Effect of inspiratory muscle loaded exercise training on peak oxygen uptake and ventilatory response during incremental exercise under normoxia and hypoxia

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Abstract

Background: Although numerous studies have reported the effect of inspiratory muscle training by attempting to improve exercise performance, the outcome of whether exercise performance is improved by inspiratory muscle training is controversial. Therefore, this study investigated the influence of inspiratory muscle-loaded exercise training (IMLET) on VO2peak, respiratory responses, and exercise performance under N and H exercise conditions. We hypothesized that IMLET enhances respiratory muscle strength and improves respiratory response, thereby VO2peak and work capacity under H condition.

Methods: Sixteen university track runners (13 men and 3 women) were randomly assigned to the IMLET (n=8) or exercise training (ET) group (n=8). All subjects underwent 4 weeks of 20-min 60%VO2peak cycling exercise training, thrice per week. IMLET loaded 50% of maximal inspiratory pressure (PImax) during exercise. At pre- and post-training periods, subjects performed exhaustive incremental cycling under normoxic (N; 20.9 ± 0%) and hypoxic (H; 15.0 ± 0.1%) conditions.

Results: Although PImax and maximal expiratory pressure significantly increased after training in both groups, the extent of PImax increase was significantly higher in the IMLET group (44.5 ± 27.2% vs. 14.9 ± 11.8%; P<0.05). In both groups, VO2peak and maximal work load (Wmax) similarly increased both under N and H conditions after training (P<0.05). Further, the extent of Wmax decrease under H condition was smaller only in the IMLET group at post-training test than at pre-training (from -14.7 ± 2.2% to -12.5 ± 1.7%; P<0.05). Maximal minute ventilation in both N and H conditions increased after training than in the pre-training period.

Conclusions: Our IMLET enhanced respiratory muscle strength, and the effect of hypoxia on work capacity was attenuated with enhancing the respiratory response.

Background

There are some difficulties in attaining success in hypoxic training for improving sea-level competitive performance among athletes because of the lack of sufficient absolute training intensity caused by a substantial decrease in aerobic capacity.(1) In the acute hypoxic (H) condition, peak oxygen consumption (VO2peak) is reduced by impairment of gas exchange, according to a reduction in the
ambient partial O$_2$ pressure. The effect of hypoxia (2,400–3,000 m) on VO$_2$peak (2, 3) is more pronounced in subjects with higher VO$_2$peak with large decrease in hemoglobin O$_2$ saturation (SaO$_2$). Notably, individuals with a large increase in maximal minute ventilation ($V_{E_{max}}$) under acute H condition relative to normoxic (N) condition show a smaller reduction in VO$_2$peak (4, 5) suggesting that a high $V_E$ under H condition is beneficial for minimizing the reduction in VO$_2$peak. However, high $V_E$ under H condition leads to higher O$_2$ cost in the respiratory muscles than that under N condition (6).

Since respiratory muscle work during heavy exercise is accounted for 10%–15% of the whole body VO$_2$ (7) even under N condition, a compromised blood flow to the active muscle during heavy exercise was observed (8, 9) Further, diaphragm fatigue has been observed following strenuous exercise (10, 11) which limits exercise performance (12, 13) In this regard, under H condition, an increase in $V_E$ with a minimum increase in respiratory muscle work may be essential to prevent a greater reduction in the VO$_2$peak and exercise performance under H condition.

Inspiratory muscle training (IMT) has confirmed the improvement of respiratory muscle strength as a consequence of an increased cross-sectional area (14) in the diaphragm, thereby reducing respiratory muscle fatigue and dyspnea after an exhaustive exercise (15, 16) Some studies reported that IMT improved exercise performance by enhancing the ventilatory response without any change in VO$_2$peak (17, 18) Although the mechanisms underlying the improvement of exercise performance without a change in VO$_2$peak remain unknown, IMT was reported to be effective in improving the ventilatory response without any change in VO$_2$peak under H condition (15, 19, 17) However, since ventilation volume plays an important role in gas exchange under H condition, it remains a possibility that IMT can improve VO$_2$peak.

Although numerous studies have reported the effect of IMT by attempting to improve exercise performance (20–25) the outcome of whether exercise performance is improved by IMT is controversial. The discrepancy in the outcomes of IMT would be influenced by study design, training maneuver, subject fitness level, and type of exercise test. With regard to training maneuver, since
IMT is often conducted at a resting condition as well as during exercise, pulmonary ventilation is not high. Indeed, isocapnic hyperventilatory training was reported to improve exercise performance.\textsuperscript{(26, 27)} Thus, it could be speculated that the combination of an inspiratory muscle load and exercise training would be effective to improve the respiratory response, $\text{VO}_2\text{peak}$, and work capacity. In fact, McEntire et al. \textsuperscript{(28)} reported that 6 weeks of 15\%PImax inspiratory load-added exercise training at three times per week greatly improves inspiratory muscle strength and exercise tolerance compared with exercise training alone.

