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Title page

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Relationship between motor corticospinal excitability and ventilatory response during intense exercise

Authors

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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_{1st} and IE_{2nd}). IE_{2nd} 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_2 output (l/min) during IE_{2nd} (28.0 ± 5.6) was significantly greater than that (25.1 ± 5.5) during IE_{1st}. Mean ESL during IE was significantly higher in IE_{2nd} (5.25 ± 0.89) than in IE_{1st} (4.67 ± 0.62). Mean MEP (normalized to maximal M-wave) during IE was significantly lower in IE_{2nd} (66 ± 22%) than in IE_{1st} (77 ± 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
$ETCO_2$	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
$PaCO_2$	Arterial carbon dioxide pressure
$PaCO_{2pred}$	Predicted arterial carbon dioxide pressure
TMS	Transcranial magnetic stimulation
T_{vent}	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_{max}	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 (± SD) age of 23.6 ± 2.4 yr, height of 172.6 ± 5.5 cm, and body mass of 69.0 ± 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_{vent}) and maximum oxygen uptake ($\dot{V}O_{2max}$). 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_{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). $\dot{V}O_{2max}$ was determined by averaging breath-by-breath $\dot{V}O_2$ over a 30-s interval. $\dot{V}O_{2max}$ of 3068 ± 568 ml/min was associated with a power output of 264 ± 49 W (maximum workload, W_{max}). The power output when T_{vent} occurred was 48.9 ± 6.2% of W_{max}, and $\dot{V}O_2$ when T_{vent} occurred was 54.5 ± 6.3% of $\dot{V}O_{2max}$.

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 ± 0.2 kp) corresponding

to 75-85% of W$_{max}$ (202 ± 34 W). After 10-min resting recovery following IE$_{1st}$, each subject performed

45-min submaximal exercise at the workload (2.1 ± 0.4 kp, 60 rpm) corresponding to T$_{vent}$ (129 ± 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 200^2, 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_{1st} 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 ± 8.1%; IE$_{2nd}$, 38.8 ± 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 ± 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·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 ± SD in the text, whereas in figures, and means ± 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_{1st} and IE_{2nd}). 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_{1st} and IE_{2nd} were 60.1 ± 0.8 and 60.0 ± 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_{2nd} than in IE_{1st}. The value of pH after the end of IE was significantly ($p < 0.01$) higher in IE_{2nd} than in IE_{1st}. As shown in Table 1, the value of [Glu] at rest was significantly ($p < 0.01$) lower before IE_{2nd} than before IE_{1st}. The highest value of [Lac] in response to IE was significantly lower in IE_{2nd} than in IE_{1st}. The lowest value of pH in response to IE was significantly higher in IE_{2nd} than in IE_{1st}.

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_{1st} and IE_{2nd} 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_{2nd} than in IE_{1st}. $PaCO_{2pred}$ during IE_{2nd} was significantly lower than that during IE_{1st} 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_{2nd} (28.0 ± 5.6) than in IE_{1st} (25.1 ± 5.5).

There was no significant difference in Mmax measured before the start of IE between the two conditions (IE_{1st}, 3.47 ± 0.31 mV; IE_{2nd}, 3.24 ± 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_{2nd} were significantly lower than those in IE_{1st},

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_{2nd} ($66 \pm 22\%$) than in IE_{1st} ($77 \pm 24\%$). 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_{2nd} (5.25 ± 0.89) than in IE_{1st} (4.67 ± 0.62). The difference in mean ESL between IE_{1st}

and IE_{2nd} 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_{2nd} than in IE_{1st}, 2) ventilatory response ($\dot{V}E/\dot{V}CO_2$ slope) during IE_{2nd}

was significantly greater than that during IE_{1st}, 3) effort sense (ESL) was significantly greater during IE_{2nd}

than during IE_{1st}, 4) MEP during IE was significantly lower in IE_{2nd} than in IE_{1st}, and 5) the decrease in

MEP from IE_{1st} to IE_{2nd} was significantly correlated with the increase in ESL from IE_{1st} to IE_{2nd}. 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_{1st} was the same as

that during IE_{2nd}, ESL during IE_{2nd} was significantly higher than that during IE_{1st}. At rest before the start

of IE, [Glu] was significantly lower in IE_{2nd} than in IE_{1st}, and also the magnitudes of increase in [Lac] and

decrease in pH during IE were significantly smaller in IE_{2nd} than in IE_{1st}. These results suggest that the

observed increase in ESL from IE_{1st} to IE_{2nd} 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_{2nd}. 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_{2nd} compared with that in IE_{1st}.

