Hypoxic effects on sympathetic vasomotor outflow and blood pressure during exercise with inspiratory resistance

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Katayama K, Yamashita S, Ishida K, Iwamoto E, Koike T, Saito M. Hypoxic effects on sympathetic vasomotor outflow and blood pressure during exercise with inspiratory resistance. Am J Physiol Regul Integr Comp Physiol 304: R374–R382, 2013. First published January 2, 2013; doi:10.1152/ajpregu.00489.2012.—The purpose of the present study was to clarify the influence of inspiratory resistive breathing during exercise under hypoxic conditions on muscle sympathetic nerve activity (MSNA) and blood pressure (BP). Six healthy males completed this study. The subjects performed a subtotal exercise test using a cycle ergometer in a semirecumbent position under normoxic [inspired oxygen fraction (FiO2 = 0.21)] and hypoxic (FiO2 = 0.12–0.13) conditions. The subjects carried out two 10-min exercises at 40% peak oxygen uptake [spontaneous breathing for 5 min and voluntary breathing with inspiratory resistance for 5 min (breathing frequency: 60 breaths/min, inspiratory and expiratory times were set at 0.5 s each)]. MSNA was recorded via microneurography of the right median nerve at the elbow. A progressive increase in MSNA burst frequency (BF) during leg-cycling exercise with inspiratory resistance in normoxia and hypoxia were accompanied by an augmentation of BP. The increased MSNA BF and mean arterial BP (MBP) during exercise with inspiratory resistive breathing in hypoxia (MSNA BF, 55.7 ± 1.4 bursts/min, MBP, 134.3 ± 6.6 mmHg) were higher than those in normoxia (MSNA BF, 39.2 ± 1.8 bursts/min, MBP, 123.6 ± 4.5 mmHg). These results suggest that an enhancement of inspiratory muscle activity under hypoxic condition leads to large increases in muscle sympathetic vasomotor outflow and BP during dynamic leg exercise.

respiratory muscle; sympathetic outflow; metaboreflex; dynamic leg exercise

IT HAS BEEN REPORTED that a progressive increase in ventilation occurs during sustained high-intensity exercise, leading to the development of inspiratory muscle (diaphragm) fatigue (22, 35). In healthy humans, this exercise-induced diaphragm fatigue does not limit the hyperventilatory response during exercise (7, 8). However, it is thought that diaphragm fatigue affects the regulation of cardiovascular regulation and blood flow distribution during exercise (7, 8, 15, 16, 35). High-intensity voluntary contraction of the inspiratory muscle against resistive loads augments an increase in muscle sympathetic nerve activity (MSNA) with corresponding increases in arterial blood pressure (BP) at rest (39, 41) and during exercise (26). It is thought that the sympathoexcitation occurs through a diaphragm fatigue-induced metaboreflex (18). The gradual increase in MSNA is accompanied by a significant decrease in limb vascular conductance and limb blood flow. Clinically, respiratory muscle activity may play an important role in limiting oxygen delivery in patients with chronic obstructive pulmonary disease (COPD) (2).

There are circumstances in which people perform exercise under hypoxic conditions, e.g., at altitude or under pathophysiological situations (2). It is well documented that the rate of limb muscle fatigue is exaggerated in hypoxia (3, 24). Additionally, respiratory muscle fatigability is exaggerated in hypoxia (13, 21, 36, 44, 45). It is also reported that the rate of lactate production during inspiratory resistive breathing in hypoxia was greater than that in normoxia (21, 44). From these findings, it is thought that large inspiratory muscle activity during exercise under hypoxic condition induces a greater sympathetically mediated vasoconstriction through the inspiratory muscle metaboreflex (8).

The purpose of the present study, therefore, was to elucidate the effect of inspiratory resistive breathing during exercise under hypoxic conditions on sympathetic vasoconstrictor outflow and BP. We recorded MSNA and cardiovascular variables during a leg-cycling exercise with inspiratory resistive breathing in normoxia and hypoxia. We hypothesized that inspiratory resistive breathing under hypoxic conditions would potentiate sympathetic vasomotor outflow and BP during dynamic leg exercise.

