-s intervals. After that, two MVC measurements were performed and the higher one was used as MVCpost. A 15-min rest interval was set between each CO_2 protocol, during which $\dot{V}E$ and $P_{ET}CO_2$ returned to baseline. The order in which the three protocols was performed was randomly balanced across participants.

Voluntary breathing and CO_2 condition

To eliminate the influences of body movement associated with breathing, breathing during the voluntary breathing period was performed as follows so that respiratory frequency (f_R) and V_T were comparable in the three CO_2 conditions. The target f_R during the voluntary breathing was 25 breaths/min in all CO_2 conditions, and participants breathed to the rhythm of a metronome. A target V_T was set for each participant so that $\dot{V}E$ was 3-4 times resting $\dot{V}E$. Participants breathed to match their

own V_T signal waveform to the V_T target line shown on the display monitor in front of them. It is difficult to maintain both breathing and force precisely at their respective target values, and prioritizing breathing may make it difficult to maintain force accurately constant and affect valid assessment of force fluctuations. Therefore, during $MVC_{30\%}$ (10 s), the V_T signal and V_T target line were blinded, and the participant was instructed to keep f_R in time with the prescribed metronome rhythm and, with respect to V_T, to continue to maintain the target value according to their subjective sense. That is, during $MVC_{30\%}$, participants only saw the force target line (30% of MVC) and their own force signal waveform shown on the display monitor.

The three CO_2 conditions were set up by adjusting the external dead space volume (CO_2 rebreathing volume): NORM condition, in which $P_{ET}CO_2$ is normal; HYPER condition, in which $P_{ET}CO_2$ is above normal; and HYPO condition, in which $P_{ET}CO_2$ is below normal.

NORM condition: 0.7 – 1.2 L of external dead space was added to the breathing mask worn by the participant so that $P_{ET}CO_2$ during voluntary breathing was as comparable as possible to $P_{ET}CO_2$ during resting breathing measured in the preliminary experiment.

HYPER condition: 1.5 L of external dead space was added to the participant's

breathing mask so that $P_{ET}CO_2$ during voluntary breathing was about 10 mmHg higher than $P_{ET}CO_2$ during resting breathing measured in the preliminary experiment.

HYPO condition: No external dead space was added so that $P_{ET}CO_2$ during voluntary breathing was about 15 mmHg lower than $P_{ET}CO_2$ during resting breathing measured in the preliminary experiment.

Measurements and data processing

Knee extension force

Knee extension force was measured using a load cell (LC1205-K500, A&D, Japan) that was connected to a wire and belt fixed over the ankle. The signal of the knee extension force was converted into digital signals at a sampling rate of 4 kHz using an analog-digital converter (PowerLab 16/35, ADInstruments, Australia). To prevent motion of the upper body and postural changes during knee extension (MVC and $MVC_{30\%}$), stoppers of the backrest were fixed on the participant's shoulders. In $MVC_{30\%}$, participants were given the following instructions: 1) not to move the head and upper body during voluntary breathing and knee extension and 2) to exert force so that the signal waveform of the self-exerted knee extension force exactly matched the force target line (30% of MVC) presented on the display monitor in front of them.

Force variability during isometric contraction originates from oscillations of the firing frequency in the motor unit and consists predominantly of low-frequency oscillations below 5 Hz (Kouzaki et al., 2004; Yoon et al., 2014; Yoshitake and Shinohara, 2013). Therefore, the force signal during $MVC_{30\%}$ was processed by an FIR low-pass filter that approximates a fourth-order Butterworth filter with a 5-Hz low-pass cut-off using LabChart's built-in software filter (ADInstruments, Australia). The magnitude of force fluctuations during each $MVC_{30\%}$ was evaluated by the coefficient of variation (CV) calculated from the mean and standard deviation of the force. A pattern of knee extension force stabilizing 2-3 s after the start of $MVC_{30\%}$ and decreasing before 10 s was observed in many trials. Therefore, in the present study, Fcv was calculated from the force during the two breaths (approximately 4.8 s) taken before the 9 s point of $MVC_{30\%}$, also considering the influence of body movements associated with breathing.

Respiratory variables

$\dot{V}E$, f_R, V_T, and $P_{ET}CO_2$ were measured by the breath-by-breath mode using a respiratory metabolism measurement system (Aeromonitor AE-310S, Minato Medical Science, Japan). Before the start of each experiment, the gas concentration sensors were calibrated

using a standard gas (O_2: 15.13%, CO_2: 5.068%). The flow sensor was also calibrated using a 2 L syringe. Respiratory data were output as averages every 10 s. Simultaneously, the respiratory flow and $P_{ET}CO_2$ during measurement were output in analog form to the AD converter (PowerLab 16/35, ADInstruments, Australia).

Additional experiment

An additional experiment was performed in 9 of the 10 participants (Fig. 1). The additional experiment followed the same protocol as that for the main experiment but without MVC and $MVC_{30\%}$, and blood pressure, transcutaneous arterial blood oxygen saturation (SpO_2) and $P_{ET}CO_2$ were measured. Mean arterial blood pressure (MAP) was measured during resting breathing (90-150 s) and voluntary breathing (330-390 s) using an automatic sphygmomanometer (TM-243, A&D, Japan). SpO_2 and $P_{ET}CO_2$ were measured during resting breathing (0-180 s) and voluntary breathing (180-400 s). SpO_2 was measured (n = 7) using a pulse oximeter (MLT321, ADInstruments, Australia). In each participant, the additional experiment was conducted at the same time of day as that in the main experiment, and the order of the CO_2 conditions was matched to the order of CO_2 conditions in the main experiment.

Statistical analysis

The normality of data was assessed using the Shapiro-Wilk test. The effects of CO_2 conditions (NORM, HYPER, HYPO) during $MVC_{30\%}$ trials (four trials) on respiratory and force variables were assessed using two-way repeated-measures analysis of variance (ANOVA). When main effects were found, the Bonferroni multiple comparisons test was used to identify significantly different pairs. If a significant interaction was indicated, one-way repeated-measures ANOVA was performed within each CO_2 condition or within each $MVC_{30\%}$ trial. A one-way repeated-measures ANOVA was also used for between-condition comparison of MVC and for comparison of respiratory and force variables averaged over four $MVC_{30\%}$ trials in each CO_2 condition. When a significant F-ratio was found, the Tukey test was used for post hoc multiple comparisons. f_R and Fcv in the main experiment and $P_{ET}CO_2$ in the additional experiment violated the assumption of normality and were therefore compared between conditions or between trials using Wilcoxon's signed-rank test with Bonferroni correction. Pearson's or Spearman's correlation coefficients (r or ρ) were used to examine correlations depending on the presence or absence of normality. All statistical analyses were performed using SPSS (v27, IBM Corporation, Armonk, NY, USA). All values are expressed as means ± standard deviation (SD). Statistical significance was defined as $p < 0.05$.