Therefore, this study aimed to investigate the influence of inspiratory muscle-loaded exercise training (IMLET) on $\text{VO}_2\text{peak}$, respiratory responses, and exercise performance under N and H exercise conditions. We hypothesized that IMLET enhances respiratory muscle strength and improves respiratory response, thereby $\text{VO}_2\text{peak}$ and work capacity under H condition.

**Methods**

**Subjects**

Sixteen healthy young, healthy volunteers who belong to the university track club (13 men and three women) were recruited for this study. Ten were middle and long-distance runners, and six were sprinters. All participants did not have any cardiac, pulmonary, and musculoskeletal disease. Subjects were asked to maintain their current diet and regular physical training during the study period. All participants were instructed to hold similar daily training. Before each testing day, subjects were instructed to avoid vigorous exercise and intake of alcohol and caffeine within 24 hours. Subjects were randomly divided into the IMLET group (7 men and 1 woman; age, 19.6 ± 0.9 years; height; 1.71 ± 0.09 m; body mass, 57.4 ± 4.9 kg) and exercise training (ET) alone group (6 men and 2 women; age, 19.5 ± 1.4 years; height; 1.68 ± 0.06 m; body mass, 54.5 ± 4.9 kg). The influence of the menstrual cycle could not be excluded in women; thus, pre- and post-exercise tests were performed at the follicular stage.

This study was approved by the Human Subjects Committees of the Osaka Kyoiku University in accordance with the guidelines set forth by the Declaration of Helsinki. All subjects provided verbal and written informed consent before participating in this study.
Experimental design
Initially, subjects were instructed and familiarized themselves to the test protocols and training protocol. As pre-training period tests, all subjects performed the pulmonary function test, respiratory strength test under N condition. Incremental cycling tests (see below for details) were performed under N (20.9 ± 0.0%) and H (15.0 ± 0.1 %) conditions in 1 week. Incremental cycling tests were separately performed in random order with at least 48-hour intervals. After the baseline test, all subjects completed 4 weeks of training period. Exercise training was conducted 3 days per week for a total of 12 sessions. Since participants conducted athletic training five times per week, IMLET and ET sessions were held at three times per week. After the training period, subjects performed the same tests as those at baseline (post-training period tests).

Exhaustive incremental exercise test
All subjects performed incremental cycling tests until exhaustion in our laboratory (placed at 132 m above sea level) under N and H conditions. The room temperature was 20.2 ± 0.4°C, and the room was continuously ventilated to minimize increases in CO₂ concentration in the air. Under both conditions, inspiratory gas was supplied through a pipe from large Douglas bags (700 L). Subjects breathed through a mouthpiece attached to a pneumotachograph flowmeter (MLT1000L; AD-Instrument, Sidney, Australia) and a two-way valve (2700; Hands Rudolph, Inc., KS, USA). Subjects started to breathe air 3 min before starting recording of measurements. The incremental cycling test was performed on a cycle ergometer (E-828; Monark, Vansbro, Sweden). Before beginning the exercise, subjects were kept at rest to obtain resting values; subsequently, subjects performed 5 min of warm-up cycling at 30 watts. The pedaling rate was set at 60 rpm. The initial pedaling load was set at 60 watts. A pedaling load was subsequently increased by 15 watts every 1 min until exhaustion. The achievement of exhaustion was assumed when the pedaling rate was below 55 rpm despite strong verbal encouragement. Metabolic and ventilatory variables were calculated using the metabolic cart (AE-310s; MINATO Medical; Osaka, Japan). The variables were measured breath-by-breath and calculated as 60 sec. All subjects accomplished two of the following three criteria for
VO₂peak: VO₂ did not increase further despite increases in work load (increase in <2.0 ml kg⁻¹ min⁻¹) (achieved by 63% of the subjects), respiratory quotient was >1.1 (achieved by 93% of the subjects), or maximal heart rate was >90% of the age-predicted value (achieved by 93% of the subjects). Moreover, all subjects have indicated 20 on the Borg scale and were exhausted despite strong encouragement.