METHODS

Subjects

Eight healthy males were enrolled; six of these completed the study (means ± SE: age = 23.8 ± 0.5 yr, height = 177.7 ± 2.0 cm, body mass = 74.5 ± 2.0 kg, forced vital capacity = 5.01 ± 0.20 liters, forced expiratory volume in 1 s = 4.58 ± 0.07 liters, 91.1 ± 1.3%). Most subjects participated in moderate-intensity exercise a couple of times a week, but none were engaged in high-intensity exercise training. Subjects were informed about the experimental procedures and potential risks involved, and written consent was obtained. This study was approved by the human research committee of the Research Center of Health, Physical Fitness and Sports at Nagoya University.

Experimental Procedures

At the preliminary visit, subjects were instructed how to laterally extend both arms and how to hold their arms during leg cycling using an electromechanically braked ergometer in a semirecumbent position (Aerobike 75XL, Combi, Tokyo, Japan) (25, 26, 37, 38). Subjects reported to the laboratory on at least five additional occasions and each visit was separated by 1 wk.

On days 1 and 2 of the experiment, the subjects carried out an incremental maximal exercise test using the ergometer while breathing a normoxic [inspired oxygen fraction (FiO2 = 0.21)] or a hypoxic
measure the PImax (PImax test) (Fig. 1). The PImax tests were done to evaluate inspiratory muscle activity by means of an oscilloscope. Subjects also practiced measuring maximal inspiratory pressure (PImax) before and immediately after exercise.

On day 3, subjects practiced submaximal exercise and were again instructed how to hold their right arm during exercise. In addition, the subjects practiced controlling their breathing during exercise with the inspiratory resistance by means of an oscilloscope. Subjects also practiced measuring maximal inspiratory pressure (PImax) before and immediately after exercise.

On day 4, two submaximal exercise tests were performed to measure the PImax (PImax test) (Fig. 1). The PImax tests were done to evaluate inspiratory muscle fatigue using inspiratory resistance during exercise in normoxia and hypoxia. Subjects arrived at the laboratory and rested for 30 min. Before the submaximal exercise, a PImax measurement was taken. The subjects first breathed normoxic gas for 5 min (rest 1) and tidal volume (VT), breathing frequency (fT), HR, BP, SpO2, end-tidal O2 fraction (FETO2), and end-tidal CO2 fraction (FETCO2) were recorded throughout the experiment. Next, the inspired gas was either maintained (FiO2 = 0.21) or switched to the hypoxic (FiO2 = 0.12 and 0.13) gas mixture, and the subjects rested for 7 min (rest 2). Then the subjects performed 10 min of submaximal exercise at the same relative exercise intensity (i.e., 40% VO2peak) while breathing a normoxic or hypoxic gas mixture. The pedaling rate was maintained at 60 rpm with the aid of a metronome. The subjects breathed spontaneously over the first 5 min of exercise (exercise 1). During the next 5 min, the subjects were asked to control their breathing with inspiratory resistance (exercise 2). Breathing frequency was maintained at 60 breaths/min, and the inspiratory and expiratory times of one breath cycle were each set at 0.5 s via auditory feedback from the metronome (26). VT was regulated to be twice the resting VT via visual feedback from an oscilloscope marked with target VT levels (26, 39). End-tidal partial pressure of CO2 (PETCO2) in normoxic and hypoxic trials and SpO2 in the hypoxic trial were maintained within ±3 mmHg and ±5% of the spontaneous breathing levels during prior exercise, i.e., exercise 1, by adding CO2 and N2 to the inspired gas mixture. Finally, measurement of PImax was performed immediately after exercise. The procedure was repeated twice for normoxia and hypoxia (normoxic and hypoxic trials), with a 30-min interval between trials. The normoxic trial was performed first, because the enhanced MSNA after exercise with inspiratory resistance under the hypoxic condition did not return to preexercise levels within 30 min. However, we confirmed that the decreased PImax and the changed respiratory and cardiovascular variables following exercise in normoxia returned to preexercise levels within 30 min of exercise cessation (26).