Results

Respiratory variables

Figure 2 shows respiratory variables during the four $MVC_{30\%}$ trials in each CO_2 condition. There was no significant difference in f_R between the four $MVC_{30\%}$ trials in any CO_2 condition and there was no significant difference in f_R between the three CO_2 conditions in any $MVC_{30\%}$ trial (Fig. 2A). The mean f_R of the four $MVC_{30\%}$ trials also did not differ significantly between the CO_2 conditions (Fig. 2E).

For V_T, there was no interaction between CO_2 condition and number of $MVC_{30\%}$ trials, with no main effect of number of trials but a main effect of CO_2 condition. V_T in the HYPER condition was significantly higher than the values in the NORM ($p = 0.007$) and HYPO ($p = 0.001$) conditions (Fig. 2B). There were also significant differences ($p < 0.01$) between the HYPER condition and the other two conditions in the mean V_T of the four $MVC_{30\%}$ trials (Fig. 2F).

For $P_{ET}CO_2$, there was a main effect of number of $MVC_{30\%}$ trials and a main effect of CO_2 condition, with no interaction. $P_{ET}CO_2$ in the first $MVC_{30\%}$ trial was significantly lower than the values in the second to fourth trials ($p < 0.05$, Fig. 2C). There were significant differences in $P_{ET}CO_2$ between all conditions ($p < 0.001$, Fig. 2C). There were

also significant differences ($p < 0.01$) between all conditions in mean $P_{ET}CO_2$ in the four $MVC_{30\%}$ trials (Fig. 2G).

MVC and force fluctuations

There was a significant difference in Fcv between the HYPER and HYPO conditions for the first $MVC_{30\%}$ trial ($p < 0.05$) and between the NORM and HYPO conditions for the fourth trial ($p < 0.05$, Fig. 2D). In the HYPER condition, there was a significant difference between Fcv values in the first and fourth trials ($p < 0.05$, Fig. 2D). For the mean Fcv of the four $MVC_{30\%}$ trials, there was a significant main effect of CO_2 condition, with a significant difference between HYPER and HYPO conditions ($p < 0.05$, Fig. 2H).

Regarding MVC, as shown in Figure 3, there was no significant difference in MVCpost between the three CO_2 conditions, but MVCpost values in the NORM and HYPO conditions were significantly lower than MVCpre. There was no significant difference between MVCpre and MVCpost in the HYPER condition.

Relationships between Fcv and respiratory variables

As shown in Figure 4, the three CO_2 conditions were pooled to examine the relationships

between Fcv and respiratory variables (both averaged over four trials in each CO_2 condition). Respiratory variable values were also calculated in the same two-breath interval as Fcv. The results showed no significant correlation between Fcv and fR or V_T, while a significant negative correlation was found between Fcv and $P_{ET}CO_2$ ($p < 0.05$, $\rho = -0.408$, Fig. 4).

As noted above, there was a trend for $P_{ET}CO_2$ and Fcv in the HYPER condition to increase with increasing number of $MVC_{30\%}$ trials. Therefore, when examining the relationship between changes in $P_{ET}CO_2$ and changes in Fcv (each from the first trial), a significant positive correlation was found in the HYPER condition ($p < 0.05$, $\rho = 0.416$, Fig. 5B). On the other hand, a negative correlation was found in the HYPO condition ($p < 0.05$, $r = -0.455$, Fig. 5C) and no correlation was found in the NORM condition ($p > 0.05$, $\rho = 0.166$, Fig. 5A). The same analysis was also performed between Fcv and fR or VT, but no significant correlation was found under any of the CO_2 conditions.

Additional experiment

Table 1 shows the data obtained from the additional experiment. $P_{ET}CO_2$ during voluntary breathing in each CO_2 condition was comparable to the values in the main experiment (Fig. 2) and significantly different between all conditions ($p < 0.05$). A small but

1 significant difference ($p < 0.01$) was found between the HYPER and HYPO conditions
2 for $P_{ET}CO_2$ during resting breathing.

3 There were no significant differences in SpO_2 during resting breathing between the
4 CO_2 conditions. Significant differences in SpO_2 during voluntary breathing were found
5 between the HYPER and NORM conditions ($p < 0.001$) and between the HYPER and
6 HYPO conditions ($p < 0.001$).

7 There were no significant differences in MAP during resting breathing between the
8 CO_2 conditions. Significant differences in MAP during voluntary breathing were found
9 between the HYPER and NORM conditions ($p < 0.001$) and between the HYPER and
10 HYPO conditions ($p < 0.001$).

11

12 **Discussion**

13 The present study was designed to examine the relationship between $P_{ET}CO_2$ and Fcv
14 during isometric contraction of lower limb muscles. Participants performed four $MVC_{30\%}$
15 trials (10 s each with 20-s rest intervals) in each of the CO_2 conditions. With respect to
16 the means of the four trials, Fcv was significantly lower in the HYPER condition than in
17 the HYPO condition (Fig. 2H) and there was a significant negative correlation between
18 $P_{ET}CO_2$ and Fcv (Fig. 4). These results appear to contradict our hypothesis that increased

$P_{ET}CO_2$ would increase Fcv. However, within the HYPER condition, a significant positive correlation was found between increases in $P_{ET}CO_2$ and increases in Fcv (Fig. 5B). These results suggest that the force output characteristics of lower limb muscles differ depending on the state of CO_2 homeostasis.

Effects of difference in $P_{ET}CO_2$ on Fcv - differences between CO_2 conditions-

In the present study, we attempted to match f_R and V_T across the three CO_2 conditions to curb the effects of body movement associated with breathing that might affect Fcv. As a result, f_R could be controlled, while V_T was significantly higher in the HYPER conditions than in the HYPO condition (Fig. 2). This result implies the possibility that body movement associated with breathing was greater in the HYPER condition than in the HYPO condition, but Fcv in the HYPER condition was rather significantly lower than Fcv in the HYPO condition (Fig. 2). Furthermore, no significant correlation was found between Fcv and f_R or V_T. Based on these results, it is reasonable to assume that Fcv decreased in the HYPER condition (and increased in the HYPO condition) regardless of the effects of body movement associated with breathing, and it is unlikely that the difference in Fcv between the two conditions could be explained by the difference in V_T.

