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Relationship between motor corticospinal excitability and ventilatory response during intense exercise

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**Abstract**

*Purpose* Effort sense has been suggested to be involved in the hyperventilatory response during intense exercise (IE). However, the mechanism by which effort sense induces an increase in ventilation during IE has not been fully elucidated. The aim of this study was to determine the relationship between effort-mediated ventilatory response and corticospinal excitability of lower limb muscle during IE.

*Methods* Eight subjects performed 3 min of cycling exercise at 75-85% of maximum workload twice (IE<sub>1st</sub> and IE<sub>2nd</sub>). IE<sub>2nd</sub> was performed after 60 min of resting recovery following 45 min of submaximal cycling exercise at the workload corresponding to ventilatory threshold. Vastus lateralis muscle response to transcranial magnetic stimulation of the motor cortex (motor evoked potentials, MEPs), effort sense of legs (ESL, Borg 0-10 scale), and ventilatory response were measured during the two IEs.

*Results* The slope of ventilation (l/min) against CO<sub>2</sub> output (l/min) during IE<sub>2nd</sub> ( $28.0 \pm 5.6$ ) was significantly greater than that ( $25.1 \pm 5.5$ ) during IE<sub>1st</sub>. Mean ESL during IE was significantly higher in IE<sub>2nd</sub> ( $5.25 \pm 0.89$ ) than in IE<sub>1st</sub> ( $4.67 \pm 0.62$ ). Mean MEP (normalized to maximal M-wave) during IE was significantly lower in IE<sub>2nd</sub> ( $66 \pm 22\%$ ) than in IE<sub>1st</sub> ( $77 \pm 24\%$ ). The difference in mean ESL between the two IEs was significantly ( $p < 0.05$ ,  $r = -0.82$ ) correlated with the difference in mean MEP between the two IEs.

*Conclusions* The findings suggest that effort-mediated hyperventilatory response to IE may be associated with a decrease in corticospinal excitability of exercising muscle.

**Keywords**

Control of breathing; Muscle glycogen; Hyperventilation; Fatigue; Central command; Effort sense

**Abbreviations**

AMT	Active motor threshold
EMG	Electromyogram
ESL	Effort sense of legs
ETCO <sub>2</sub>	End-tidal carbon dioxide
IE	Intense exercise
iEMG	Integrated electromyogram
MEPs	Motor evoked potentials
MNS	Magnetic motor nerve stimulation
Mmax	Maximal M-wave
MVC	Maximal voluntary contraction
PaCO <sub>2</sub>	Arterial carbon dioxide pressure
PaCO <sub>2pred</sub>	Predicted arterial carbon dioxide pressure
TMS	Transcranial magnetic stimulation
T <sub>vent</sub>	Ventilatory threshold
$\dot{V}CO_2$	Carbon dioxide output
$\dot{V}E$	Ventilation
VL	Vastus lateralis
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximum oxygen uptake
W <sub>max</sub>	Maximum workload

## Introduction

Ventilatory kinetics during intense exercise (IE) is characterized by a disproportionate increase in ventilation ( $\dot{V}_E$ ) relative to carbon dioxide output ( $\dot{V}_{CO_2}$ ) and a resulting decrease in arterial  $CO_2$  pressure ( $PaCO_2$ ). This hyperventilatory response is considered to be respiratory compensation to minimize a decrease in blood pH (Kowalchuk et al. 1988; Rausch et al. 1991; Ward 2007; Wasserman et al. 1986; Yunoki et al. 1999), and the decrease in blood pH *per se* has been regarded as an important factor enhancing ventilation during exercise above the lactate threshold (Stringer et al. 1992; Wasserman et al. 1975). However, by using a glycogen-reduction procedure (Gollnick et al. 1974; Heigenhauser et al. 1983; Sabapathy et al. 2006), which can manipulate the degree of metabolic acidosis, we found that hyperventilatory response to IE is not dependent on blood pH but is associated with effort sense of exercising muscle (Yamanaka et al. 2011; Yamanaka et al. 2012).

Effort-mediated ventilatory response during exercise is generally explained by a feedforward mechanism involving activation of the cardiorespiratory center, which is induced, in parallel, by activation of the motor cortex (central motor command) (Goodwin et al. 1972; Heigenhauser et al. 1983; Krogh and Lindhard 1913; Marcora et al. 2008). However, it has been confirmed that during IE performed after muscle glycogen reduction, integrated electromyogram (iEMG) activity recorded in exercising muscle, which reflects central motor command (Amann et al. 2006; Amann and Dempsey 2008), does not increase while ventilation is elevated (Osborne and Schneider 2006; Perrey et al. 2003; Yamanaka et al. 2012). These findings indicate the possibility that although ventilatory response to IE is related to effort sense, an increase in motor drive from the motor cortex to exercising muscles, which may produce effort sense via efference copy/corollary discharge mechanisms (Marcora 2009; Sperry 1950; Von Holst 1954), is not always required for the augmented ventilatory response to IE (Yamanaka et al.

2012; Yunoki 2012). Thus, the mechanism of effort-mediated ventilatory response during IE remains to be elucidated.

Recent studies have suggested that group III/IV muscle afferents originating in exercising muscle play an important role in the development of fatigue during exercise [for review see (Amann et al. 2015)]. For example, Amann et al. (2013) demonstrated that group III/IV muscle afferent feedback associated with exhaustive endurance exercise reduced the output of spinal motoneurons and endurance performance. It has also been suggested that reductions in the output from spinal motoneurons are attributed to the inhibitory effect of group III/IV muscle afferents on excitability of the corticospinal tract consisting of the motor cortex and spinal motoneurons (Hilty et al. 2011; Martin et al. 2006; Sidhu et al. 2014). In this kind of situation, there is a possibility that activation of upstream regions of the motor cortex might be involved in the generation of a greater effort sense and consequently an increase in ventilation in order to maintain a required power output during IE (Amann et al. 2013). Therefore, in the present study, using the glycogen-reduction procedure adopted in our previous study (Yamanaka et al. 2012), we examined the relationship between effort-mediated ventilatory response and corticospinal excitability of lower limb muscle during IE. It has been shown that moderate-intensity exercise decreased muscle glycogen content to half of the pre-exercise level in about 45 min (Gollnick et al. 1974). In the present experiment, IE was repeated twice, and 45-min moderate-intensity exercise was performed between the two IEs in order to reduce the muscle glycogen content. We hypothesized that muscle glycogen reduction would induce increases in effort sense and ventilation with a decrease in corticospinal excitability of lower limb muscle during IE. Corticospinal excitability was assessed using transcranial magnetic stimulation (TMS) over the motor cortex to induce motor evoked potentials (MEPs) in the vastus lateralis (VL) during intense cycling exercise.