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, $PaCO_{2pred}$ during IE_{2nd} was significantly lower than that during IE_{1st}. 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_{2nd} compared with that during IE_{1st}, $\dot{V}E$ during IE_{2nd} was maintained at the same level as that during IE_{1st}. This indicates that factors other than humoral feedback and muscle afferent feedback mentioned above could have contributed to the ventilatory response during IE_{2nd}. 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_{1st} to IE_{2nd}. Therefore, increase in ventilatory response ($\dot{V}E/\dot{V}CO_2$ slope) observed in IE_{2nd} 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_{1st} to IE_{2nd}. 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_{2nd}. 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 Mmax 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_{2nd} compared with that in IE_{1st}, 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 13C 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_{2nd} was significantly lower than that before the start of IE_{1st}. The value of

[Glu] at rest before the start of IE_{2nd} was close to the level observed at exhaustion in a prolonged

submaximal exercise for about 2 h (Zinker et al. 1990). Therefore, in IE_{2nd}, 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_{1st} to IE_{2nd} showed a significant correlation with increase in ESL from

IE_{1st} to IE_{2nd} (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_{1st} to IE_{2nd}. 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% Wmax) 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 Mmax assessed before the start of IE between the two conditions, we cannot exclude the possibility that Mmax decreased during IEs and that the decrease in Mmax 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 Mmax (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.

References

Amann M, Dempsey JA (2008) Locomotor muscle fatigue modifies central motor drive in healthy humans and imposes a limitation to exercise performance. J Physiol 586:161–173

Amann M, Eldridge MW, Lovering AT, Stickland MK, Pegelow DF, Dempsey JA (2006) Arterial oxygenation influences central motor output and exercise performance via effects on peripheral locomotor muscle fatigue in humans. J Physiol 575:937–952

Amann M, Sidhu SK, Weavil JC, Mangum TS, Venturelli M (2015) Autonomic responses to exercise: Group III/IV muscle afferents and fatigue. Auton Neurosci 188:19–23

Amann M, Venturelli M, Ives SJ, McDaniel J, Layec G, Rossman MJ, Richardson RS (2013) Peripheral fatigue limits endurance exercise via a sensory feedback-mediated reduction in spinal motoneuronal output. J Appl Physiol 115:355–364

Beaver WL, Wasserman K, Whipp BJ (1986) A new method for detecting anaerobic threshold by gas exchange. J Appl Physiol 60:2020–2027

Borg GA (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14:377–381

Brown AM (2004) Brain glycogen re-awakened. J Neurochem 89:537–552

Busse MW, Maassen N, Konrad H (1991) Relation between plasma K^+ and ventilation during incremental exercise after glycogen depletion and repletion in man. J Physiol 443:469–476

Decety J, Jeannerod M, Germain M, Pastene J (1991) Vegetative response during imagined movement is proportional to mental effort. Behav Brain Res 42:1–5

Gollnick PD, Piehl K, Saltin B (1974) Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. J Physiol 241:45–57

Goodwin GM, McCloskey DI, Mitchell JH (1972) Cardiovascular and respiratory responses to changes in

central command during isometric exercise at constant muscle tension. J Physiol 226:173–190

Gruet M, Temesi J, Rupp T, Levy P, Millet GY, Verges S (2013) Stimulation of the motor cortex and corticospinal tract to assess human muscle fatigue. Neurosci 231: 384–399