To estimate the work of breathing (WOB), esophageal and mouth pressures were measured using a pressure transducer catheter (MicroSensor Basic Kit: Codman & Shurtleff, Inc., MA, USA). Airflow was measured using a pneumotachograph meter (MLT1000L; AD-Instrument; Sidney, Australia). The variables were recorded at a sampling rate of 200 Hz using Power Lab (Power Lab 8/35; AD-Instrument, Sydney, Australia) and analysis software (LabChart 7; AD-Instrument, Sydney, Australia). Before and after the test measurements, a pressure transducer catheter was carefully calibrated. To obtain calibration signal, a pressure transducer catheter was immersed to 0–60 cm depth in a darken pipe with water. A pneumotachograph meter was calibrated by using a 3L calibration syringe. An esophageal pressure catheter was inserted from the nasal passage to a distance 1/4 of the height minus 9 cm through a nasal cavity and subsequently further inserted to the stomach where the esophageal pressure was confirmed to be changing from negative to positive. Thereafter, the catheter was carefully withdrawn to keep the negative pressure at rest condition (within −1 to −10 cmH₂O). The mouth pressure catheter was fixed to the mouthpiece. Trans-pulmonary pressure was calculated as a difference between the esophageal pressure and mouth pressure. Since the participants often have a failure to inspiratory capacity maneuver, the drift in the system of a lung volume was not followed. Thus, the lung volume was reset at the end of the expiration flow. Estimated WOB was calculated as an integration of trans-pulmonary pressure and volume curve. We assessed the tidal volume (VT), respiratory frequency (fR), Vₑ, trans-pulmonary pressure, peak expiratory flow rate (PEFR), and WOB every 60 sec.

Heart rate was measured using the three-lead electrocardiogram (FE132: AD-Instrument; Sidney, Australia). SaO₂ was measured using a forehead pulse oximeter (N−560; Covidien; CA, USA). Maximal
achieved work load ($W_{max}$) and time to exhaustion were measured to assess the exercise performance.

**Pulmonary function and respiratory muscle strength tests**

Pulmonary function and respiratory muscle strength were assessed by spirometry (AS–507, MINATO Medical, Osaka, Japan) according to manufacturer instruction. The subjects were familiarized with the test maneuver before the test day to avoid the learning effect. The vital capacity (VC), forced vital capacity (FVC), and forced expiratory volume in 1 second ($FEV_{1.0}$) were measured at least three trials. The subjects performed the test at a standing position wearing a nose clip. The highest value of 3–4 trials was taken as the value for each parameter. The pulmonary function assessment also included the maximal voluntary ventilation performed at 12 sec of maximal ventilatory effort. Respiratory muscle strength was assessed using a handheld mouth pressure meter (AS–507, MINATO Medical, Osaka, Japan). The respiratory muscle strength was assessed as the static maximal inspiratory and expiratory mouth pressure. All maneuvers were performed in the standing position. The subjects were familiarized with the test maneuver before each testing day to avoid the learning effect. The inspiratory muscle strength was evaluated by maximal inspiratory mouth pressure ($P_{Imax}$) measurement. Initially, subjects expired slowly to residual volume (RV) and then performed maximal inspiratory effort from RV. The expiratory muscle strength was evaluated by the maximal expiratory mouth pressure ($P_{Emax}$) measurement. Initially, subjects inspired slowly to total inspiratory capacity (IC) and then performed maximal expiratory effort from IC. Both $P_{Imax}$ and $P_{Emax}$ values were calculated as a mean value of 1 sec including the highest value when maximal pressure was held at least 1.5 sec. The values were assigned as the best of at least five satisfactory efforts. Subjects were given verbal encouragement for performing the maximal effort.

**Training protocol**

The subjects were assigned to the IMLET group or ET group. At the training day, the IMT group performed 20 min of cycling exercise at 60%$VO_{2peak}$ with inspiratory pressure load breathing at
50%PImax by using inspiratory muscle trainer (POWERbreatheK5; IMT Technologies, Birmingham, UK) through the exercise. POWERbreatheK5 requires a predetermined inspiratory pressure throughout the inspiration for opening the electrically controlled shutter, while expiration was not resisted. The subjects were not given any instruction about breathing depth, frequency, or volume during IMT exercise. Previous studies have reported that a load of the 50%PImax of IMT elicits an adaption to the respiratory muscles.(21, 23) Inspiratory load was calculated from the pre-training period muscle strength test of PImax, and this load was used throughout the IMLET. Meanwhile, the ET group performed 20 min of cycling exercise at 60%VO2peak without any resistive inspiratory pressure.

Statistical analysis
Data are expressed as mean ± standard deviation. SPSS 25 (IBM, Armonk, NY, USA) was used for all statistical analyses. Variables obtained by respiratory function test were analyzed with a two-way repeated-measures analysis of variance (ANOVA) with training period variables (pre vs. post-training) and subject groups (IMLET vs. ET). Variables obtained during the incremental exercise test were analyzed with three-way repeated-measures ANOVA with factors of the experimental conditions (H vs. N), training period, and subject groups. The comparisons of the extent of change by conditions or training period were analyzed with two-way repeated-measures ANOVA with the training period variables and subject groups. After detecting the main effect, post-hoc Bonferroni multiple comparisons were performed. Paired t-tests were used to compare the variables of pairwise comparison. Effect size (ES) was calculated when a significant difference was found. P values < 0.05 were considered statistically significant.