## Methods

### Subjects

Eight healthy males, with a mean ( $\pm$  SD) age of  $23.6 \pm 2.4$  yr, height of  $172.6 \pm 5.5$  cm, and body mass of  $69.0 \pm 7.1$  kg, participated in this study. Prior to participating in this study, each subject was fully informed of the experimental procedures and potential risks involved, and then they provided informed consent and completed a health-screening questionnaire for participation in an experiment involving TMS. The subjects were requested to abstain from strenuous physical activity, drinking alcohol, and taking caffeine for 24 h prior to each test. This study was approved by the Human Research Ethics Committee of the Graduate School of Education, Hokkaido University. Each subject participated in two tests, a graded-exercise test (GXT) and an IE test (IET), which were conducted on separate days.

### Graded-exercise test (GXT)

On the first test day, the subjects carried out a GXT on an electronically braked cycle ergometer (Ergometer 232 CXL, Combi, Japan) to determine their ventilatory threshold ( $T_{\text{vent}}$ ) and maximum oxygen uptake ( $\dot{V}O_{2\text{max}}$ ). The test began with 0 W for 2 min, after which power output was increased in a ramp function (20 W/min) until the subject could not maintain the required pedaling frequency (60 rpm).  $T_{\text{vent}}$  was determined by identifying a breakpoint in the slope of the  $\dot{V}O_2 - \dot{V}CO_2$  relationship based on the V-slope method (Beaver et al. 1986).  $\dot{V}O_{2\text{max}}$  was determined by averaging breath-by-breath  $\dot{V}O_2$  over a 30-s interval.  $\dot{V}O_{2\text{max}}$  of  $3068 \pm 568$  ml/min was associated with a power output of  $264 \pm 49$  W (maximum workload,  $W_{\text{max}}$ ). The power output when  $T_{\text{vent}}$  occurred was  $48.9 \pm 6.2\%$  of  $W_{\text{max}}$ , and  $\dot{V}O_2$  when  $T_{\text{vent}}$  occurred was  $54.5 \pm 6.3\%$  of  $\dot{V}O_{2\text{max}}$ .

### Intense exercise test (IET)

A few days after the GXT, each subject performed an IET on an electronically braked recumbent cycle ergometer with a backrest (Cateye ergociser EC-3700, Cateye Co. Ltd., Japan). This ergometer was used to prevent the upper body and head from swaying when TMS was carried out during IE. As shown in Figure 1, 3-min IE was performed twice ( $IE_{1st}$  and  $IE_{2nd}$ ). During the two IEs, the subjects were constrained to maintain pedaling frequency at 60 rpm against the workload ( $3.4 \pm 0.2$  kp) corresponding to 75-85% of  $W_{max}$  ( $202 \pm 34$  W). After 10-min resting recovery following  $IE_{1st}$ , each subject performed 45-min submaximal exercise at the workload ( $2.1 \pm 0.4$  kp, 60 rpm) corresponding to  $T_{vent}$  ( $129 \pm 26$  W) in order to reduce muscle glycogen content (Gollnick et al. 1974). Pedaling frequency during the two IEs and a submaximal exercise was averaged over a 30-s interval. A further resting recovery was provided for 60 min before the start of the second IE trial ( $IE_{2nd}$ ) in order to restore the disturbance of physiological parameters (i.e., blood acid-base parameters, muscle temperature, and cardiopulmonary parameters) (Yamanaka et al. 2012). The subjects were permitted to drink only water ad libitum during the 45-min submaximal exercise and subsequent 60-min resting recovery.

### **Electromyogram recording**

A surface electromyogram (EMG) was recorded via a bipolar EMG sensor (interelectrode distance of 20 mm; SX230, Biometrics Ltd., UK) positioned over the muscle belly of the right vastus lateralis (VL). Before attachment of the EMG sensor, the skin was shaved, abraded, and cleaned with alcohol in order to reduce skin impedance. The ground electrode was placed over the styloid process of the left wrist. The EMG signal was amplified using an amplifier imbedded in the EMG sensor (bandwidth = 20–450 Hz; common mode rejection ratio, CMRR > 96 dB; input impedance >  $10^{13}\Omega$ ; gain = 1,000) and converted into a digital signal at a sampling rate of 2 kHz using an analog–digital converter (MacLab/8s, ADInstruments, Australia). Then EMG data were processed offline by using analysis software (LabChart

7, ADInstruments, Australia). Raw data were filtered using a band-pass filter with cutoff frequencies of 20 and 450 Hz for later analysis. The filtered data were used to assess (a) motor evoked potentials (MEPs) induced by magnetic stimulation of the motor cortex (TMS) and (b) maximal compound action potential (maximal M-wave;  $M_{\max}$ ) induced by magnetic stimulation of the peripheral motor nerve (MNS).

### **Transcranial magnetic motor cortex stimulation (TMS)**

Prior to the IET, the subjects performed 5-s isometric maximal voluntary contraction (MVC) of the knee extensors against a load cell (LC1205-K500, A&D, Japan) that was connected to a noncompliant strap placed around the right leg just superior to the ankle malleoli. The MVC was carried out at 90 degrees of knee joint angle on a chair with a backrest that has stoppers to fix the upper body and head. Each subject performed two MVCs interspersed with a 3-min rest period, and the higher value was adopted as MVC. Then maximum EMG ( $EMG_{\max}$ ) during the MVC was established. A transcranial magnetic stimulator (Magstim 200<sup>2</sup>, Magstim, UK) with a double-cone coil (110 mm in diameter) was used to elicit MEPs in the VL. The intersection of the TMS coil was aligned tangentially with the sagittal plane, with the center of the coil being < 2 cm to the left on the vertex (Cz). The coil was oriented so that the induced current flow within the cortex was in a posterior-to-anterior direction. During this procedure, the magnetic stimulator was set at about 45% of maximal output and the coil was moved over the vertex until the position evoking the largest MEP in the VL was found while each subject was performing isometric knee extension at the intensity matching 20% of  $EMG_{\max}$  (Sidhu et al. 2012). Visual feedback of the EMG signal was displayed on a computer monitor so that the subjects could produce the required EMG (20% of  $EMG_{\max}$ ) during the submaximal isometric contraction. The optimal location for the stimulation was determined as the position where the largest MEP was observed. The position was

marked on a tight-fitting swimming cap that covered the subject's head to ensure constant positioning of the coil throughout the experiment. Subsequently, active motor threshold (AMT) intensity (mean intensity:  $40.1 \pm 6.4\%$  of maximum stimulator output) was determined. AMT was defined as the lowest intensity of the stimulator that elicited an MEP clearly distinguishable from the background EMG in three out of five pulses during the submaximal isometric contraction.