In the HYPER condition, $P_{ET}CO_2$ was significantly higher than the values in the NORM and HYPO conditions (Fig. 2). It is therefore inferred that the involuntary ventilatory drive induced by stimulation of central chemoreceptors was greater in the HYPER condition than in the other conditions. On the other hand, in the HYPO condition, $P_{ET}CO_2$ was reduced by an average of 14 mmHg compared to that in the NORM condition. It is therefore likely that in the HYPO condition, the ventilatory response via central chemoreceptors was attenuated and an increase in cortex-controlled voluntary breathing was required to compensate for the attenuated involuntary ventilation in order to maintain target f_R and V_T. In other words, regarding the contributions of the brainstem and cortex as respiratory centers, the degree of reliance on the latter would have been greater in the HYPO condition than in the HYPER condition. This difference may have resulted in differences in Fcv for two possible reasons. One relates to the fact that $MVC_{30\%}$ was imposed simultaneously with voluntary controlled breathing. This is a kind of dual task in that both required precise tasking; in the HYPO condition, as noted above, cortical involvement in voluntary breathing may have been greater than that in the HYPER condition, which may have reduced the ability to precisely control knee extension force due to the dispersion of attention and motor commands (Turner, 2002). This would have resulted in a significantly higher Fcv in the HYPO condition than in the HYPER condition.

A second possibility is that in the HYPO condition, activation of cortical respiratory muscle areas facilitated adjacent cortical lower limb muscle areas. Previous studies have suggested that voluntary breathing increased cortical excitability innervating hand muscles (Li and Rymer, 2011; Ozaki and Kurata, 2015) and lower limb muscles (Shirakawa et al., 2015) muscles. Although the mechanism was not revealed in the present study, in any case, the difference in Fcv observed between the HYPER and HYPO conditions is likely to be due to differences in the degree of involvement of cortical respiratory muscle regions. The significant negative correlation between $P_{ET}CO_2$ and Fcv (Fig. 4: the lower the $P_{ET}CO_2$, the higher the Fcv) seems to support this possibility. In the HYPO condition, decreased $P_{ET}CO_2$ may have increased cortical involvement and increased Fcv.

Exercise-induced fatigue is known to decrease force steadiness (Wu et al., 2019; Enoka and Farina, 2021). Hence, it is also possible that factors related muscle fatigue might have given rise to the difference in Fcv between the HYPER and HYPO conditions. In the present study, MVCpost values in the NORM and HYPO conditions were significantly reduced compared to MVCpre, but no such reduction was observed in the HYPER condition (Fig. 3). This indicates that the degree of muscle fatigue was smaller in the HYPER condition than in the other conditions. Regarding fatigability, increased

blood pressure has been suggested to reduce muscle fatigue by increasing muscle blood flow (Wright et al., 2000). In the present study, MAP in the HYPER condition was higher than MAPs in the other conditions, and the increase in MAP in the HYPER condition was approximately 12 mmHg (14%) (Table 1). Wright et al. (2000) in their study have demonstrated that 18% of the contraction force lost through fatiguing contractions was recovered for each 10% increase in systemic blood pressure. On the other hand, it has been shown that hyperventilation-induced hypocapnia causes a decrease in cerebral blood flow and concomitant reductions in MVC force and cortical VA, and that these reductions in MVC and VA are quickly restored towards baseline values by inhalation of 5% CO_2 (Ross et al., 2012) (although an experiment in a hot environment). Taken together, the suppression of muscle fatigue associated with the increased MAP may have led to the suppression of the decrease in MVC and the increase in Fcv under the HYPER condition, while the decrease in cerebral blood flow may have led to the decrease in MVC and the increase in Fcv under the HYPO condition. Hypoxia may also affect the circulatory response, but the decrease in SpO_2 in the HYPER condition was very small (Table 1). Thus, large shifts in $P_{ET}CO_2$ may influence control of muscle force through effects on the circulatory system, even without concomitant hypoxia.

Effects of hypercapnia on Fcv

As stated above, in the present study, contrary to our hypothesis, the mean Fcv of the four $MVC_{30\%}$ trials was significantly higher in the HYPO condition than in the HYPER condition (Fig. 2H), but the difference was not consistent across trials, with error bars significantly overlapping in the 4th trial (Fig. 2D). This may be because Fcv under the HYPER condition was higher in the 4th trial than in the 1st trial. Within the HYPER condition, $P_{ET}CO_2$ also tended to increase with the number of trials, resulting in a significant positive correlation between ΔFcv and $\Delta P_{ET}CO_2$ (Fig. 5B). Such a correlation was not found in the NORM condition. This result implies that in situations where $P_{ET}CO_2$ is higher than normal, force fluctuations increase with increasing $PaCO_2$. In contrast, in the HYPO condition, given the discussion in the previous section, it is likely that Fcv decreases as the dependence of respiration on cortical motor control decreases when $P_{ET}CO_2$ increases. Consequently, a negative correlation is presumed to have been shown such that Fcv decreases with increasing $P_{ET}CO_2$ in the HYPO condition. On the other hand, in the NORM condition, there was no significant correlation between $\Delta P_{ET}CO_2$ and ΔFcv, but the range of $\Delta P_{ET}CO_2$ was so much greater despite the normocapnia condition (Fig. 5A). This large range of $\Delta P_{ET}CO_2$ values in the NORM condition may be related to the fact that the mean of $P_{ET}CO_2$ values in the 1st trial tended to be lower and the SD of

$P_{ET}CO_2$ values in the 3rd trial tended to be larger (Fig. 2C). This would be due to the limitations of the method used in the present study, that is, attempting to maintain normocapnia by regulating the external dead space to be added, rather than by automatic CO_2 addition through precise feedback control. However, despite these problems, the data obtained (Fig. 5A) implies that Fcv does not significantly change if the increase in $P_{ET}CO_2$ from normal level is up to approximately 5 mmHg.

What is the mechanism of the positive correlation between $P_{ET}CO_2$ and Fcv in the HYPER condition?

In the present study, it is not possible to elucidate the definitive mechanism by which Fcv increases with increasing $P_{ET}CO_2$ in situations where $P_{ET}CO_2$ is markedly higher than normal (e.g., the HYPER condition). However, it may be possible to speculate the involvement of 5-HT, since increases in $PaCO_2$ are assumed to stimulate the brainstem raphe serotonergic neurons (Nattie et al., 2004; Richerson, 2004; Wang et al., 2001).

Force production by limb muscles depends on motor output generated in spinal motoneurons. Spinal motoneurons have nerve projections from the brainstem (raphe nuclei) that contains serotonergic neurons. The release of 5-HT induces PICs and greatly increases the input-output excitability of spinal motoneurons (Heckman et al., 2003;

Hultborn et al., 2004). Indeed, the availability of 5-HT has been suggested to affect maximal effort contractions (Kavanagh and Taylor, 2022; Thorstensen et al., 2021). Furthermore, it has been reported that drugs that augment/block 5-HT increase/decrease force fluctuations during isometric contraction (Wei et al., 2014). These findings suggest that 5-HT is involved in force regulation and that 5-HT-induced PICs increases force fluctuations. Although 5-HT was not measured in the present study, PICs in lower limb muscles have been suggested to be enhanced by hyperpnea (Hatano et al., 2018; Hatano et al., 2022; Kirkwood et al., 2002; Kirkwood et al., 2005). Furthermore, it has been suggested that hypercapnia induced by CO_2-rebreathing increases 5-HT, albeit indirectly (Rojas Vega et al., 2003; Rojas Vega et al., 2011). In the HYPER condition, $P_{ET}CO_2$ was set higher (about +10 mmHg) than the normal level (NORM), and $PaCO_2$ would have increased progressively with the time course of CO_2 rebreathing. Therefore, in the HYPER condition, 5-HT-derived PICs may have been activated with increasing $P_{ET}CO_2$, resulting in increased Fcv (Fig. 5B).