Results
Participant characteristics are shown in Table 1. No differences in physical and fitness at the pre-training measurement variables were found between the groups. Moreover, no significant differences in age, height, weight, PImax, and VO2peak at pre-test were observed between the IMLET and ET groups.

Respiratory function and respiratory muscle strength
The results of the pulmonary function test are shown in Table 2. Only VC significantly increased by
4.7± 4.2% after post-training vs at pre-training in the IMELT group ($P < 0.05$, $d = 0.48$). Further, FVC significantly increased in both training groups (2.2% in IMLET and 2.5% in ET) (ANOVA, $P < 0.05$, partial $\eta^2_p = 0.34$).

The results of the Plmax and PEmax are depicted in Figure 1. Plmax significantly increased after post-training vs pre-training in both the IMLET group (44.6 ± 27.3%; $P < 0.05$, $d = 1.96$) and ET group (14.9 ± 11.8%; $P < 0.05$, $d = 0.68$) (ANOVA, $P < 0.05$, $\eta^2_p = 0.77$). The extent of increase in Plmax following training was significantly greater in the IMLET group than in the ET group ($P < 0.05$, $d = 1.41$). PEmax significantly increased at post-training vs pre-training in both the IMLET group (21.6 ± 25.4%; $P < 0.05$, $d = 0.82$) and the ET group (15.6 ± 12.1%; $P < 0.05$, $d = 0.81$) (ANOVA, $P < 0.05$, $\eta^2_p = 0.49$). No difference in the extent of increase of PEmax was found between the IMLET and ET groups.

**Incremental exercise test under normoxia**

Table 3 and Figure 2 show the variables of the exhaustive incremental test under N condition. At post-training, $\text{VO}_2\text{peak}/\text{w}$ under N condition increased by 9.2 ± 7.8% in the ET group compared with that at pre-training ($P < 0.05$, $d = 0.67$, Fig. 2), whereas in the IMLET group, it increased by 11.0 ± 13.5%, but failed to reach significance ($P = 0.058$, $d = 0.97$) (ANOVA, $P < 0.05$, $\eta^2_p = 0.51$, Fig. 2). The percentage of improvement of $\text{VO}_2\text{peak}/\text{w}$ by the training was not significantly different between the training groups ($P = 0.46$, $\eta^2_p = 0.04$). Both $W_{\text{max}}$ (6.4 ± 3.7% in the IMLET; 6.4 ± 7.0% in ET, $P < 0.05$, $\eta^2_p = 0.75$) and time to exhaustion (5.4 ± 1.7% in IMLET; 4.5 ± 5.6% in ET, $P < 0.05$, $\eta^2_p = 0.75$) significantly increased at post-training compared those at pre-training (Fig. 2). $V_{\text{Emax}}$ significantly increased by 16.7 ± 9.7% in the IMLET group at post-training vs pre-training ($P < 0.05$, $d = 0.82$). Further, the extent of increase in $V_{\text{Emax}}$ at post-training vs pre-training was significantly greater in the IMLET group than in the ET group ($P < 0.05$, $\eta^2_p = 0.48$). No significant difference in WOB was found between before and after training in both training groups. Both Plpeak and PEpeak were not
changed by the training in both training groups, while PEFR significantly increased by 20.5 ± 23.4% after the training in the IMLET group ($P < 0.05, d = 0.76$).

**Incremental exercise test under hypoxia**

Table 4 and Figure 2 show the variables of the exhaustive incremental test under H condition. $VO_{2\text{peak}}/w$, $W_{\text{max}}$, and time to exhaustion were significantly lower in the H condition than in the N condition, both at pre- and post-test in all subjects (Fig. 2). Especially, the percentage of decrease in $W_{\text{max}}$ under H condition ($%dW_{\text{max}}$) at pre- and post-training tests for the IMLET groups was smaller at post-training than that pre-training (-14.7 ± 2.2% at pre vs. -12.5 ± 1.7% at the post, Fig. 3) ($P < 0.05, d = 1.14$). All subjects of IMLET group showed reduced $%dW_{\text{max}}$ at post-training compared with that at pre-training. In the ET group, six of eight subjects showed a greater $%dW_{\text{max}}$ at post-training than at pre-training (-11.7± 6.4 % in pre-test vs. -12.1 ± 7.0% in post-test). $VO_{2\text{peak}}$ under H condition increased after the training period in both groups (12.9 ± 13.8% in the IMLET group, 7.4 ± 8.8% in the ET group) (ANOVA, $P <0.05, \eta_{p}^{2} = 0.51$). The extent of improvement of $VO_{2\text{peak}}$ by the training was not significantly different between the training groups. Both maximal $W_{\text{max}}$ (9.3 ± 3.6% in IMLET, 5.9 ± 5.2%; $P < 0.05$) (ANOVA, $P < 0.05, \eta_{p}^{2} = 0.75$) and time to exhaustion (6.2 ± 4.0% in IMLET, 39. ± 3.4% in CONT; $P < 0.05$) (ANOVA, $P < 0.05, \eta_{p}^{2} = 0.75$) under H condition significantly increased after training compared to those at baseline. $V_{E\text{max}}$ under H condition was significantly higher only in the IMLET group at post-training than at pre-training (+16.4 ± 8.2%; $P < 0.05, d = 0.72$). WOB was increased by 21.9 ± 21.1% at post-training than at pre-training in the ET groups ($P = 0.05, d = 0.84$). The extent of increase in WOB in the IMLET group was 22.5 ± 39.5%, but there was no significance ($P = 0.18, d = 0.71$). Both $P_{\text{Ipeak}}$ and $P_{\text{Epeak}}$ values were not changed by the training in both training groups, while PEFR significantly increased by 23.9 ± 31.2% post-training in the IMLET group ($P < 0.05, d = 1.07$).