In the IET (Fig. 1), the TMS intensity was set at 130% of the AMT. During each 3-min IE, TMS was manually (visually) delivered during the knee extension phase (pedal pushing period) of the right leg at about every 10 s (18 TMSs in total). The size of MEPs was defined as EMG peak-to-peak amplitude within 50 ms after TMS application (Li and Rymer 2011). For further data analysis, the obtained MEPs were averaged at 60-s intervals, in which the MEP that was farthest from the median value was excluded. Background integrated EMG (iEMG) was calculated over a 100-ms window prior to TMS delivery during the IE.

A goniometer (SG 150, Biometrics Ltd., UK) was attached to the right knee joint to check the knee joint angle position at which TMS was delivered during IE. The goniometer signal was converted into a digital signal at a sampling rate of 2 kHz using an analog–digital converter (MacLab/8s, ADInstruments, Australia). Since TMS was manually (visually) delivered, we cannot eliminate the possibility that the state of muscle contraction when TMS was delivered was not constant. However, by checking a goniometer signal, we confirmed that all TMS that induced MEPs used in data analysis was delivered during the extension phase of the right leg except for TMS during IE<sub>1st</sub> for one subject in whom the angle signal was not recorded due to a technical problem (breaking of the wire). When maximal flexion position and maximal extension position of the right knee joint during pedaling were defined as 0% and 100%, respectively, for each subject ( $n = 7$ ) except for the above subject, it was confirmed that TMS was

delivered at about 40% of the maximal extension position ( $IE_{1st}$ ,  $39.3 \pm 8.1\%$ ;  $IE_{2nd}$ ,  $38.8 \pm 12.2\%$ , N.S.).

In addition, the subjects were pedaling at a constant cadence. Therefore, we assume that position variability would have been very minor.

### **Magnetic motor nerve stimulation (MNS)**

Prior to the IET, the femoral nerve of the right thigh was stimulated using the aforementioned magnetic stimulator and coil while each subject remained in a resting supine position on an examination table. The coil was placed within the femoral triangle and repositioned to determine the best location evoking the largest M-wave in the VL. Intensity of the magnetic stimulation was increased gradually until the size of the M-wave demonstrated no further increase (i.e., maximal M-wave;  $M_{max}$ ). In the IET (Fig. 1), the stimulation intensity was then increased by a further 20% (mean intensity:  $72.0 \pm 7.2\%$  of maximum stimulator output) to ensure maximal activation of the muscle. This stimulation was delivered three times before the start of each IE trial, and the highest value was used as  $M_{max}$ . The stimulation position was marked on the right thigh to ensure constant positioning of the coil.

### **Respiratory variables**

$\dot{V}E$ ,  $\dot{V}CO_2$ ,  $\dot{V}O_2$ , and end-tidal carbon dioxide ( $ETCO_2$ ) were measured in the GXT and IET using a respiratory gas analyzer (AE-280S, Minato Medical Science, Japan). For each 10-second interval, the average of each respiratory variable was calculated. Inspired and expired flows were measured using a hot-wire flow meter that is linear with respect to a flow range of 0–600  $l \cdot \text{min}^{-1}$ . A zirconium sensor and infrared absorption analyzer were used to analyze the inspired and expired fractions of  $O_2$  and  $CO_2$ , respectively. The flow meter and gas analyzers were calibrated prior to each test with a standard 2-l syringe and precision reference gas ( $O_2$  15.0%,  $CO_2$  5.0%). Predicted  $PaCO_2$  ( $PaCO_{2pred}$ ) was calculated from  $ETCO_2$  (Jones et al. 1979).

**Blood variables**

The hand of each subject was pre-warmed in 40–45°C water prior to each IE, and a glove containing a heater was utilized during the exercise and subsequent recovery in order to arterialize capillary blood (Yamanaka et al. 2011, Yamanaka et al. 2012). Blood sampling was performed from fingertips using capillary tubes at rest before each IE and at 1 and 3 min after the end of each IE. Samples of 100 µl were analyzed by using a blood gas analyzer (i-STAT, i-STAT Corporation, USA) to measure lactate concentration ([Lac]) and pH. The blood gas analyzer was calibrated by a known calibration solution before each test. Blood glucose concentration ([Glu]) at rest before each IE was measured from 1-µl samples by using a blood glucose meter (OneTouch Ultra, Johnson & Johnson Medical, USA).

**Effort sense**

By using the modified Borg scale (Borg 1982), we asked each subject to assess effort sense of legs (ESL) at rest before each IE and at 1, 2, and 3 min during each IE.

**Statistical analysis**

Group data are presented as means  $\pm$  SD in the text, whereas in figures, and means  $\pm$  SE are shown in figures. A paired t-test was used to compare [Glu] and Mmax at rest before each IE and  $\dot{V}E/\dot{V}CO_2$  slope during IE between the two conditions (IE<sub>1st</sub> and IE<sub>2nd</sub>). Differences in variables (pH, [Lac], background iEMG, MEP, respiratory variables, ESL, pedaling frequency) between the two conditions with time were evaluated by two-way repeated-measures ANOVA. After ANOVA, the Bonferroni post hoc test was performed for multiple comparisons. If a significant interactive effect was indicated, one-way repeated-measures ANOVA and the paired t-test were used to examine the time and condition effects, respectively. Pearson's correlation coefficient was determined in order to examine the correlation between ESL and MEP. Statistical significance was set at  $P < 0.05$ .

## Results

There was no significant difference in pedaling frequencies (rpm) between the two IEs, and no significant main effect of time was found. On average, pedaling frequencies during IE<sub>1st</sub> and IE<sub>2nd</sub> were  $60.1 \pm 0.8$  and  $60.0 \pm 1.2$  rpm, respectively.