On day 5, the subjects performed two submaximal exercise tests to measure MSNA (MSNA test) and clarify the influence of respiratory muscle activity under normoxic and hypoxic conditions on MSNA and BP during exercise. Subjects arrived at the laboratory and rested for 30 min. The procedures from rest 1 to exercise 2 were identical to those of the PImax test, as shown in Fig. 1. After exercise 2, subjects rested for 5 min (recovery). The same procedure was repeated twice for normoxia and hypoxia, with a 30-min interval between trials. The order of the normoxic and hypoxic trials was identical to that in the PImax test.

MSNA recordings during two exercise tests were successful in five of eight subjects. MSNA recording failed in the other three subjects during exercise because of electrode displacement from the muscle sympathetic nerve or bursts from electromyographic, efférent, and afferent nerve activities that covered MSNA bursts due to arm or body movement. In these three subjects, MSNA testing was repeated 1 mo later, and MSNA recording was successful in one of the three subjects. Consequently, six subjects from whom we obtained nerve recordings were used in the analysis.

**Inspiratory Resistance**

To increase inspiratory resistance during exercise, an inspiratory muscle training device (Threshold IMT, Phillips Respironics, Andover, MA) was connected to the inspiratory side via a tube. Inspiratory resistance was set at 40 cmH2O (26).

**Inspiratory Muscle Strength**

PImax, the index of inspiratory muscle strength (9, 46), was measured using a handheld mouth pressure meter (AAM377, Minato Ikagaku, Osaka, Japan) connected to a computerized spirometry system (AS-507, Minato Ikagaku). All measurements were taken from the residual volume (9, 26, 46). For each measurement, five trials were completed, and the highest of three measurements with less than 5% variability was averaged and used as the PImax (46, 47).
Respiratory Variables

Subjects breathed through a mouthpiece with their nose occluded. The mouthpiece was attached to a hot wire flowmeter (RF-H, Minato Ikagaku), which was connected to a device equipped with a one-way low-resistance valve. The dead space in this ventilatory system was ~130 ml. The flow signal from the flowmeter was connected to an oscilloscope, which indicated the target $V_T$ as a horizontal line for visual feedback. Sample gas was drawn through a sampling tube connected to the mouthpiece for measurement of $FE_{CO_2}$ and $FE_{O_2}$, and was used in a computer software (PC-9821RA40, NEC, Tokyo, Japan). $SpO_2$ was measured using a finger pulse oximeter (Biox 3740, Ohmeda, Madison, WI) throughout the test. The signals from the flowmeter, gas analyzer, and pulse oximeter were sampled at a frequency of 200 Hz through an analog-to-digital converter (CBI-3133B, Interface, Hiroshima, Japan) and stored in a computer (CF-F8, Panasonic, Osaka, Japan). The signals were analyzed afterward using our own computer software.