**Discussion**

To the best of our knowledge, this study is the first to assess the effect of IMLET on ventilatory
response and VO$_{2peak}$ under hypoxia. We hypothesized that IMLET would improve the respiratory response and WOB under H condition, resulting in improved gas exchange. Thus, we also hypothesized that IMLET could improve VO$_{2peak}$ and work capacity under H condition. First, we found that although either IMLET or ET enhanced the inspiratory and expiratory muscle strength (Figure 1), the magnitude of improvement in inspiratory muscle strength was greater in the IMLET group than in the ET group. Second, under both N and H conditions, VO$_{2peak}$ and W$_{max}$ increased in both training groups (Figure 2); however, V$_{E_{\text{max}}}$ increased only in the IMLET group. Under H condition exercise, although similar changes in W$_{max}$ in both the training groups were seen, the magnitude of decrease in W$_{max}$ under H condition was significantly smaller only in the IMLET groups (Figure 3). Our results suggest that IMLET changes the ventilatory response under H condition and suppress the extent of decrease in W$_{max}$ in H condition.

**Respiratory muscle strength**

Previous studies have reported that 4–10 weeks of the respiratory muscle training enhances PImax by approximately 20%–30%.(15, 20–22, 29) Our IMLET caused a 44% increase in PImax, suggesting that the respiratory muscle strength much improved within a short period. McEntire et al. (28) observed an increase in PImax by 28% following moderate-intensity exercise training with 15%PImax inspiratory loads, although PImax also significantly increased by 6 weeks of 30-min cycling exercise training at 70% peak work rate alone. Compared with that of McEntire et al. (28), we observed approximately three times greater increase in PImax by 50%PImax inspiratory-loaded exercise training. Both McEntire et al. (28) and the present study observed an improvement of PImax in both the IMLET group and ET group. Some previous studies observed increasing respiratory muscle strength caused by strenuous exercise training itself.(30–32) However, we found greater increases in PImax in the IMLET groups than in the ET group, implying that more than 50%PImax inspiratory load needs to highlight the additional influence of inspiratory load on respiratory muscle strength. Interestingly, PEmax also improved after training in both the IMLET and ET groups. Thus, it is speculated that the increase in PEmax after the training period test is caused by the exercise training per se. More likely, although
we did not add expiratory load on the exercise training, the diameter becomes smaller because the mouth is squeezed to hold the inspiratory muscle trainer; thus, a possibility that slight pressure was applied to the expiration was present. Increasing respiratory muscle endurance was observed in respiratory muscle training, especially with hyperventilatory training,(24, 26, 27, 24) although we did not evaluate the respiratory muscle endurance.

Effect of IMLET on normoxic exercise

With regard to exercise performance under N condition, numerous studies have observed that the exercise performance improved following the respiratory muscle training (21–23, 26, 33), while others disagreed.(15, 25) Since there was no significant difference in the extent of improvement in $W_{\text{max}}$ between the IMLET group and the ET group, the improvement in $W_{\text{max}}$ caused by the exercise training would not augment, even applying an inspiratory load during the training.