Figure 2 shows the changes in [Lac] and pH in the two IE trials. There was no significant difference in [Lac] and pH at rest before the start of IE between the two conditions. The value of [Lac] after the end of IE was significantly ( $p < 0.01$ ) lower in IE<sub>2nd</sub> than in IE<sub>1st</sub>. The value of pH after the end of IE was significantly ( $p < 0.01$ ) higher in IE<sub>2nd</sub> than in IE<sub>1st</sub>. As shown in Table 1, the value of [Glu] at rest was significantly ( $p < 0.01$ ) lower before IE<sub>2nd</sub> than before IE<sub>1st</sub>. The highest value of [Lac] in response to IE was significantly lower in IE<sub>2nd</sub> than in IE<sub>1st</sub>. The lowest value of pH in response to IE was significantly higher in IE<sub>2nd</sub> than in IE<sub>1st</sub>.

Figure 3 shows the time courses of  $\dot{V}O_2$ ,  $\dot{V}CO_2$ ,  $\dot{V}E$ , and  $PaCO_{2pred}$  during IE in the two conditions. No significant exercise condition effect was found for  $\dot{V}O_2$  or  $\dot{V}E$ . The values of peak  $\dot{V}O_2$  during IE<sub>1st</sub> and IE<sub>2nd</sub> were  $90.4 \pm 15.2\%$  and  $91.9 \pm 16.4\%$  of  $\dot{V}O_{2max}$ , respectively (N.S.).  $\dot{V}CO_2$  during the middle part of IE was significantly lower in IE<sub>2nd</sub> than in IE<sub>1st</sub>.  $PaCO_{2pred}$  during IE<sub>2nd</sub> was significantly lower than that during IE<sub>1st</sub> between 1 and 3 min of IE. Figure 4 shows the relationship between  $\dot{V}E$  and  $\dot{V}CO_2$  for the group. The slope of the  $\dot{V}E$  (l/min) -  $\dot{V}CO_2$  (l/min) relationship obtained individually was significantly ( $p < 0.05$ ) higher in IE<sub>2nd</sub> ( $28.0 \pm 5.6$ ) than in IE<sub>1st</sub> ( $25.1 \pm 5.5$ ).

There was no significant difference in Mmax measured before the start of IE between the two conditions (IE<sub>1st</sub>,  $3.47 \pm 0.31$  mV; IE<sub>2nd</sub>,  $3.24 \pm 0.47$  mV). There was no significant difference in background iEMG between the two exercise conditions, and no significant main effect of time was found for background iEMG. MEPs (normalized to Mmax) in IE<sub>2nd</sub> were significantly lower than those in IE<sub>1st</sub>,

while no significant main effect of time was found (Fig. 5). Mean MEP during IE (for 3 min) was significantly ( $p < 0.05$ ) lower in IE<sub>2nd</sub> ( $66 \pm 22\%$ ) than in IE<sub>1st</sub> ( $77 \pm 24\%$ ). There was a significant difference in ESL between the two IEs (Fig. 6). Mean ESL during IE (for 3 min) was significantly ( $p < 0.05$ ) greater in IE<sub>2nd</sub> ( $5.25 \pm 0.89$ ) than in IE<sub>1st</sub> ( $4.67 \pm 0.62$ ). The difference in mean ESL between IE<sub>1st</sub> and IE<sub>2nd</sub> was significantly ( $p < 0.05$ ,  $r = -0.82$ ) correlated with the difference in mean MEP between the two IEs (Fig. 7).

## Discussion

The major findings of the present study were as follows: 1) changes in [Lac] and pH in response to IE were significantly smaller in IE<sub>2nd</sub> than in IE<sub>1st</sub>, 2) ventilatory response ( $\dot{V}E/\dot{V}CO_2$  slope) during IE<sub>2nd</sub> was significantly greater than that during IE<sub>1st</sub>, 3) effort sense (ESL) was significantly greater during IE<sub>2nd</sub> than during IE<sub>1st</sub>, 4) MEP during IE was significantly lower in IE<sub>2nd</sub> than in IE<sub>1st</sub>, and 5) the decrease in MEP from IE<sub>1st</sub> to IE<sub>2nd</sub> was significantly correlated with the increase in ESL from IE<sub>1st</sub> to IE<sub>2nd</sub>. These findings support our hypothesis that an increase in effort sense would increase ventilatory response with a decrease in corticospinal excitability of exercising limb muscle during IE.

Although power output kinetics (workload and pedaling frequency) during IE<sub>1st</sub> was the same as that during IE<sub>2nd</sub>, ESL during IE<sub>2nd</sub> was significantly higher than that during IE<sub>1st</sub>. At rest before the start of IE, [Glu] was significantly lower in IE<sub>2nd</sub> than in IE<sub>1st</sub>, and also the magnitudes of increase in [Lac] and decrease in pH during IE were significantly smaller in IE<sub>2nd</sub> than in IE<sub>1st</sub>. These results suggest that the observed increase in ESL from IE<sub>1st</sub> to IE<sub>2nd</sub> was not due to intramuscular metabolic byproducts (lactate and hydrogen ions) but predominantly due to decrease in muscle glycogen content, which was probably accelerated by a prolonged exercise prior to IE<sub>2nd</sub>. Previous studies (Busse et al. 1991; Heigenhauser et al.

1983; Osborne and Schneider 2006) have shown lower blood lactate concentration and higher blood pH during exercise at the same work rate after muscle glycogen reduction. Although we did not conduct direct measurement of muscle glycogen content, we assume that the obtained data (e.g., blood lactate and pH) would have reflected decreased muscle glycogen content in IE<sub>2nd</sub> compared with that in IE<sub>1st</sub>.

Similar to our previous study (Yamanaka et al. 2012), metabolic acidosis (increase in [Lac] and decrease in pH) was attenuated in the second IE trial compared with that in the first IE trial. In addition, PaCO<sub>2pred</sub> during IE<sub>2nd</sub> was significantly lower than that during IE<sub>1st</sub>. Although these observations concerning blood acid-base balance suggest that ventilatory chemoreflex via central and peripheral chemoreceptors (Rausch et al. 1991; Ward 1994; Ward 2007) and perhaps via muscle afferents (Oelberg et al. 1998) would have been attenuated during IE<sub>2nd</sub> compared with that during IE<sub>1st</sub>,  $\dot{V}E$  during IE<sub>2nd</sub> was maintained at the same level as that during IE<sub>1st</sub>. This indicates that factors other than humoral feedback and muscle afferent feedback mentioned above could have contributed to the ventilatory response during IE<sub>2nd</sub>. In the present study, since  $\dot{V}O_2$  during IE was similar in the two conditions, it is possible that  $\dot{V}E$  depended on metabolic demand during IE. However, our previous study confirmed the premise that during repeated IE performed after glycogen depression,  $\dot{V}E$  increases in accordance with the augmentation of ESL even though  $\dot{V}O_2$  remains unchanged (Yamanaka et al. 2012). We observed a significant increase in ESL from IE<sub>1st</sub> to IE<sub>2nd</sub>. Therefore, increase in ventilatory response ( $\dot{V}E/\dot{V}CO_2$  slope) observed in IE<sub>2nd</sub> can be explained, at least in part, by increase in ESL.