Muscle Sympathetic Nerve Activity

Multunit muscle sympathetic nerve discharges were recorded by the microneurographic technique using a recording system similar to that in our previous study (25, 26). A tungsten microelectrode with a shaft diameter of 0.1 mm (impedance 1–5 MΩ) was inserted manually by an experimenter into the right median nerve at the cubital fossa (25, 26, 37). The right arm was fixed using equipment to prevent arm movement artifacts during the leg cycling exercise. After insertion, the electrode was adjusted until MSNA was recorded. Identification of MSNA was based on the following criteria: spontaneous burst discharge synchronized with heartbeats and enhanced by the Valsalva maneuver or breath holding, but showing no change in response to sensory stimuli such as a loud noise or cutaneous touch (6, 10, 38, 43). Additionally, we asked the subjects to hold their breath to identify MSNA at the middle phase during the rest 1, exercise 1, and recovery sessions (at least 15 s at rest and 5 s during exercise). The neurogram was fed to a differential amplifier and amplified 100,000 times through a band-pass filter (700–2,000 Hz). The neurogram was full-wave rectified and integrated by a capacitance-integrated circuit with a time constant of 0.1 s. The mean voltage neurogram was continuously digitized through an analog-to-digital converter with a computer program-assisted inspection (25, 26), which accounted for the latency from the ECG-R wave to the sympathetic burst (10). MSNA was quantified as burst frequency (BF, bursts/min) (25, 26, 37, 38). The right arm was fixed using equipment to prevent arm movement artifacts during the leg cycling exercise. After insertion, the electrode was adjusted until MSNA was recorded. Identification of MSNA at the middle phase during the

Cardiovascular Variables

An ECG was obtained using a three-lead electrocardiograph (AB-621, Nihon Koden, Tokyo, Japan), and HR was calculated from each R-R interval obtained from the ECG. Beat-by-beat arterial BP was acquired using finger photoplethysmography from the middle finger of the left hand (Finometer, Finapres Medical Systems BV, Amsterdam, The Netherlands). ECG and BP signals were sampled and analyzed using a method similar to that for respiratory variables. Arterial systolic and diastolic BP (SBP and DBP) were determined from the BP waveform signal, and mean arterial BP (MBP) was calculated using the following equation: $MBP = (SBP - DBP)/3 + DBP$.

Statistical Analysis

Values are expressed as means ± SE. The respiratory and cardiovascular variables and MSNA BF values were averaged every 1 min throughout the experiment. For all data, the assumption of normal distribution was verified using a Kolmogorov-Smirnov test. Changes in variables during the experiment in each trial were analyzed using a Dunnett test. In comparisons of the data following 5 min of exercise, we indicate significance only when values were higher during exercises 2 and recovery. Comparisons of parameters between the normoxic and hypoxic trials were performed using paired t-test (parametric test) if the distribution was regular. When the distribution was not regular, the Wilcoxon test (nonparametric test) was used. The SPSS (11.5, SPSS) statistical package was used only to execute the Kolmogorov-Smirnov test; the Statview (5.0, SAS Institute) software was utilized for other statistical analyses. A value of $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Maximal Exercise Test

Table 1 summarizes the cardiorespiratory variables at exhaustion during the maximal exercise test. $V_O_2$, $SpO_2$, and workload at exhaustion in hypoxia were lower ($P < 0.05$) than those in normoxia.

Submaximal Exercise Test

Baseline descriptive data before the submaximal exercise. There were no significant differences among the variables at rest or during exercise between the $P_{max}$ and MSNA tests. Thus we are only reporting the data we collected during the MSNA test. No differences in these variables were found at rest 1 between the normoxic and hypoxic trials. Workload was higher in the normoxic trial than in the hypoxic trial ($93.0 ± 8.6 W vs. 65.0 ± 5.9 W, P < 0.05$).

Inspiratory muscle strength. $P_{max}$ was decreased significantly after exercise in the normoxic (122.9 ± 8.6 to 112.2 ± 10.5 cmH2O, $-9.2 ± 4.1%, P < 0.05$) and hypoxic trials (123.2 ± 8.3 to 110.1 ± 10.7 cmH2O, $-11.3 ± 4.4%, P < 0.05$).