As discussed above, HYPER seemed to suppress the decline in MVC (Fig. 3). This is inconsistent with the findings of Smith et al. (2008), who demonstrated that hypercapnia had no effect on MVC and VA of the elbow flexor muscles (Smith et al., 2008). This discrepancy is probably due to difference in contraction tasks used in the two

studies. In their study, a 2-min sustained MVC was performed, during which MVC decreased by more than 60%. It has been suggested that inhibition of spinal motoneuron excitability due to a spillover of 5-HT (Cotel et al., 2013; D'Amico et al. 2017) can occur during sustained maximal effort contractions (Kavanagh et al., 2019; Kavanagh and Taylor, 2022). Thus, in the study by Smith et al. (2008), even if raphe serotonergic neurons were activated by hypercapnia, it may have had an inhibitory effect on spinal motoneurons and force generation. On the other hand, such inhibition by 5-HT spillover does not appear to occur with prolonged low-intensity contraction (Kavanagh and Taylor, 2022; Thorstensen et al., 2020). Therefore, in the present study, it is speculated that the activation of 5-HT system by hypercapnia had an excitatory effect on spinal motoneurons, and as a result, the decrease in MVC was suppressed in the HYPER condition.

Study limitations and future directions

The results of the present study showed that the motor output characteristics differ depending on the state of CO_2 homeostasis, but only one contraction intensity (30% MVC) was examined in this study. It is therefore unclear whether our findings also apply to contractions of lower or higher intensity. Despite such a limitation, given that quadriceps muscle activity in daily activities such as stairway locomotion and chair-rise

movement is approximately 30% MVC (Hortobágyi et al., 2003), the results of the present study are at least applicable to such activities. It has been reported that muscle exertion effort in daily life is greater in the elderly than in the young as muscle mass declines with aging (Hortobágyi et al., 2003). Furthermore, reduced PICs activity has been suggested as a contributing factor to age-related muscle weakness (Hassan et al., 2021; Orssatto et al., 2021a). As put forward by Orssatto et al. (2021b), although speculative at present, breathing interventions such as those used in the present study (e.g., dead space ventilation) may lead to chronic adaptations in the serotonergic system and attenuate the age-related decline in PICs activity. However, we have no direct evidence for the release of 5-HT from the brainstem to the spinal cord. Therefore, in order to apply the findings of the present study to exercise prescription and rehabilitation, the above possibilities need to be rigorously tested by more detailed (5-HT assessment) and larger-scale (sample size, duration) studies.

Conclusion

Force variability during isometric knee extension was found to be influence by differences in $P_{ET}CO_2$ independently of breathing pattern variables, suggesting that disturbances in CO_2 homeostasis themselves affect the force output characteristics of

lower limb muscles. Furthermore, sustained above-normal increases in $P_{ET}CO_2$ can increase Fcv, and the mechanism was speculated to involve 5-HT release from the brainstem to spinal motoneurons.

Conflict of interest

The authors declare no conflict of interest.

Data Availability

Data will be made available on request.

Acknowledgments

The authors are grateful Associate Professor Yujiro Yamanaka, Hokkaido University for their technical assistance and constructive advice.

Funding

This work was supported by JSPS KAKENHI Grant Numbers JP17K01612 and JP22K11626.

Credit authorship contribution statement

Takahiro Yunoki: Study design, Experimental procedures, Data analysis, Manuscript preparation, Review of the manuscript, Funding acquisition, Project administration. **Zang Kejun**: Experimental procedures, Data analysis, Manuscript preparation, Review of the manuscript, **Kei Hatano**: Study design, Experimental procedures, Review of the manuscript, **Ryouta Matsuura**: Study design, Review of the manuscript. **Yoshinori Ohtsuka**: Study design, Project administration, Review of the manuscript.

References

Balzamo, E., Vuillon-Cacciuttolo, G., Burnet, H., Jammes, Y., 1997. Influence of respiratory afferents upon the proprioceptive reflex of skeletal muscles in healthy humans. Neurosci. Lett. 236, 127–130. https://doi.org/10.1016/S0304-3940(97)00782-9.

Collins, D.F., Burke, D., Gandevia, S.C., 2001. Large involuntary forces consistent with plateau-like behavior of human motoneurons. J. Neurosci. 21, 4059–4065. https://doi.org/10.1523/JNEUROSCI.21-11-04059.2001.

Cotel, F., Exley, R., Cragg, S.J., Perrier, J.F., 2013. Serotonin spillover onto the axon initial segment of motoneurons induces central fatigue by inhibiting action potential initiation. Proc. Natl. Acad. Sci. U.S.A. 110, 4774–4779. https://doi.org/10.1073/pnas.1216150110.

D'Amico, J.M., Butler, A.A., Heroux, M.E., Cotel, F., Perrier, J.M., Butler, J.E., Gandevia, S.C., Taylor, J.L., 2017. Human motoneurone excitability is depressed by activation of serotonin 1A receptors with buspirone. J. Physiol. 595, 1763–1773. https://doi.org/10.1113/JP273200.

David, P., Laval, D., Terrien, J., Petitjean, M., 2012. Postural control and ventilatory drive during voluntary hyperventilation and carbon dioxide rebreathing. Eur. J.

Appl. Physiol. 112, 145–154. https://doi.org/10.1007/s00421-011-1954-8.

Dempsey, J.A., Romer, L., Rodman, J., Miller, J., Smith, C., 2006. Consequences of exercise-induced respiratory muscle work. Respir. Physiol. Neurobiol. 151, 242–250. https://doi.org/10.1016/j.resp.2005.12.015.

Enoka, R.M., Duchateau, J., 2017. Rate coding and the control of muscle force. Cold Spring Harb. Perspect. Med. 7, a029702. https://doi.org/10.1101/cshperspect.a029702

Enoka, R.M., Farina, D., 2021. Force steadiness: From motor units to voluntary actions. Physiol. (Bethesda) 36, 114–130. https://doi.org/10.1152/physiol.00027.2020.

Hassan, A.S., Fajardo, M.E., Cummings, M., McPherson, L.M., Negro, F., Dewald, J.P.A., Heckman, C.J., Pearcey, G.E.P., 2021. Estimates of persistent inward currents are reduced in upper limb motor units of older adults. J. Physiol. 599, 4865-4882. https://doi.org/10.1113/JP282063.