In addition to the increase in ESL, a significant decrease in MEPs was observed from IE<sub>1st</sub> to IE<sub>2nd</sub>. Since no significant change in MEPs with time was observed in either of the IE trials, the observed difference in MEPs between the two conditions was probably due to the prolonged exercise performed before IE<sub>2nd</sub>. Sidhu et al. (2012) measured MEPs and cervicomedullary evoked potentials (CMEPs) from

exercising muscles (VL and rectus femoris) during 30 min of cycling at 75% maximal aerobic workload. They normalized MEP and CMEP amplitudes to cycling EMG, and they found that CMEPs were not different throughout the exercise, while MEPs were significantly reduced from 10 min after the start of the exercise. Therefore, they mentioned the possibility that there was a tendency toward reduced cortical excitability from 10 min after the start of the exercise, and some factors such as temperature regulation, glucose availability, and catecholamine concentration have been assumed to influence the responses of cells in the motor cortex and within the corticospinal tract during whole-body fatiguing exercise (Sidhu et al. 2012, Gruet et al. 2013). However, in their study (Sidhu et al. 2012), post hoc tests of differences in modulation of MEPs vs. CMEPs are not strictly justified due to the lack of an interaction effect. In addition, when normalized to  $M_{max}$  as was done in the present study, changes in MEP and CMEP size were not observed throughout the exercise. Thus, it is reasonable to consider that any suggestion of a purely cortical involvement is unsupported in the previous study by Sidhu et al. (2012). On the other hand, Group III/IV muscle afferent feedback associated with intramuscular metabolic perturbation has been suggested to have an inhibitory effect on the central nervous system (i.e., central fatigue), limiting the output of spinal motoneurons during exhaustive locomotor exercise (Amann et al. 2013; Amann et al. 2015; Hilty et al. 2011; Sidhu et al. 2014). Moreover, it has been suggested that decrease in the output from spinal motoneurons is attributed to the inhibitory effect of group III/IV muscle afferents on voluntary descending drive 'upstream' of the motor cortex (Taylor et al., 2006) and/or an afferent-mediated depression of excitability of the corticospinal tract (Hilty et al. 2011, Martin et al. 2006; Martin et al. 2008, Sidhu et al. 2014). In the present study, MEP was significantly lower in  $IE_{2nd}$  than in  $IE_{1st}$ , while  $M_{max}$  was similar in the two conditions. This suggests that there was no difference in sarcolemmal excitability between the two conditions. Therefore, it is likely that the decrease in MEP

would have been due to a reduction of the net excitability that occurred in the pathway from the motor cortex to the muscle. Since metabolic acidosis was attenuated in IE<sub>2nd</sub> compared with that in IE<sub>1st</sub>, low muscle glycogen concentrations rather than muscle acidosis might have exerted inhibitory effects on supraspinal areas of the central nervous system (Rauch et al. 2005), causing the difference in MEP between the two IE trials.

Another possible mechanism accounting for the observed difference in MEP between the two conditions is a decrease in brain glycogen. Hypoglycaemia induced during prolonged exercise has been shown to cause a decrease in glycogen levels in various brain regions including the motor cortex (Matsui et al. 2011). Brain glycogen is known to localize in astrocytes and to be utilized as an important energy source for neurons (Brown 2004). Oz et al. (2009) showed by using <sup>13</sup>C NMR that brain glycogen supports energy metabolism when glucose supply from the blood is inadequate in humans. In the present study, [Glu] before the start of IE<sub>2nd</sub> was significantly lower than that before the start of IE<sub>1st</sub>. The value of [Glu] at rest before the start of IE<sub>2nd</sub> was close to the level observed at exhaustion in a prolonged submaximal exercise for about 2 h (Zinker et al. 1990). Therefore, in IE<sub>2nd</sub>, decreases in [Glu] might have directly caused central fatigue (i.e., decrease in MEP) through a decrease in brain glycogen.

Decrease in MEP from IE<sub>1st</sub> to IE<sub>2nd</sub> showed a significant correlation with increase in ESL from IE<sub>1st</sub> to IE<sub>2nd</sub> (Fig. 7). This indicates the possibility that increase in ESL was associated with decrease in corticospinal excitability, in other words, ESL increased to compensate for the depressed excitability of the corticospinal tract. Indeed, Amann et al. (2013) pointed out the possibility that in order to maintain a given workload under the development of central fatigue, the central nervous system needs to increase voluntary drive (i.e., a greater effort) to the motor cortex, and the increased voluntary drive compensates for the reduced motor pathway excitability and at the same time would be responsible for increases in

cardioventilatory response during fatiguing exercise. In the present study, we observed a significant decrease in MEP from IE<sub>1st</sub> to IE<sub>2nd</sub>. Therefore, the findings in the present study not only support the above concept (Amman et al. 2013) but also suggest that effort-mediated ventilatory response during fatiguing IE cannot be explained by the conventional framework of central command that drives breathing via neural mechanisms consisting of parallel activation of motor and respiratory centers (Goodwin et al. 1972; Heigenhauser et al. 1983; Krogh and Lindhard 1913; Marcora et al. 2008). Recent studies (Decety et al. 1991; Thornton et al. 2001; Williamson et al. 2002, 2006; Yunoki et al. 2009) using a cognitive approach to dissociate peripheral neural signals from central command have suggested that central command-mediated response does not necessarily require parallel activation of central motor command. Thornton et al. (2001) showed by using positron emission tomography (PET) that breathing during imagination of effortful exercise was increased with significant activations of the dorsolateral prefrontal cortex, supplementary motor areas, premotor area, and cerebellum. Likewise, Williamson et al. (2002) localized the insular and anterior cingulate cortices as important brain sites related to cardiovascular response induced by imagined handgrip exercise with effort sense. Although we cannot verify the neuroanatomical structure, our results suggest that effort-mediated hyperventilatory response to IE does not require an increase in central motor command from the primary motor cortex to exercising muscle.