Hyperventilation helps maintain $\text{SaO}_2$ during intensive exercise even under normoxia among the subjects with exercise-induced arterial hypoxemia,(34) implying that the gas exchange partly limits the $\text{VO}_2\text{peak}$. Some studies reported that IMT improved exercise performance related to enhanced ventilatory response.(17,18). Our result of the increasing $V_{\text{Emax}}$ under normoxia following IMLET is consistent with those of previous studies.(19, 25, 35) However, this increase in $V_{\text{Emax}}$ by IMLET did not contribute to the improvement of $\text{SaO}_2$ and $\text{VO}_2\text{peak}$, hence the exercise performance, because our subjects did not express arterial desaturation during maximal exercise. For instance, under N condition, $\text{VO}_2\text{peak}$ did not change even if subjects were breathing helium-$\text{O}_2$ mixtures with lowered air flow resistances.(36)

Effect of IMLET on hypoxic exercise

Esposito et al. (19) reported that $V_{\text{Emax}}$ under H condition (11% $\text{O}_2$) was increased following IMT compared with pre-training test, which is in agreement with our results. On the contrary, Downey et al. (15) reported that $V_{E}$ under H condition (14% $\text{O}_2$) during $\sim$85%$V_{\text{O}_2}\text{peak}$ submaximal running was reduced following IMT. This disagreement of response in $V_{E}$ following IMT might be due to a difference
in test exercise intensity (maximal vs. submaximal). We observed that $V_E$ was not significantly
different between pre- and post-exercise tests when compared with the same absolute work load (at
$W_{max}$ in pre-test) (not shown in the results; 104.8±22.0 L min$^{-1}$ in pre vs. 100.5±26.6 L min$^{-1}$ in post;
IMLET group). With this, we can speculate that an increase in $V_{E_{max}}$ after IMLET was a result of by an
increase in work load after IMLET.[]
A high VE under H condition appears to be beneficial for minimizing the reduction in $VO_2$peak,(4, 5)
while WOB and oxygen cost of breathing should be higher with an increase in $V_E$ (6) compared to
those under N condition. Further, higher WOB would elicit respiratory muscle fatigue (37, 38).
Accordingly, we hypothesized that the benefit of IMLET would be emphasized under hypoxic exercise
condition rather than under normoxic exercise condition. Previous studies have reported that the IMT
did not change the $VO_2$peak under H condition.(15, 19) We observed an increase in $VO_2$peak in both
training groups following the training period. Similar to the N condition, we cannot deny that the
increase in $VO_2$peak in the H condition was caused by the exercise training itself. Interestingly,
$VO_2$peak increases without any increase in SaO$_2$ in both groups after training, which indicates that the
magnitude of increase in $V_E$ after IMLET is inadequate for improving the alveoli gas exchange. Thus,
the increase in $VO_2$peak may be the result of exercise training causing circulatory function and
peripheral adaptation. In fact, %d$VO_2$peak under H condition did not change even after IMLET. We
should speculate one possibility that increase in $V_{E_{max}}$ by IMLET was a result of an increase in
exhaustion intensity by the training.
High $V_E$ under H condition leads to higher O$_2$ cost in respiratory muscles with an increase of
inspiratory flow resistive work and expiratory flow-limitation (37). This would lead to a compromised
blood flow to active muscles during heavy exercise.(8, 9) Recently, we have reported that in
exhaustive incremental running, higher $V_{E_{max}}$ and exercise performance without any change in
$VO_2$peak under hypobaric normoxic condition (492 mmHg with 32.2% O$_2$ gas inhalation) than
normobaric normoxia (760 mmHg) and estimated respiratory muscle VO$_2$ reduced by 23% under
Hypobaric normoxia, suggesting that lower air density-related reduction of respiratory load affects exercise performance. (39) Further, Downey et al. (40) reported that during 85% VO_{2peak} running under H condition (14% O_2), VO_2 and cardiac output reduced after IMT. In this line, we hypothesized that if IMLET reduced WOB against hyperventilation, the reduction in exercise performance under H condition would be attenuated. Our results that %dW_{max} was significantly smaller after training in the IMLET group partly supports the hypothesis that IMLET increased oxygen transport, such as increased blood flow to active muscles. Further, our result of an increase in V_{Emax} without any significant increase in WOB in H condition following IMLET, not seen in the ET group, implies that the participants can more hyperventilate with similar ventilatory effort after IMLET. Indeed, PEFR and VT at post-training test were higher than those at pre-training test, implying that breathing pattern is altered and increased with elastic work (i.e., recoiled energy work by chest wall inflation). However, we must emphasize the fact that WOB increased by 16% after IMLET. It is suspected that reduced %dW_{max} after IMLET is a result of reduced limb muscle fatigue due to unloading at submaximal intensity; eventually, the distribution of blood flow in the whole body VO_2 at maximal intensity was not different before and after training. Further investigation is warranted to clarify this point.

**Limitations**

As a limitation in the present study, we did not fully record subject’s daily workout habits. However, subjects were instructed not to change their usual daily activities and not to participate in strenuous exercises. In addition, most subjects belonged to the university track club, and similar exercise training was performed in both the IMLET group and ET group, except that in the laboratory.

**Conclusions**

Our results suggest that the exercise training with 50%PImax inspiratory load could improve inspiratory muscle strength and ventilatory response during hypoxic exercise. The extent of W_{max} decrease under H condition was smaller only in the IMLET group. This implied that IMLET can prevent an excessive decline in exercise performance under acute H conditions. MLET will be advantageous to attain successful altitude training and prevent altitude sickness. The application of both IMLET and
altitude training will be the focus of a future study.