Heigenhauser GJ, Sutton JR, Jones NL (1983) Effect of glycogen depletion on the ventilatory response to exercise. J Appl Physiol 54: 470-474

Hilty L, Lutz K, Maurer K, Rodenkirch T, Spengler CM, Boutellier U, Jäncke L, Amann M (2011) Spinal opioid receptor-sensitive muscle afferents contribute to the fatigue-induced increase in intracortical inhibition in healthy humans. Exp Physiol 96:505–517

Jones NL, Robertson DG, Kane JW (1979) Difference between end-tidal and arterial PCO_2 in exercise. J Appl Physiol 47:954–960

Kalmar JM, Cafarelli E (2004) Central fatigue and transcranial magnetic stimulation: effects of caffeine and the confound of peripheral transmission failure. J Neurosci Methods 138: 15–26

Kowalchuk JM, Heigenhauser GJF, Lindinger MI, Obminski G, Sutton JR, Jones NL (1988) Role of lungs and inactive muscle in acid-base control after intense exercise. J Appl Physiol 65:2090–2096

Krogh A, Lindhard J (1913) The regulation of respiration and circulation during the initial stages of muscular work. J Physiol 47:112–136

Marcora S (2009) Perception of effort during exercise is independent of afferent feedback from skeletal muscles, heart, and lungs. J Appl Physiol 106:2060–2062

Marcora SM, Bosio A, Morree HM (2008) Locomotor muscle fatigue increases cardiorespiratory responses and reduces performance during intense cycling exercise independently from metabolic stress. Am J Physiol Regul Intedr Comp Physiol 294:R874–R883

Martin PG, Smith JL, Butler JE, Gandevia SC, Taylor JL (2006) Fatigue-sensitive afferents inhibit

extensor but not flexor motoneurons in humans. J Neurosci 26:4796–4802

Martin PG, Weerakkody N, Gandevia SC, Taylor JL (2008) Group III and IV muscle afferents differentially affect the motor cortex and motoneurones in humans. J Physiol 586: 1277–1289

Matsui T, Soya S, Okamoto M, Ichitani Y, Kawanaka K, Soya H (2011) Brain glycogen decreases during prolonged exercise. J Physiol 589:3383–3393

Oz Gulin, Kumar A, Rao JP, Kodl CT, Chow L, Eberly LE, Seaquist ER (2009) Human brain glycogen metabolism during and after hypoglycemia. Diabetes 58: 1978–1985

Oelberg DA, Evans AB, Hrovat MI, Pappagianopoulos PP, Patz S, Systrom DM (1998) Skeletal muscle chemoreflex and pHi in exercise ventilatory control. J Appl Physiol 84:676–682

Osborne MA, Schneider DA (2006) Muscle glycogen reduction in man: relationship between surface EMG activity and oxygen uptake kinetics during heavy exercise. Exp Physiol 91: 179–189

Perrey S, Candau R, Rouillon JD, Hughson RL (2003) The effect of prolonged submaximal exercise on gas exchange kinetics and ventilation during heavy exercise in humans. Eur J Appl Physiol 89:587–594

Rauch HG, St Clair Gibson A, Lambert EV, Noakes TD (2005) A signalling role for muscle glycogen in the regulation of pace during prolonged exercise. Br J Sports Med 39:34–38

Rausch SM, Whipp BJ, Wasserman K, Huszczuk A (1991) Role of the carotid bodies in the respiratory compensation for the metabolic acidosis of exercise in humans. J Physiol 444:567–578

Sabapathy S, Morris NR, Schneider DA (2006) Ventilatory and gas-exchange responses to incremental exercise performed with reduced muscle glycogen content. J Sci Med Sport 9:267–273

Sidhu SK, Cresswell AG, Carroll TJ (2012) Motor cortex excitability does not increase during sustained cycling exercise to volitional exhaustion. J Appl Physiol 113:401–409

Sidhu SK, Weavil JC, Venturelli M, Garten RS, Rossman MJ, Richardson RS, Gmelch BS, Morgan DE, Amann M (2014) Spinal μ-opioid receptor-sensitive lower limb muscle afferents determine corticospinal responsiveness and promote central fatigue in upper limb muscle. J Physiol 592:5011–5024