Hatano, K., Matsuura, R., Ohtsuka, Y., Yunoki, T., 2022. Enhancement of self-sustained muscle activity through external dead space ventilation appears to be associated with hypercapnia. Respir. Physiol. Neurobiol. 295, 103777. https://doi.org/10.1016/j.resp.2021.103777.

Hatano, K., Shirakawa, K., Usuda, N., Matsuura, R., Ohtsuka, Y., Yunoki, T., 2018. Effect of hypercapnia on self-sustained muscle activity. Respir. Physiol. Neurobiol.

250, 24–30. https://doi.org/10.1016/j.resp.2018.02.002.

Heckman, C.J., Lee, R.H., Brownstone, R.M., 2003. Hyperexcitable dendrites in motoneurons and their neuromodulatory control during motor behavior. Trends. Neurosci. 26, 688–695. https://doi.org/10.1016/j.tins.2003.10.002.

Hortobágyi, T., Mizelle, C., Beam, S., DeVita, P., 2003. Old adults perform activities of daily living near their maximal capabilities. J. Gerontol. A. Biol. Med. Sci. 58, 453–460. https://doi.org/10.1093/gerona/58.5.M453.

Hultborn, H., Brownstone, R.B., Toth, T.I., Gossard, J.P., 2004. Key mechanisms for setting the input-output gain across the motoneuron pool. Prog. Brain. Res. 143, 75–95. https://doi.org/10.1016/S0079-6123(03)43008-2.

Kavanagh, J.J., McFarland, A.J., Taylor, J.L., 2019. Enhanced availability of serotonin increases activation of unfatigued muscle but exacerbates central fatigue during prolonged sustained contractions. J. Physiol. 597, 319–332. https://doi.org/10.1113/JP277148.

Kavanagh, J.J., Taylor, J.L., 2022. Voluntary activation of muscle in humans: does serotonergic neuromodulation matter? J. Physiol. 600, 3657–3670. https://doi.org/10.1113/JP282565.

Kirkwood, P., Denton, M.E., Wienecke, J., Nielsen, J., Hultborn, H., 2005.

Physiological roles for persistent inward currents in motoneurones: insights from the central respiratory drive. Biocybern. Biomed. Eng. 25, 31–38.

Kirkwood, P.A., Lawton, M., Ford, T.W., 2002. Plateau potentials in hindlimb motoneurones of female cats under anaesthesia. Exp. Brain. Res. 146, 399–403. https://doi.org/10.1007/s00221-002-1163-0.

Kouzaki, M., Shinohara, M., Masani, K., Fukunaga, T., 2004. Force fluctuations are modulated by alternate muscle activity of knee extensor synergists during low-level sustained contraction. J. Appl. Physiol. 97, 2121–2131. https://doi.org/10.1152/japplphysiol.00418.2004.

Li, S., Rymer, W.Z., 2011. Voluntary breathing influences corticospinal excitability of nonrespiratory finger muscles. J. Neurophysiol. 105, 512–521. https://doi.org/10.1152/jn.00946.2010.

Li, S., Yasuda, N., 2007. Forced ventilation increases variability of isometric finger forces. Neurosci. Lett. 412, 243–247. https://doi.org/10.1016/j.neulet.2006.11.013.

Mesquita, R.N.O., Taylor, J.L., Kirk, B., Blazevich, A.J., 2021. Involuntary sustained firing of plantar flexor motor neurones: effect of electrical stimulation parameters during tendon vibration. Eur. J. Appl. Physiol. 121, 881–891. https://doi.org/10.1007/s00421-020-04563-7.

Nattie, E.E., Li, A., Richerson, G., Lappi, D.A., 2004. Medullary serotonergic neurones and adjacent neurones that express neurokinin-1 receptors are both involved in chemoreception *in vivo*. J. Physiol. 556, 235–253. https://doi.org/10.1113/jphysiol.2003.059766.

Nozaki, D., Kawashima, N., Aramaki, Y., Akai, M., Nakazawa, K., Nakajima, Y., Yano, H., 2003. Sustained muscle contractions maintained by autonomous neuronal activity within the human spinal cord. J. Neurophysiol. 90, 2090–2097. https://doi.org/10.1152/jn.00200.2003.

Orssatto, L.B.R., Borg, D.N., Blazevich, A.J., Sakugawa, R.L., Shield, A.J., Trajano, G.S., 2021a. Intrinsic motoneuron excitability is reduced in soleus and tibialis anterior of older adults. GeroSci. 43, 2719–2735. https://doi.org/10.1007/s11357-021-00478-z.

Orssatto, L.B.R., Mesquita, R.N.O., Phillips, K.M., 2021b. Looking at the bigger PICture: understanding and counteracting the decline of persistent inward currents in older adults. J. Physiol. 599, 5137–5139. https://doi.org/10.1113/JP282370.

Ozaki, I., Kurata, K., 2015. The effects of voluntary control of respiration on the excitability of the primary motor hand area, evaluated by end-tidal CO_2 monitoring. Clin. Neurophysiol. 126, 2162–2169.

https://doi.org/10.1016/j.clinph.2014.12.032.

Richerson, G.B., 2004. Serotonergic neurons as carbon dioxide sensors that maintain ph homeostasis. Nat. Rev. Neurosci. 5, 449–461. https://doi.org/10.1038/nrn1409.

Rojas Vega, S., Hollmann, W., Strüder, H.K., 2011. Influences of exercise and training on the circulating concentration of prolactin in humans. Neuroendocrinol. 24, 395–402. https://doi.org/10.1111/j.1365-2826.2011.02266.x.

Rojas Vega, S., Strüder, H.K., Hollmann, W., 2003. Plasma prolactin concentration increases after hypercapnia acidosis. Horm. Metab. Res. 35, 598-602. https://doi.org/10.1055/s-2003-43506.

Ross, E.Z., Cotter, J.D., Wilson, L., Fan, J-L., Lucas, S.J.E., Ainslie, P.N., 2012. Cerebrovascular and corticomotor function during progressive passive hyperthermia in humans. J. Appl. Physiol. 112, 748–758. https://doi.org/10.1152/japplphysiol.00988.2011.

Shirakawa, K., Yunoki, T., Afroundeh, R., Lian, C-S., Matsuura, R., Ohtsuka, Y., Yano, T., 2015. Voluntary breathing increases corticospinal excitability of lower limb muscle during isometric contraction. Respir. Physiol. Neurobiol. 217, 40-45. https://doi.org/10.1016/j.resp.2015.07.003.

Smith, J.L., Butler, J., Martin, P.G., McBain, R.A., Taylor, J.L., 2008. Increased

ventilation does not impair maximal voluntary contractions of the elbow flexors. J. Appl. Physiol. 104, 1674–1682. https://doi.org/10.1152/japplphysiol.01358.2007.