Abbreviations
IMLET Inspiratory muscle loaded exercise training
IMT Inspiratory muscle training
PEmax Maximal expiratory mouth pressure
PEpeak Peak expiratory pressure
\( P_{ETCO_2} \) End-tidal partial pressure of \( CO_2 \)
\( SaO_2 \) Arterial oxyhemoglobin saturation.
\( V_E \) Minute ventilation
\( V_{Emax} \) Maximal minute ventilation
\( VO_2 \) Oxygen uptake
\( VO_2peak \) Peak oxygen uptake
\( VTT \) Tidal volume
WOB Work of breathing
\( W_{max} \) Maximal work load

Declarations

Ethics approval and consent to participate
This study was approved by the Human Subjects Committees of the Osaka Kyoiku University in accordance with the guidelines set forth by the Declaration of Helsinki. All subjects provided verbal and written informed consent before participating in this study.

Consent for publication
Not applicable

Availability of data and materials
Not applicable

Competing interests
The authors declare that they have no competing interests.

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Authors’ contributions

T. O. and M. N. conceived and designed the experiments. T. O. and M. N. performed the data analysis.

T. O. and N. F. drafted the manuscript. All authors contributed to the data collection and interpreted the experimental results. All authors edited and revised the manuscript and approved the final version of the manuscript.

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Tables
Table 1: Variables of the characteristics of subjects

|                  | IMLET (n=8) | ET (n=8) | P value |
|------------------|-------------|----------|---------|
| sex [M / W]      | 6/2         | 5/3      | 0.82    |
| Age [years]      | 19.6 ± 0.9  | 19.5 ± 1.4 | 0.82   |
| Height [cm]      | 171.3 ± 8.9 | 167.5 ± 5.5 | 0.31   |
| Weight [kg]      | 57.4 ± 4.9  | 54.5 ± 4.9 | 0.30   |
| PI_{max} [cmH2O]| 101 ± 15    | 104 ± 22  | 0.64    |
| VO_{2peak} [ml kg^{-1} min^{-1}] | 55.1 ± 7.2 | 53.4 ± 7.7 | 0.45   |

Values are mean ± SD. PI_{max}: maximal inspiratory pressure, VO_{2max}: maximal oxygen consumption.

Table 2 Variables of pulmonary function test
|                          | IMLET (n=8) | ET (n=8) |
|--------------------------|------------|----------|
|                          | pre        | post     | pre      | post     |
| Vital capacity [L]       | 4.1 ± 0.4  | 4.3 ± 0.5* | 4.2 ± 0.7 | 4.2 ± 0.7 |
| Force vital capacity [L] | 4.1 ± 0.4  | 4.2 ± 0.5* | 4.0 ± 0.6 | 4.1 ± 0.6* |
| Force expiratory volume  | 3.7 ± 0.4  | 3.7 ± 0.3  | 3.7 ± 0.5  | 3.7 ± 0.5  |
| in one second [L]        |            |          |          |          |
| Maximal voluntary        | 143 ± 31   | 148 ± 33  | 120 ± 35  | 134 ± 26  |
| ventilation by 12        |            |          |          |          |
| seconds [L min⁻¹]        |            |          |          |          |

Values are mean ± SD. * significantly different from pre-training.

Table 3 Maximal values during incremental test under normoxia

|                          | IMLET(n=8) | ET(n=8) |
|--------------------------|------------|----------|
|                          | pre        | post     | pre      | post     |
| $V_{Emax}$ [L min⁻¹]     | 109.6 ± 20.6 | 127.6 ± 23.1* | 106.2 ± 16.7 | 109.9 ± 19.7 |
| $f_R$ [breath min⁻¹]     | 50.9 ± 9.7  | 53.7 ± 11.7 | 49.2 ± 8.5  | 48.3 ± 9.3  |
| $VT$ [L]                 | 2.47 ± 0.33 | 2.81 ± 0.45 | 2.30 ± 0.38 | 2.42 ± 0.41 |
| $VE$ VO₂⁻¹               | 34.58 ± 2.6 | 36.63 ± 2.86 | 37.08 ± 5.33 | 34.69 ± 5.04 |
| $VE$ VCO₂⁻¹              | 31.9 ± 2.9  | 33.9 ± 2.2* | 32.4 ± 3.3  | 32.8 ± 3.4  |
| $P_{ETCO₂}$ [mmHg]       | 40.9 ± 2.2  | 37.2 ± 2.3* | 40.0 ± 3.7  | 38.7 ± 3.1  |
| $WOB$ [J min⁻¹]          | 276 ± 87    | 318 ± 98   | 258 ± 66   | 288 ± 97   |
| $PEpeak$ [cmH₂O]         | 24.5 ± 11.2 | 23.2 ± 7.0  | 26.8 ± 9.6  | 26.4 ± 11.2 |
| $Plpeak$ [cmH₂O]         | -27.9 ± 22.8 | -37.9 ± 9.7 | -35.6 ± 5.3 | -38.2 ± 10.0 |
| $PEFR$ [L sec⁻¹]         | 6.39 ± 1.56 | 7.54 ± 1.46* | 6.66 ± 1.65 | 6.63 ± 1.39 |
| $HR_{max}$ [beat min⁻¹]  | 185.5 ± 7   | 187.7 ± 7.5 | 184.3 ± 10.4 | 184.5 ± 14.4 |
| $SaO₂$ [%]               | 96 ± 3      | 96 ± 2     | 96 ± 2     | 97 ± 2     |