Sperry RW (1950) Neural basis of the spontaneous optokinetic response produced by visual neural inversion. J Comp Physiol Psychol 43:482–489

Stringer W, Casaburi R, Wasserman K (1992) Acid-base regulation during exercise and recovery in humans. J Appl Physiol 72: 954–961

Thornton JM, Guz A, Murphy K, Griffith AR, Pedersen DL, Kardos A, Leff A, Adams L, Casadei B, Paterson DJ (2001) Identification of higher brain centres that may encode the cardiorespiratory response to exercise in humans. J Physiol 533:823–836

Von Holst E (1954) Relations between the central nervous system and peripheral organs. Br J Anim Behav 2:89–94

Ward SA (1994) Peripheral and central chemoreceptor and control of ventilation during exercise in humans. Can J Appl Physiol 19:305–333

Ward SA (2007) Ventilatory control in humans: constraints and limitations. Exp Physiol 92:357–366

Wasserman K, Whipp BJ, Casaburi R (1986) Respiratory control during exercise. In: Cherniack NS, Widdicombe JG (eds) Handbook of physiology, vol 2, Chap. 17. American Physiological Society, Bethesda, pp 595–619

Wasserman K, Whipp BJ, Kotal SN, Cleary MG (1975) Effect of cardio body resection on ventilatory and acid-base control during exercise. J Appl Physiol 39:354–358

Williamson JW, Fadel PJ, Mitchell JH (2006) New insights into central cardiovascular control during

exercise in humans: a central command update. Exp Physiol 91:51–58

Williamson JW, McColl R, Mathws D, Mitchell JH, Raven PB, Morgan WP (2002) Brain activation by

central command during actual and imagined handgrip under hypnosis. J Appl Physiol 92:1317–

1324

Yamanaka R, Yunoki T, Arimitsu T, Lian CS, Afroundeh R, Matsuura R, Yano T (2012) Relationship

between effort sense and ventilatory response to intense exercise performed with reduced muscle

glycogen. Eur J Appl Physiol 112: 2149–2162

Yamanaka R, Yunoki T, Arimitsu T, Lian CS, Yano T (2011) Effects of sodium bicarbonate ingestion on

EMG, effort sense and ventilatory response during intense exercise and subsequent active recovery.

Eur J Appl Physiol 111:851–858

Yunoki T (2012) Mental processes and breathing during exercise. J Phys Fitness Sports Med 3: 357–362.

Yunoki T, Horiuchi M, Yano T (1999) Kinetics of excess CO_2 output during and after intensive exercise.

Jpn J Physiol 49:139–144

Yunoki T, Matsuura R, Arimitsu T, Yamanaka R, Kosugi S, Lian CS, Yano T (2009) Effects of

awareness of change in load on ventilatory response during moderate exercise. Respir Physiol

Neurobiol 169:69–73

Zinker BA, Britz K, Brooks GA (1990) Effects of a 36-hour fast on human endurance and substrate

utilization. J Appl Physiol 69: 1849–1855

Figure Legends

Fig. 1. Overview of the intense exercise test (IET). Subjects performed an intense exercise (IE) twice (IE$_{1st}$ and IE$_{2nd}$) 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$_{1st}$ trial (open circles) and IE$_{2nd}$ trial (filled circles). Data presented are means ± SE. * Significant difference vs. IE$_{1st}$ (P < 0.01).

Fig. 3. Changes in O$_2$ uptake ($\dot{V}O_2$), CO$_2$ output ($\dot{V}CO_2$), ventilation ($\dot{V}E$), and predicted arterial CO$_2$ pressure ($PaCO_{2pred}$) during intense exercise (IE) in the IE$_{1st}$ trial (open circles) and IE$_{2nd}$ trial (filled circles). Data presented are means ± SE. * Significant difference vs. IE$_{1st}$ (P < 0.05).