Thorstensen, J.R., Taylor, J.L., Kavanagh, J.J., 2021. Human corticospinal-motoneuronal output is reduced with 5-HT_2 receptor antagonism. J. Neurophysiol. 125, 1279–1288. https://doi.org/10.1152/jn.00698.2020.

Thorstensen, J.R., Taylor, J.L., Tucker, M.G., Kavanagh, J.J., 2020. Enhanced serotonin availability amplifies fatigue perception and modulates the TMS-induced silent period during sustained low-intensity elbow flexions. J. Physiol. 598, 2685–2701. https://doi.org/10.1113/JP279347.

Trajano, G.S., Seitz, L.B., Nosaka, K., Blazevich, A.J., 2014. Can passive stretch inhibit motoneuron facilitation in the human plantar flexors? J. Appl. Physiol. 117, 1486–1492. https://doi.org/10.1152/japplphysiol.00809.2014.

Turner, D.L., 2002. Expiratory resistive loaded breathing in humans increases fluctuations of force production in submaximal isometric quadriceps contractions. Neurosci. Lett. 328, 13–16. https://doi.org/10.1016/S0304-3940(02)00420-2.

Walton, C., Kalmar, J.M., Cafarelli, E., 2002. Effect of caffeine on self-sustained firing in human motor units. J. Physiol. 545, 671–679. https://doi.org/10.1113/jphysiol.2002.025064.

Wang, W., Tiwari, J.K., Bradley, S.R., Zaykin, R.V., Richerson, G.B., 2001. Acidosis stimulated neurons of the medullary raphe are serotonergic. J. Neurophysiol. 85, 2224–2235. https://doi.org/10.1152/jn.2001.85.5.2224.

Wei, K., Glaser, J.I., Deng, L., Thompson, C.K., Stevenson, I.H., Wang, Q., Hornby, T.G., Heckman, C.J., Kording, K.P., 2014. Serotonin affects movement gain control in the spinal cord. J. Neurosci. 34, 12690–12700. https://doi.org/10.1523/JNEUROSCI.1855-14.2014.

Wright, J.R., McCloskey, D.I., Fitzpatrick, R.C., 2000. Effects of systemic arterial blood pressure on the contractile force of a human hand muscle. J. Appl. Physiol. 88, 1390–1396. https://doi.org/10.1152/jappl.2000.88.4.1390.

Wu, R., Delahunt, E., Ditroilo, M., Ferri Marini, C., De Vito, G., 2019. Torque steadiness and neuromuscular responses following fatiguing concentric exercise of the knee extensor and flexor muscles in young and older individuals. Exp. Gerontol. 124, 110636. https://doi.org/10.1016/j.exger.2019.110636.

Yoon, T., Vanden Noven, M.L., Nielson, K.A., Hunter, S.K., 2014. Brain areas associated with force steadiness and intensity during isometric ankle dorsiflexion in men and women. Exp. Brain. Res. 232, 3133–3145. https://doi.org/10.1007/s00221-014-3976-z.

Yoshitake, Y., Shinohara, M., 2013. Low-frequency component of rectified EMG is temporally correlated with force and instantaneous rate of force fluctuations during steady contractions. Muscle. Nerve. 47, 577-584. https://doi.org/10.1002/mus.23628.

Figure legends

Figure 1. Schematic diagram of the main experimental protocol

The main experiment was consisted of a resting breathing period and a voluntary breathing period. During the voluntary breathing period, each participant was instructed to perform tidal volume (V_T) at a respiratory frequency (f_R) of 25 breaths/min so that the minute ventilation ($\dot{V}E$) was 3-4 times resting $\dot{V}E$. The voluntary breathing was performed under one of three CO_2 conditions: normocapnia (NORM), hypercapnia (HYPER), and hypocapnia (HYPO). Each participant participated in all three CO_2 condition protocols. A 15-min rest interval was set between each protocol. Each participants performed four sets of isometric knee extension ($MVC_{30\%}$) at an intensity of 30% of the pre-determined maximal voluntary contraction (MVC). Each $MVC_{30\%}$ was held for 10 s followed by 20-s rest. After the four $MVC_{30\%}$ trials, MVC was measured twice. The four $MVC_{30\%}$ trials and two MVC measurements were performed during the voluntary breathing period. In the main experiment, respiratory and force parameters were measured. Bold horizontal arrows indicate the variables (mean arterial pressure (MAP), transcutaneous arterial blood oxygen saturation (SpO_2) and end-tidal CO_2 partial pressure ($P_{ET}CO_2$)) measured in the additional experiment. The additional

experiment followed the same protocol as that in the main experiment but without MVC and $MVC_{30\%}$.

Figure 2. Respiratory frequency (f_R, A), tidal volume (V_T, B), end-tidal CO_2 partial pressure ($P_{ET}CO_2$, C), and coefficient of variation of force (Fcv, D) in each of the four $MVC_{30\%}$ trials under each CO_2 condition (normocapnia, NORM; hypercapnia, HYPER; hypocapnia, HYPO)

The means of four trials under each CO_2 condition for f_R, V_T, $P_{ET}CO_2$, and Fcv are shown in E to H, respectively. *Different from NORM and HYPO ($p < 0.01$); $^{\#}$ different from 2nd - 4th trials ($p < 0.05$); $^{\$}$ difference between all CO_2 conditions ($p < 0.001$); $^{¥}$different from HYPER in 1st trial and NORM in 4th trial ($p < 0.05$); †different from 1st trial ($p < 0.05$); ‡difference between HYPER and HYPO conditions ($p < 0.05$).

Figure 3. Maximum voluntary contraction before (MVCpre) and after (MVCpost) the four $MVC_{30\%}$ trials in the three CO_2 conditions (normocapnia, NORM; hypercapnia, HYPER; hypocapnia, HYPO)

*Different from Pre ($p < 0.01$).

Figure 4. Relationship between end-tidal CO_2 partial pressure ($P_{ET}CO_2$) and coefficient of variation of force (Fcv)

Both variables are the average of four trials in each CO_2 condition (normocapnia, NORM; hypercapnia, HYPER; hypocapnia, HYPO).

Figure 5. Relationship between changes in end-tidal CO_2 partial pressure ($\Delta P_{ET}CO_2$) and changes in coefficient of variation of force (ΔFcv) in each CO_2 condition (normocapnia, NORM [A]; hypercapnia, HYPER [B]; hypocapnia, HYPO [C])

Both changes are the difference between the values of the 2nd, 3rd, and 4th trials and the value of the 1st trial. The horizontal and vertical dashed lines represent the respective reference (first trial).

Table 1. End-tidal CO_2 partial pressure ($P_{ET}CO_2$), transcutaneous arterial blood oxygen saturation (SpO_2), and mean arterial blood pressure (MAP) during resting breathing and voluntary breathing in the additional experiment.