### **Limitations**

Exercise intensity (75-85% Wmax) that had been obtained from the GXT was prescribed in the IET. Although an upright ergometer was used for the GXT, the IET was conducted by using a recumbent ergometer in order to perform TMS as accurately as possible during cycling. Consequently, as suggested

from the value of peak  $\dot{V}O_2$  during IE, there is a possibility that the true intensity during IE was slightly higher than that (75-85%  $W_{max}$ ) estimated from GXT.

In the present study, MNS was delivered in the relaxed muscle before each IE, whereas TMS was delivered in the contracting muscle during each IE. Although there was no significant difference in  $M_{max}$  assessed before the start of IE between the two conditions, we cannot exclude the possibility that  $M_{max}$  decreased during IEs and that the decrease in  $M_{max}$  was greater during  $IE_{2nd}$  than during  $IE_{1st}$ . This can lead to an overestimation of the contribution of diminished corticospinal excitability to the MEP depression during  $IE_{2nd}$ . Thus, to differentiate the corticospinal and peripheral mechanisms, it would be essential to systematically normalize MEP to concomitant  $M_{max}$  (Gruet et al. 2013, Kalmar and Cafarelli 2004).

### **Conclusions**

The results imply that an increase in effort sense increases ventilatory response with a decrease in corticospinal excitability of exercising limb muscle during IE. It is likely that effort-mediated hyperventilatory response to IE is independent of an increase in motor command from the primary motor cortex to exercising limb muscle.

### **Acknowledgments**

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### Figure Legends

**Fig. 1.** Overview of the intense exercise test (IET). Subjects performed an intense exercise (IE) twice (IE<sub>1st</sub> and IE<sub>2nd</sub>) at 75-85% of maximum workload for 3 min. MNS, magnetic motor nerve stimulation; TMS, transcranial magnetic motor cortex stimulation.

**Fig. 2.** Changes in blood lactate concentration ([Lac]) and blood pH during and after intense exercise (IE) in the IE<sub>1st</sub> trial (open circles) and IE<sub>2nd</sub> trial (filled circles). Data presented are means  $\pm$  SE. \* Significant difference vs. IE<sub>1st</sub> (P < 0.01).

**Fig. 3.** Changes in O<sub>2</sub> uptake ( $\dot{V}O_2$ ), CO<sub>2</sub> output ( $\dot{V}CO_2$ ), ventilation ( $\dot{V}E$ ), and predicted arterial CO<sub>2</sub> pressure ( $PaCO_{2pred}$ ) during intense exercise (IE) in the IE<sub>1st</sub> trial (open circles) and IE<sub>2nd</sub> trial (filled circles). Data presented are means  $\pm$  SE. \* Significant difference vs. IE<sub>1st</sub> (P < 0.05).

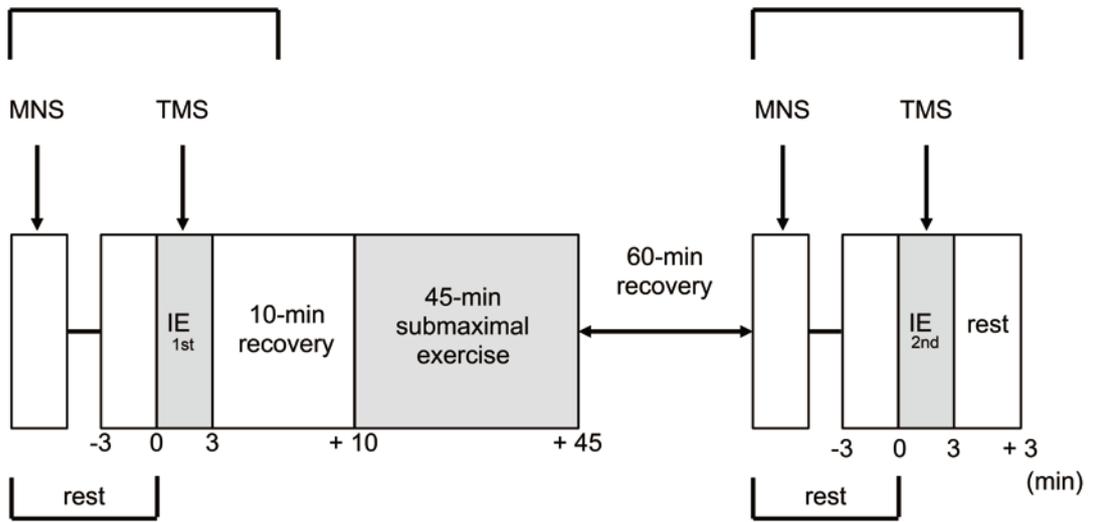
**Fig. 4.** Relationship between ventilation ( $\dot{V}E$ ) and CO<sub>2</sub> output ( $\dot{V}CO_2$ ) during intense exercise (IE) in the IE<sub>1st</sub> trial (open circles) and IE<sub>2nd</sub> trial (filled circles). The relationship was obtained from mean values of  $\dot{V}E$  and  $\dot{V}CO_2$  for the group.