Fig. 4. Relationship between ventilation ($\dot{V}E$) and CO$_2$ output ($\dot{V}CO_2$) during intense exercise (IE) in the IE$_{1st}$ trial (open circles) and IE$_{2nd}$ trial (filled circles). The relationship was obtained from mean values of $\dot{V}E$ and $\dot{V}CO_2$ for the group.
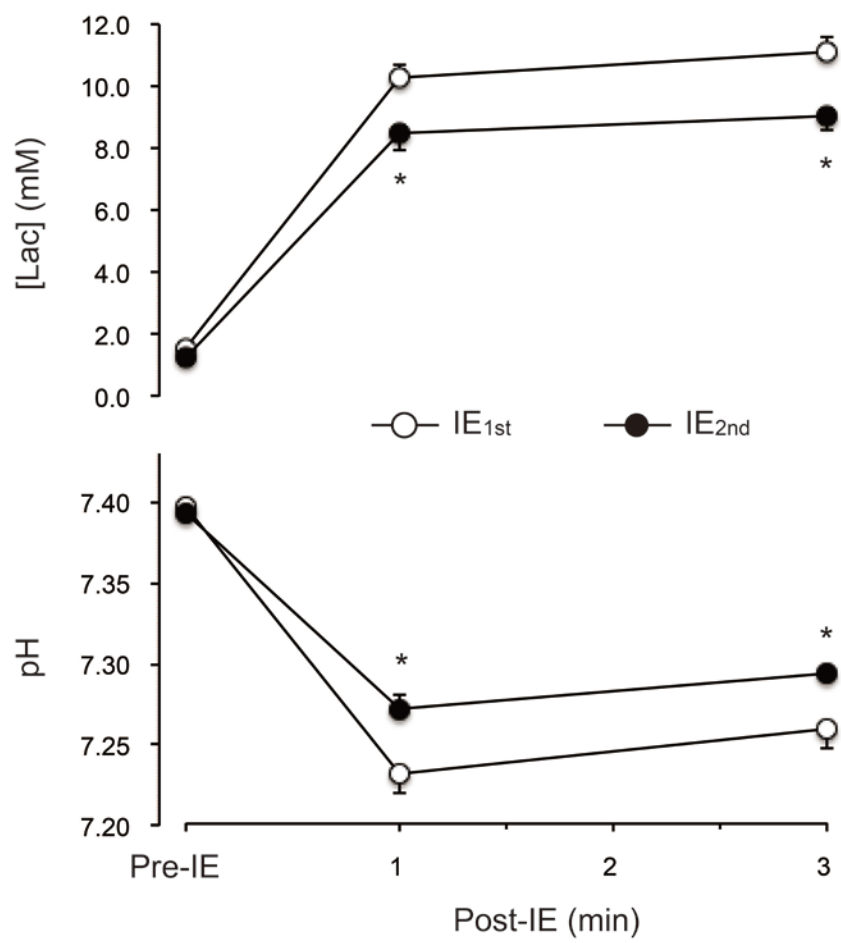
Fig. 5. Changes in motor evoked potentials (MEPs) induced by transcranial magnetic stimulation (TMS) during intense exercise (IE) in the IE$_{1st}$ trial (open circles) and IE$_{2nd}$ trial (filled circles). MEPs