		Resting breathing	Voluntary breathing
Variable (units)	CO_2 condition		
$P_{ET}CO_2$ (mmHg)	NORM	38.4 ± 2.2	37.7 ± 2.8*
	HYPER	37.9 ± 2.2#	45.9 ± 2.3*
	HYPO	38.8 ± 2.6	25.1 ± 2.0*
SpO_2 (%)	NORM	96.7 ± 0.6	97.7 ± 0.7
	HYPER	96.8 ± 0.4	93.6 ± 2.4$
	HYPO	96.9 ± 0.6	98.4 ± 0.2
MAP (mmHg)	NORM	84.1 ± 7.9	85.4 ± 7.5
	HYPER	83.7 ± 8.0	95.8 ± 11.3$
	HYPO	83.4 ± 9.3	82.4 ± 6.6

Values are mean ± SD. *Significantly different between conditions, $P < 0.05$. #Significantly different from HYPO, $P < 0.01$. $Significantly different from HYPO and NORM, $P < 0.001$.

Figure 1.

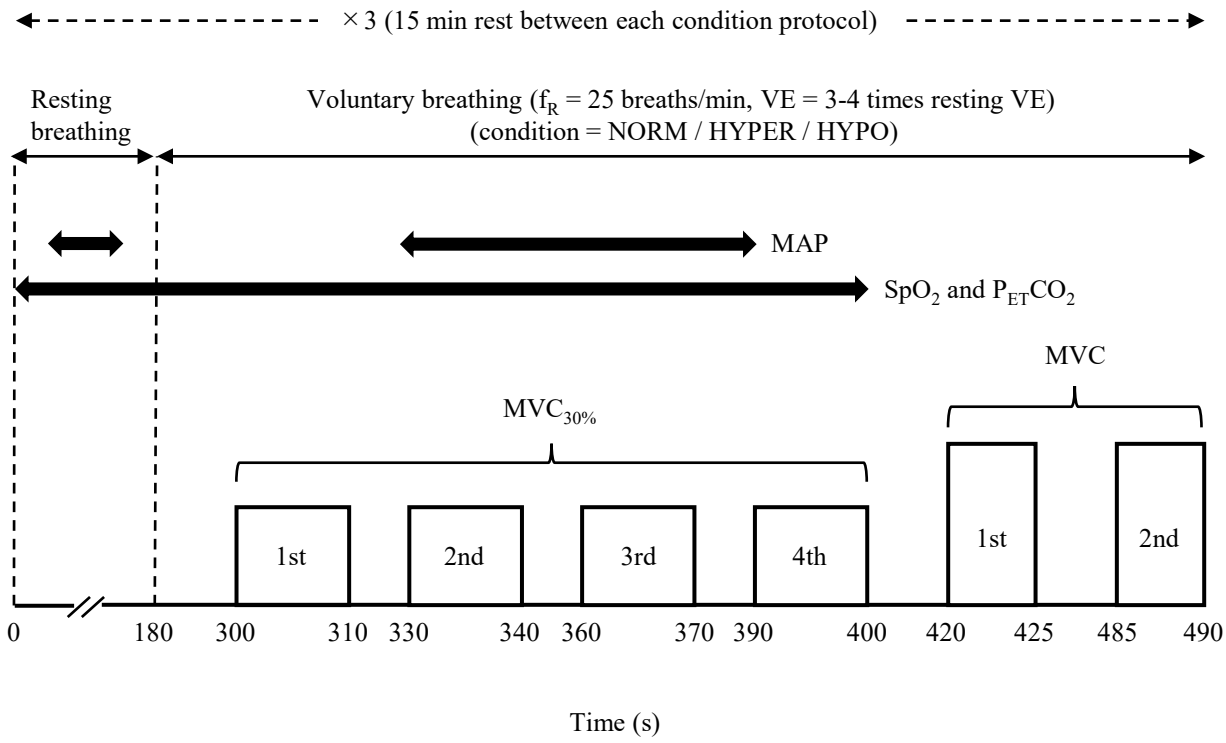

× 3 (15 min rest between each condition protocol)
Resting breathing
Voluntary breathing (f_R = 25 breaths/min, VE = 3-4 times resting VE)
(condition = NORM / HYPER / HYPO)
MAP
SpO_2 and $P_{ET}CO_2$
MVC
$MVC_{30\%}$
1st
2nd
3rd
4th
1st
2nd
0
180
300
310
330
340
360
370
390
400
420
425
485
490
Time (s)

Figure 2.