Table 1. Cardiorespiratory parameters and workload at exhaustion in normoxia and hypoxia

<table>
<thead>
<tr>
<th></th>
<th>$V_t$, l/min</th>
<th>$V_O_2$, l/min</th>
<th>$V_O_2$/BM, ml·kg⁻¹·min⁻¹</th>
<th>HR, beats/min</th>
<th>$SpO_2$, %</th>
<th>Workload, Watts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td>144.7 ± 7.2</td>
<td>3.29 ± 0.10</td>
<td>44.3 ± 1.7</td>
<td>185.0 ± 3.1</td>
<td>97.0 ± 0.4</td>
<td>272.5 ± 9.0</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>138.7 ± 4.3</td>
<td>2.45 ± 0.12*</td>
<td>32.8 ± 1.2*</td>
<td>180.3 ± 2.3</td>
<td>73.2 ± 1.9*</td>
<td>220.0 ± 7.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. $V_t$ expired minute ventilation; $V_O_2$, oxygen uptake; $V_O_2$/BM, oxygen uptake per body mass; HR, heart rate; $SpO_2$, arterial oxygen saturation. *Significant from normoxia, $P < 0.05$.
There was no significant difference in $P_{\text{Imax}}$ following exercise between the normoxic and hypoxic trials.

**Respiratory variables.** Respiratory variables are shown in Table 2. At rest 2, $V_e$ and $V_T$ tended to increase and $S_{\text{PO}_2}$, $P_{\text{ETO}_2}$, and $P_{\text{ETO}_2}$ decreased significantly in hypoxia. $V_e$ and $f_b$ increased significantly during exercise 1, and further significant increases in $V_e$ and $f_b$ appeared during exercise with resistive breathing (exercise 2) in each trial. $V_e$, $V_T$, and $f_b$ during exercise 2 in the hypoxic trial did not differ from those in the normoxic trial, whereas $S_{\text{PO}_2}$ and $P_{\text{ETO}_2}$ in the hypoxic trial were lower ($P < 0.05$) than those in the normoxic trial.

Muscle sympathetic nerve activity. Representative MSNA recordings and changes in MSNA BF are presented in Figs. 2 and 3A. MSNA BF increased significantly at rest 2 and during exercise 1 in the hypoxic trial. During exercise with inspiratory resistance (exercise 2), a progressive increase ($P < 0.05$) in MSNA BF occurred in both trials. MSNA BF values at rest 2, during exercises 1 and 2, and during recovery in the hypoxic trial were higher ($P < 0.05$) than those in the normoxic trial.

The magnitude of percent changes in MSNA BF ($\Delta$MSNA BF) were calculated individually as the difference between those obtained at 5 min during exercise 1 and at 1–5 min during exercise 2 (Fig. 4A). There was no significant difference in percent change in $\Delta$MSNA BF between the normoxic and hypoxic trials.

**Cardiovascular variables.** HR at rest 2 increased significantly in the hypoxic trial. In both trials, HR increased significantly during exercise 1. During exercise with inspiratory resistance (exercise 2), further increases in HR ($P < 0.05$) occurred during in both trials (Table 2).

A typical BP recording is shown in Fig. 2, and SBP, DBP, and MBP values are shown in Fig. 3, B and C, and Table 2. SBP and DBP increased significantly during exercise 1 in both trials. During exercise 2, a progressive increase in BP in both trials was observed. SBP and DBP during the latter part of exercise 2 in the hypoxic trial were higher ($P < 0.05$) than those in the normoxic trial.

The mean percent changes in SBP and DBP ($\Delta$SBP and $\Delta$DBP) during exercise 2 were shown in Fig. 4, B and C. The magnitude of $\Delta$SBP and $\Delta$DBP was calculated individually as the difference between those obtained at 5 min during exercise 1 and 1–5 min during exercise 2. $\Delta$SBP at 3 and 4 min and $\Delta$DBP at 4 min during exercise 2 in the hypoxic trial were higher ($P < 0.05$) than those in the normoxic trial.

**Discussion**

The primary findings of the present study were that 1) the progressive increase in MSNA BF during leg-cycling exercise with inspiratory resistive breathing under normoxic and hypoxic conditions, accompanied by an augmentation of BP, and 2) the increased BP during exercise with inspiratory resistance in hypoxia were greater than those in normoxia. To our knowledge, this is the first study to clarify MSNA and cardiovascular responses during dynamic leg exercise with inspiratory resistance under hypoxic conditions. The results from this study should provide information regarding the significance of inspiratory muscle activity on circulatory regulation during dynamic exercise under hypoxic conditions.