were expressed as percentage of maximal M-wave (Mmax) induced by magnetic motor nerve stimulation (MNS). Data presented are means ± SE. * Significant difference vs. IE$_{1st}$ (P < 0.05).
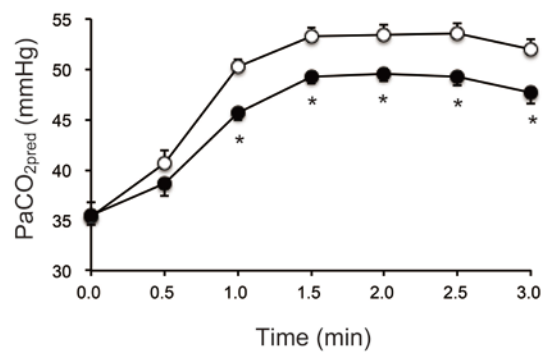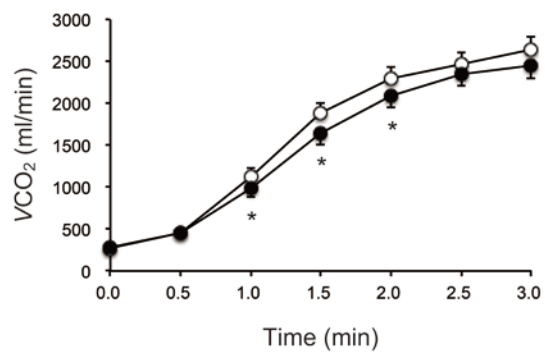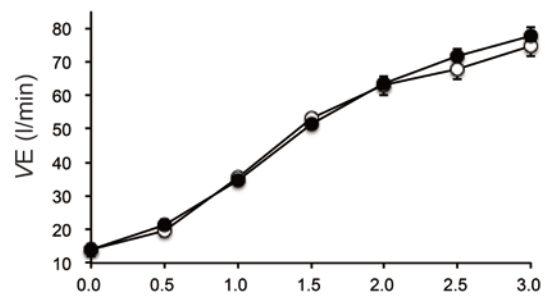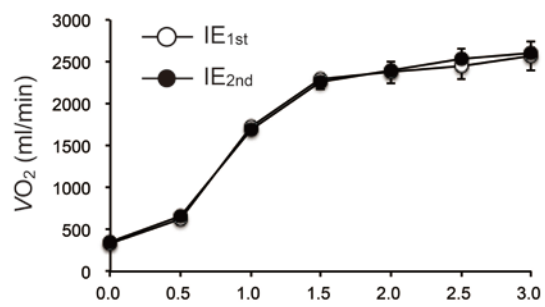
Fig. 6. Changes in effort sense of legs (ESL) during intense exercise (IE) in the IE_{1st} trial (open circles) and IE_{2nd} trial (filled circles). Data presented are means ± SE. * Significant difference vs. IE_{1st} (P < 0.05).