Values are mean ± SD.

$f_R$, respiratory frequency; $HR_{max}$, maximal heart rate; $PEFR$, peak expiratory flow rate; $PEpeak$, peak expiratory pressure; $P_{ETCO₂}$, end-tidal partial pressure of CO₂; $Plpeak$, peak inspiratory pressure;
pressure; SaO₂, arterial oxyhemoglobin saturation; Vₑₘₐₓ, maximal minute ventilation; VT, tidal volume; WOB, work of breathing. * significantly different from pre-training.

Table 4 Maximal values of incremental test under hypoxia

|                     | IMLET (n=8)     | ET (n=8)      |
|---------------------|-----------------|---------------|
|                     | pre             | post          | pre            | post          |
| Vₑₘₐₓ [L min⁻¹]    | 104.8 ± 22.0    | 121.8 ± 25.0* | 103.6 ± 16.4   | 107.5 ±19.7   |
| fᵣ [breath min⁻¹]  | 48.9 ± 12.1     | 50.3 ± 11.8   | 50.4 ± 8.6     | 47.6 ± 9.3    |
| VT [L]              | 2.46 ± 0.24     | 2.85 ± 0.44*  | 2.27 ± 0.43    | 2.66 ± 0.41*  |
| VE VO₂⁻¹            | 39.61 ± 3.06#   | 40.83 ± 3.80# | 40.89 ± 3.58#  | 39.80 ± 5.04# |
| VE VCO₂⁻¹           | 35.6 ± 3.7#     | 36.2 ± 2.7#   | 35.7 ± 2.9#    | 35.3 ± 3.4#   |
| Pₑ₇₇CO₂ [mmHg]      | 36.6 ± 3.0#     | 34.0 ± 2.1*   | 36.4 ± 2.8     | 35.9 ± 2.9#   |
| WOB [J min⁻¹]       | 235 ± 59#       | 276 ± 85#     | 231 ± 64#      | 299 ± 97*#    |
| PEpeak [cmH₂O]      | 22.1 ± 6.3      | 22.3 ± 12.6   | 21.6 ± 5.3     | 21.5 ± 11.2   |
| PIpeak [cmH₂O]      | -33.8 ± 4.6     | -32.7 ± 8.7   | -34.0 ± 7.3    | -39.1 ±8.3    |
| PEFR [L sec⁻¹]      | 5.81 ± 1.29     | 7.00 ± 1.53*  | 5.35 ± 0.51    | 6.54 ± 1.39   |
| HRₘₐₓ [beat min⁻¹]  | 175.1 ± 9.4#    | 178.0 ± 8.0#  | 180.4 ± 11.7#  | 181.0 ± 14.4# |
| SaO₂ [%]            | 82 ± 3#         | 82 ± 4#       | 83 ± 2#        | 84 ± 2#       |

Values are mean ± SD. fᵣ, respiratory frequency; HRₘₐₓ, maximal heart rate; PEFR, peak expiratory flow rate; PEpeak, peak expiratory pressure; Pₑ₇₇CO₂, end-tidal partial pressure of CO₂; PIpeak, peak inspiratory pressure; SaO₂, arterial oxyhemoglobin saturation; Vₑₘₐₓ, maximal minute ventilation; VT, tidal volume; WOB, work of breathing. *significantly different from pre-training. # significantly different from normoxia.

Figures
Figure 1

Respiratory muscle strength before and after training period. Following 4 weeks of training, both PImax and PEmax improved after the training period compared with those at baseline in both the IMLET and ET groups. * significantly different from pre-training.
Figure 2

VO₂max and Wmax before and after the training period. * P < 0.05 vs. pre-training test.

Figure 3

Extent of decrease in Wmax under H condition (%dWmax) before and after the training period. Following 4 weeks of training, the IMLET group had reduced %dWmax compared that at pre-training (P<0.05). * P < 0.05 vs. pre-training test.