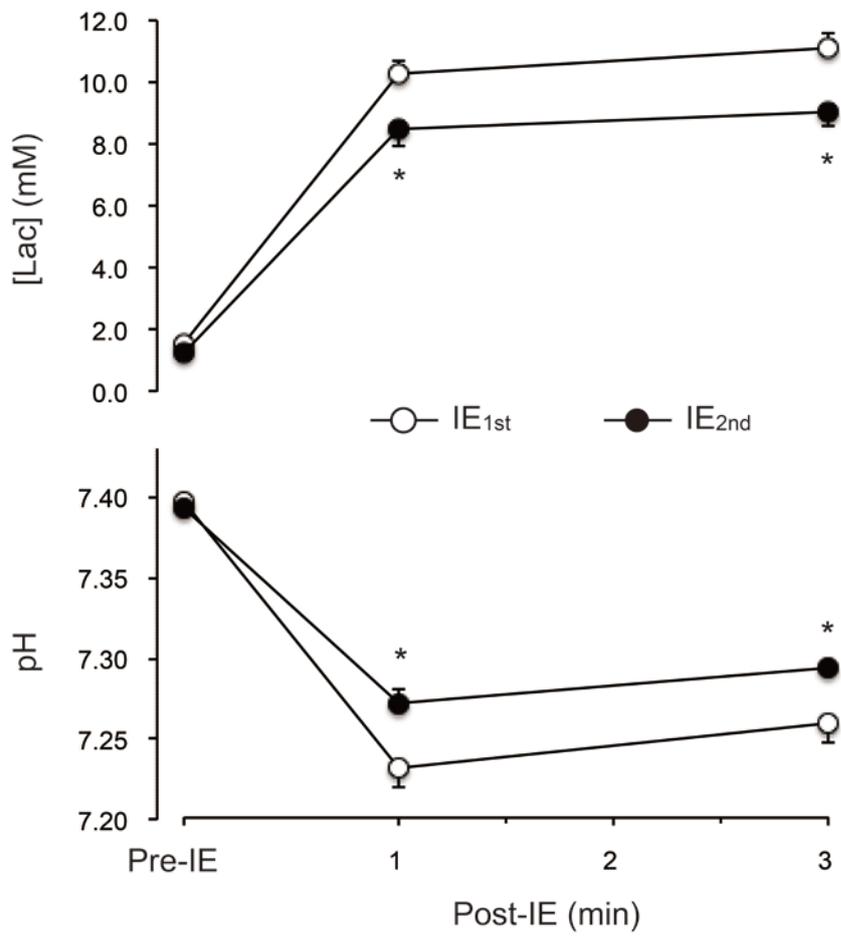
**Fig. 5.** Changes in motor evoked potentials (MEPs) induced by transcranial magnetic stimulation (TMS) during intense exercise (IE) in the IE<sub>1st</sub> trial (open circles) and IE<sub>2nd</sub> trial (filled circles). MEPs were expressed as percentage of maximal M-wave (Mmax) induced by magnetic motor nerve stimulation (MNS). Data presented are means  $\pm$  SE. \* Significant difference vs. IE<sub>1st</sub> (P < 0.05).

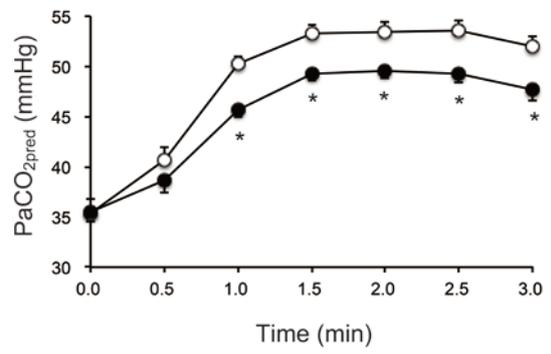
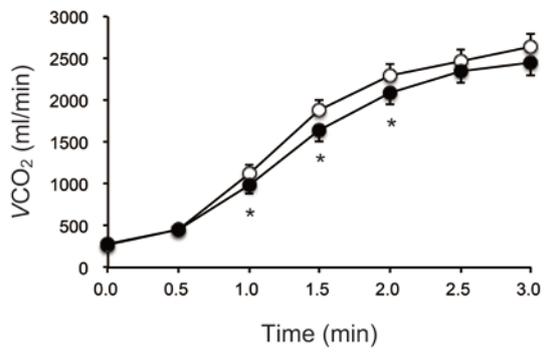
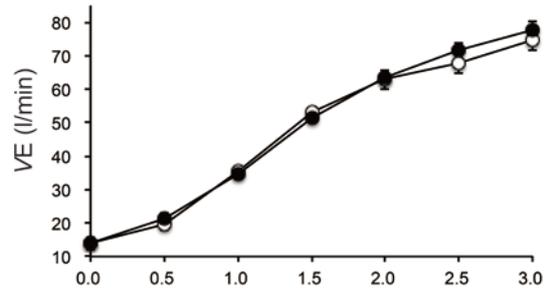
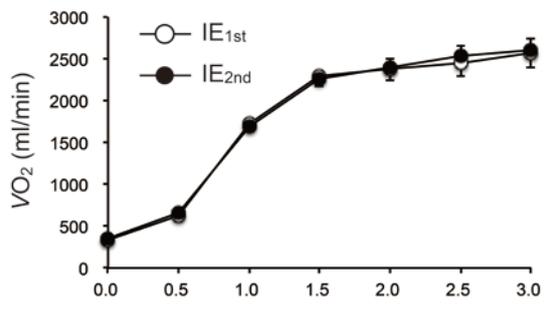
**Fig. 6.** Changes in effort sense of legs (ESL) during intense exercise (IE) in the IE<sub>1st</sub> trial (open circles) and IE<sub>2nd</sub> trial (filled circles). Data presented are means  $\pm$  SE. \* Significant difference vs. IE<sub>1st</sub> ( $P < 0.05$ ).

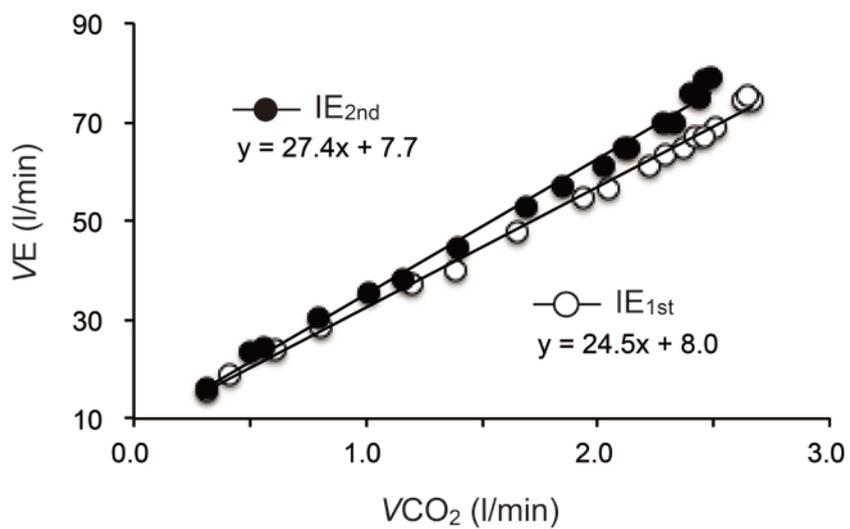
**Fig. 7.** Relationship between motor evoked potentials (MEPs) and effort sense of legs (ESL) during intense exercise (IE). MEPs were expressed as percentage of maximal M-wave (Mmax).  $\Delta$ MEP denotes the difference in mean MEPs during IE between the two conditions. Similarly,  $\Delta$ ESL denotes the difference in mean ESL during IE between the two conditions. The obtained relationship suggests that increase in ESL was significantly ( $p < 0.05$ ,  $r = -0.82$ ) correlated with decrease in MEPs.