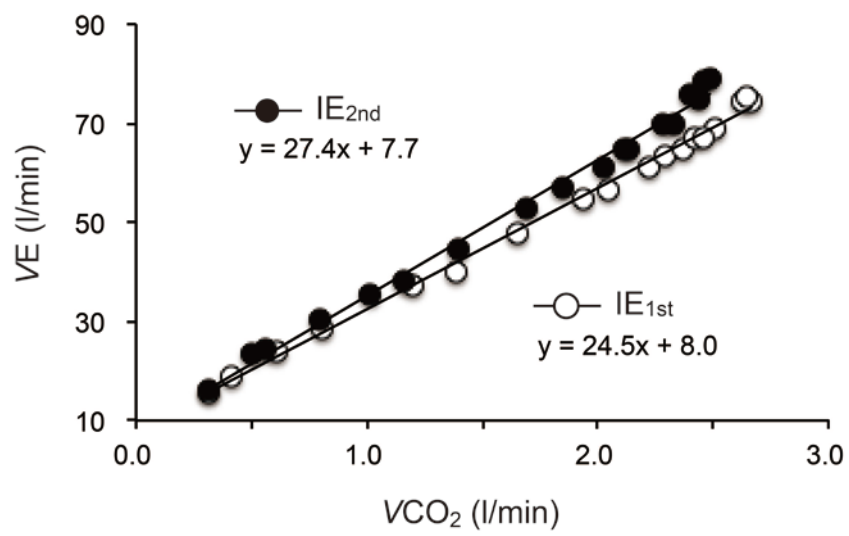
Fig. 7. Relationship between motor evoked potentials (MEPs) and effort sense of legs (ESL) during intense exercise (IE). MEPs were expressed as percentage of maximal M-wave (Mmax). ΔMEP denotes the difference in mean MEPs during IE between the two conditions. Similarly, ΔESL denotes the difference in 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.
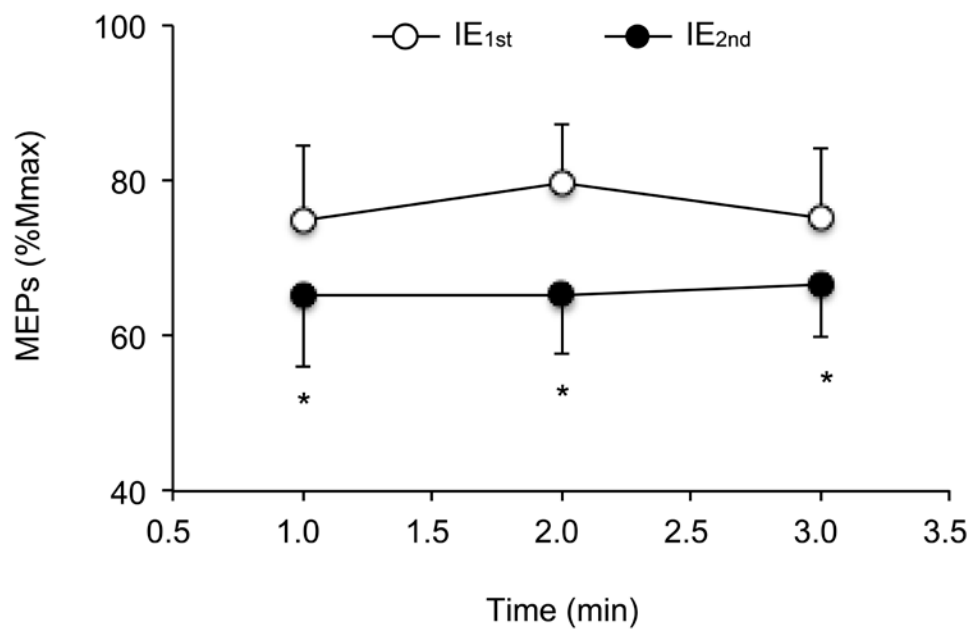
Measurement of physiological
and psychological data