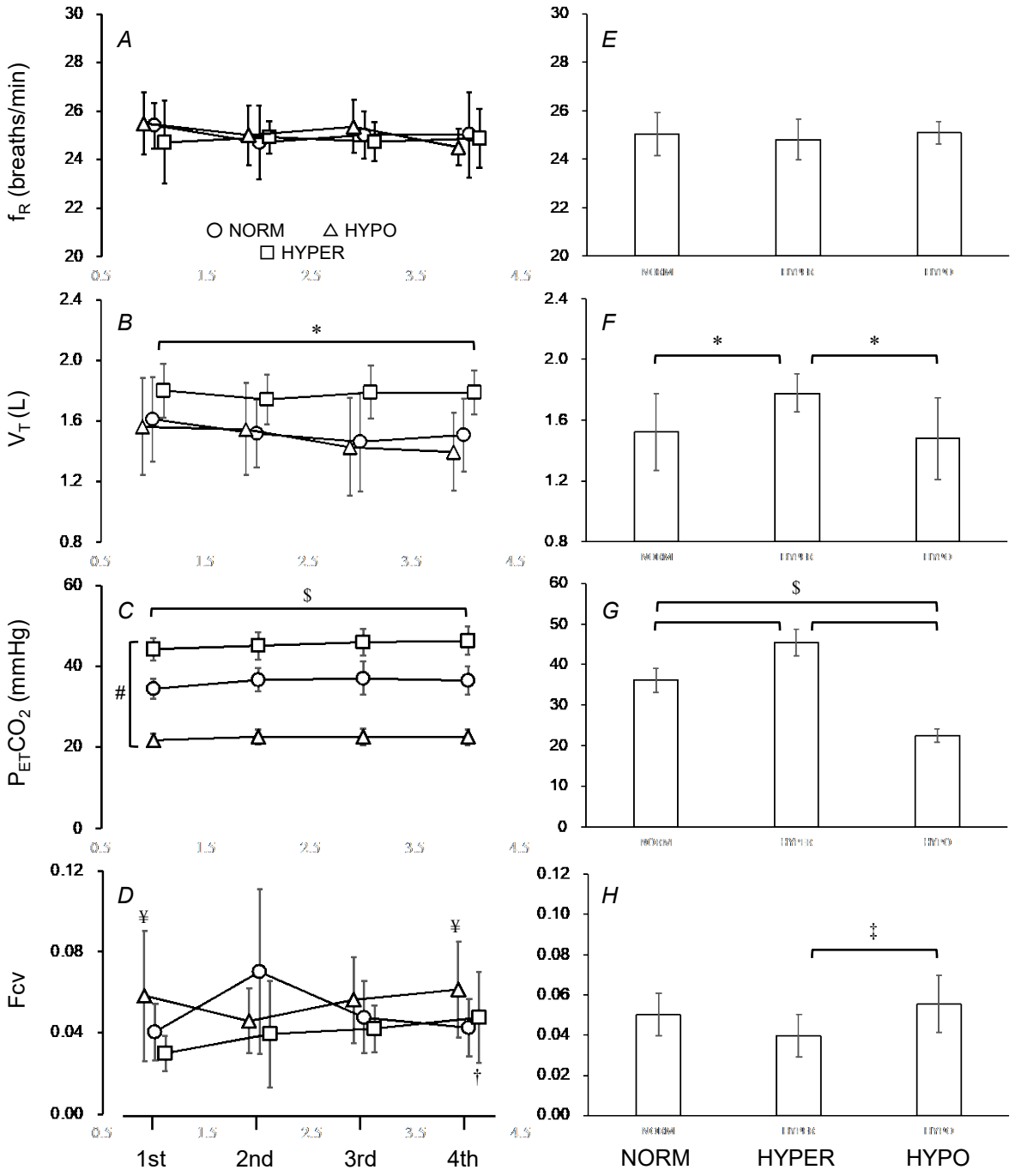

A
B
C
D
E
F
G
H
f_R (breaths/min)
V_T (L)
$P_{ET}CO_2$ (mmHg)
Fcv
○ NORM △ HYPO
□ HYPER
*
$
#
¥
†
‡
1st
2nd
3rd
4th
NORM
HYPER
HYPO

Figure 3.

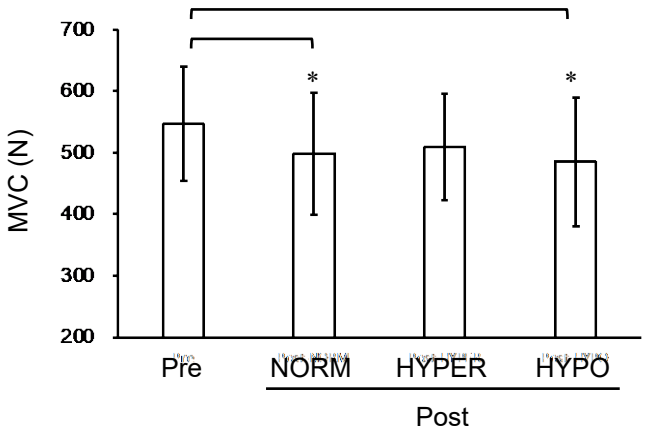


Figure 4.

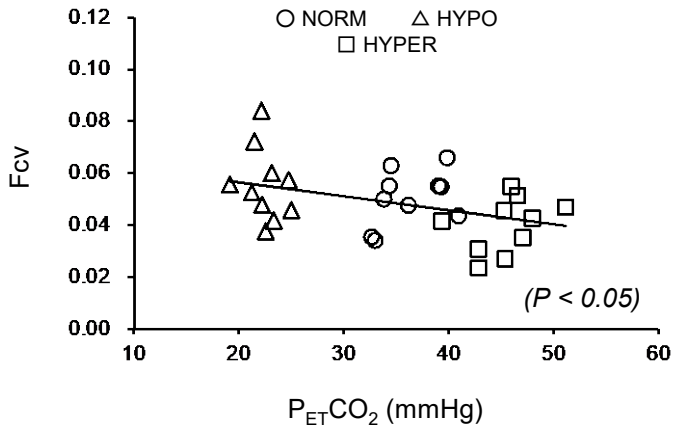
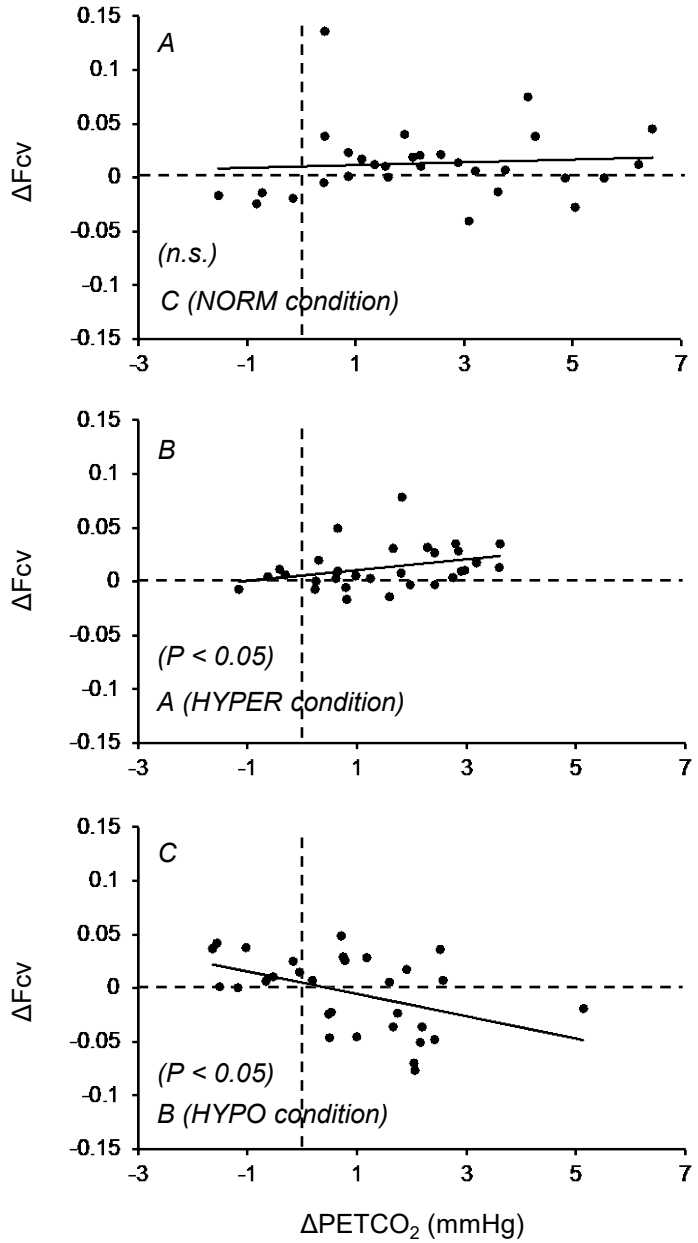

○ NORM △ HYPO
□ HYPER
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Fcv
10
20
30
40
50
60
(P < 0.05)
P_ETCO_2 (mmHg)

Figure 5.


A
(n.s.)
C (NORM condition)
B
(P < 0.05)
A (HYPER condition)
C
(P < 0.05)
B (HYPO condition)
ΔFcv
ΔPETCO2 (mmHg)
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-3
-1
1
3
5
7