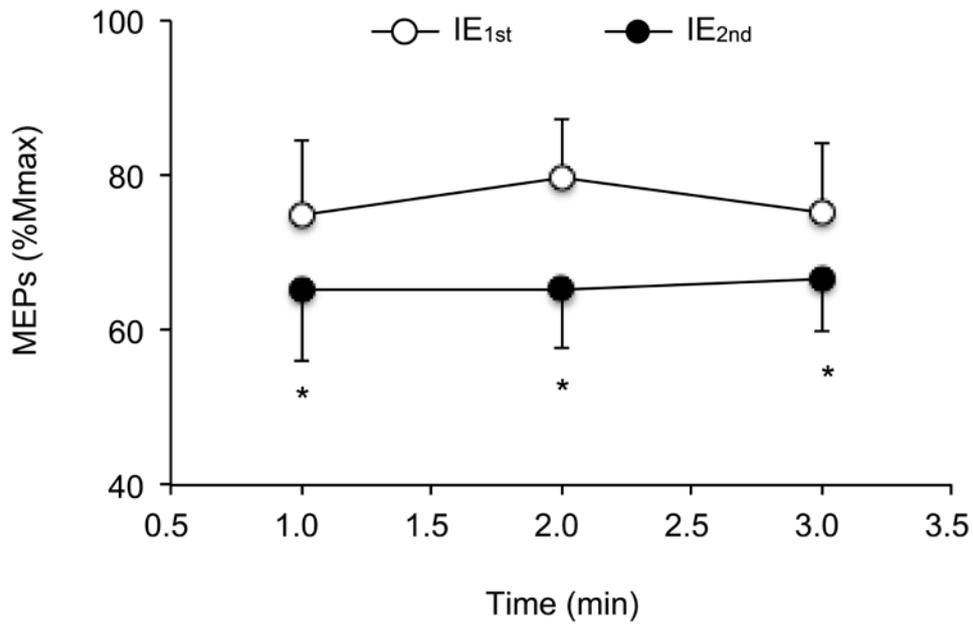
Measurement of physiological and psychological data

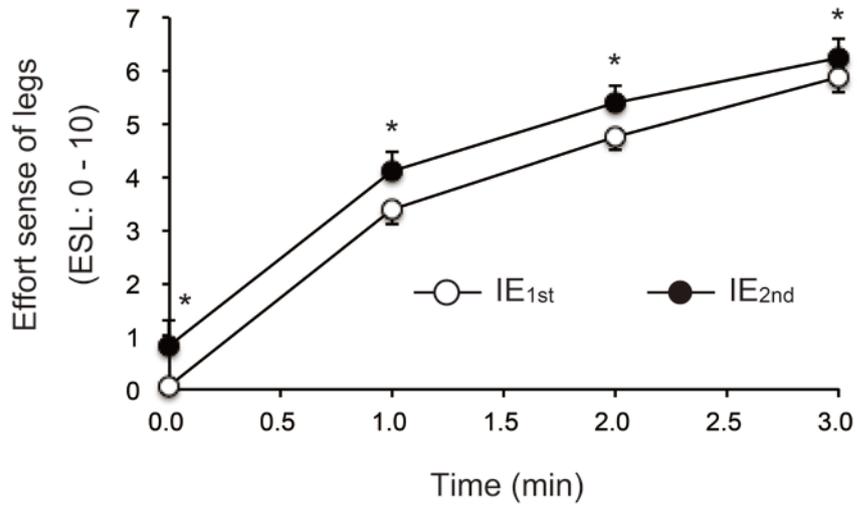












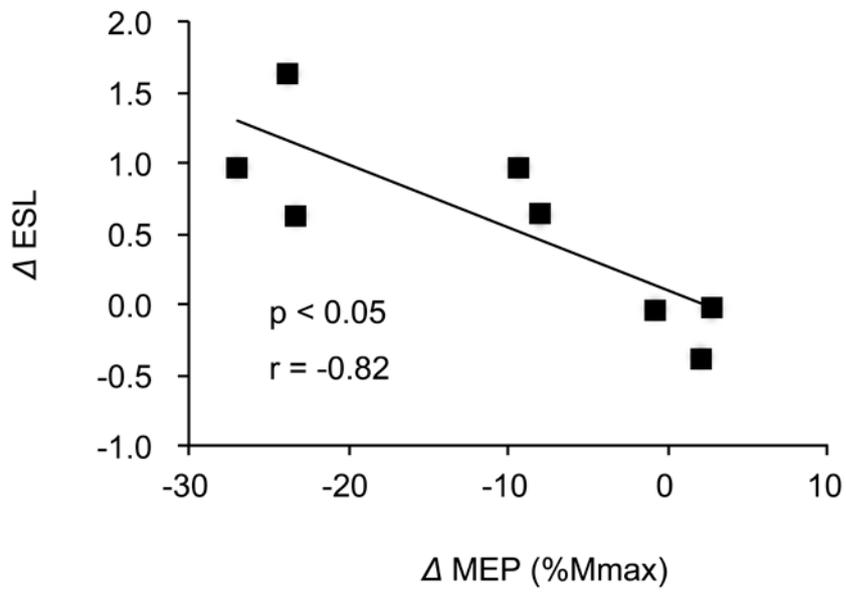


Table 1. Group mean data of blood variables

	IE <sub>1st</sub>	IE <sub>2nd</sub>
[Glu] <sub>rest</sub> (mg/dl)	111.6 ± 6.1	86.8 ± 4.8 *
[Lac] <sub>highest</sub> (mM)	11.1 ± 0.5	9.1 ± 0.5 *
pH <sub>lowest</sub>	7.232 ± 0.012	7.272 ± 0.008 *

Values are means ± SE. [Glu]<sub>rest</sub>, glucose level at rest before IE;  
[Lac]<sub>highest</sub>, the highest value of lactate level in response to IE;  
pH<sub>lowest</sub>, the lowest value of pH in response to IE.

\* Significant difference vs. IE<sub>1st</sub> (p < 0.01).