MNS   TMS   MNS   TMS

| | | IE 1st | 10-min recovery | 45-min submaximal exercise | | 60-min recovery | | | IE 2nd | rest |

-3   0   3   + 10   + 45   -3   0   3   + 3

rest   rest   (min)
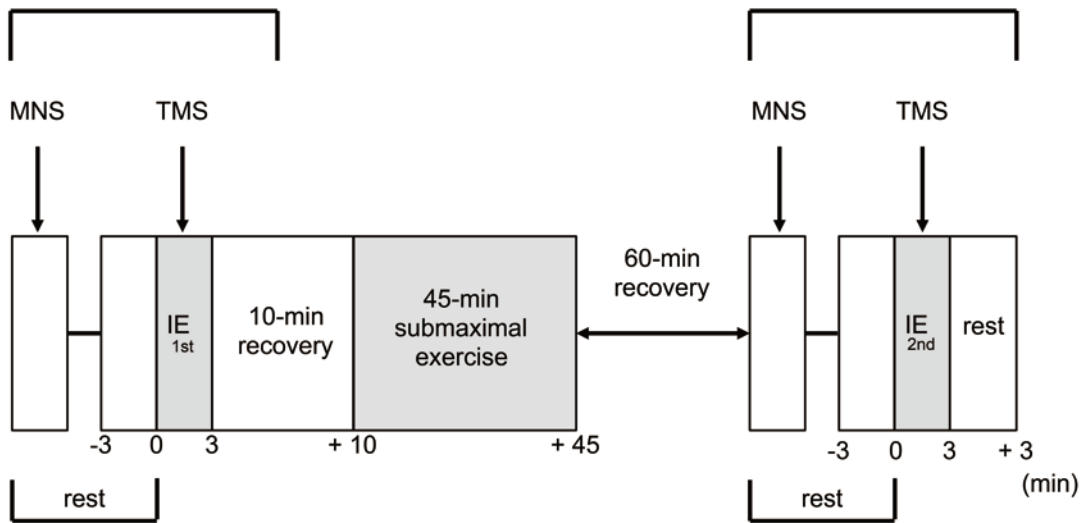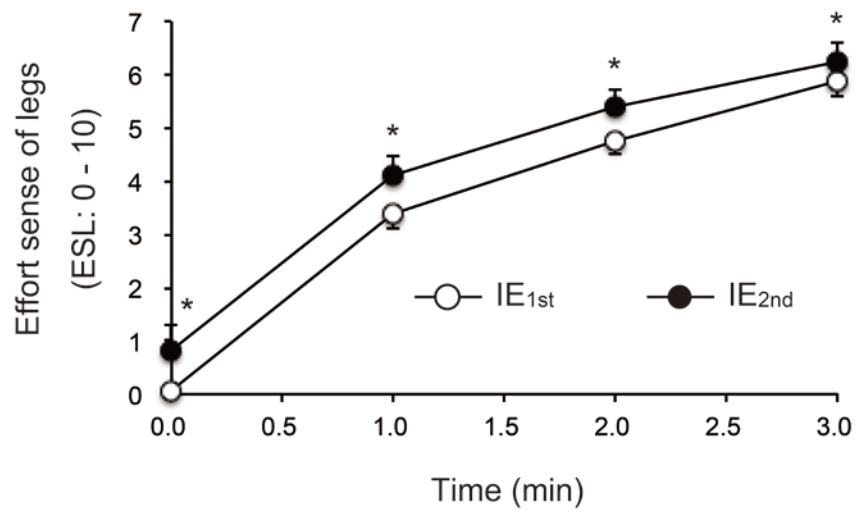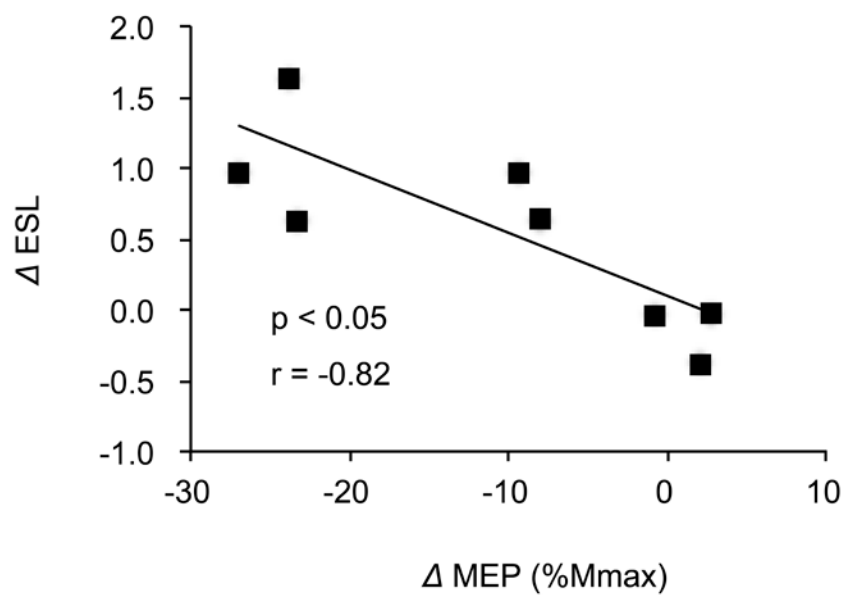
Table 1. Group mean data of blood variables

	IE_{1st}	IE_{2nd}
$[Glu]_{rest}$ (mg/dl)	111.6 ± 6.1	86.8 ± 4.8 *
$[Lac]_{highest}$ (mM)	11.1 ± 0.5	9.1 ± 0.5 *
pH_{lowest}	7.232 ± 0.012	7.272 ± 0.008 *

Values are means ± SE. $[Glu]_{rest}$, glucose level at rest before IE; $[Lac]_{highest}$, the highest value of lactate level in response to IE; pH_{lowest}, the lowest value of pH in response to IE.

* Significant difference vs. IE_{1st} ($p < 0.01$).