**Effect of Inspiratory Resistance on Inspiratory Muscle Strength**

To examine the effect of inspiratory resistance on inspiratory muscle strength, we determined $P_{\text{Imax}}$ as an index of inspiratory muscle strength before and after submaximal exercise with resistive breathing under hypoxic condition. The results from this study should indicate significance only when values are higher during exercises 2 and recovery.

### Table 2. Respiratory, cardiovascular, and MSNA variables during experiment

<table>
<thead>
<tr>
<th>Trials</th>
<th>Rest 1</th>
<th>Rest 2</th>
<th>Exercise 1</th>
<th>Exercise 2</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_e$, l/min</td>
<td>7.9 ± 0.5</td>
<td>8.2 ± 0.5</td>
<td>31.0 ± 1.9</td>
<td>61.4 ± 2.7</td>
<td>9.5 ± 4</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>7.6 ± 0.3</td>
<td>10.6 ± 1.0</td>
<td>31.9 ± 1.8</td>
<td>60.8 ± 3.1</td>
<td>11.2 ± 0.8</td>
</tr>
<tr>
<td>$V_T$, liter</td>
<td>0.65 ± 0.08</td>
<td>0.56 ± 0.01</td>
<td>1.45 ± 0.10</td>
<td>1.02 ± 0.04</td>
<td>0.67 ± 0.02</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>0.53 ± 0.03</td>
<td>0.65 ± 0.03</td>
<td>1.36 ± 0.10</td>
<td>1.01 ± 0.05</td>
<td>0.67 ± 0.03</td>
</tr>
<tr>
<td>$f_b$, breaths/min</td>
<td>13.1 ± 1.6</td>
<td>14.6 ± 0.7</td>
<td>21.9 ± 1.9</td>
<td>59.9 ± 0.9</td>
<td>14.3 ± 0.8</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>14.6 ± 0.9</td>
<td>16.2 ± 1.1</td>
<td>23.7 ± 1.1</td>
<td>60.0 ± 0.2</td>
<td>16.7 ± 1.0</td>
</tr>
<tr>
<td>$S_{\text{PO}_2}$, %</td>
<td>97.5 ± 0.1</td>
<td>97.6 ± 0.2</td>
<td>97.8 ± 0.3</td>
<td>98.0 ± 0.4</td>
<td>97.9 ± 0.3</td>
</tr>
<tr>
<td>Normoxia</td>
<td>97.5 ± 0.2</td>
<td>84.0 ± 2.1</td>
<td>76.1 ± 1.2</td>
<td>75.2 ± 1.0</td>
<td>83.5 ± 0.7</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>110.8 ± 0.9</td>
<td>110.6 ± 1.1</td>
<td>121.8 ± 2.4</td>
<td>130.7 ± 1.7</td>
<td>120.7 ± 1.5</td>
</tr>
<tr>
<td>$P_{\text{ETO}_2}$, mmHg</td>
<td>109.6 ± 1.6</td>
<td>58.9 ± 2.6</td>
<td>58.5 ± 1.5</td>
<td>64.3 ± 3.5</td>
<td>55.8 ± 1.2</td>
</tr>
<tr>
<td>Normoxia</td>
<td>108.6 ± 1.6</td>
<td>58.9 ± 2.6</td>
<td>58.5 ± 1.5</td>
<td>64.3 ± 3.5</td>
<td>55.8 ± 1.2</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>42.6 ± 0.7</td>
<td>42.7 ± 1.3</td>
<td>43.8 ± 1.0</td>
<td>43.9 ± 1.0</td>
<td>41.0 ± 1.4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>62.9 ± 2.9</td>
<td>62.7 ± 2.2</td>
<td>106.6 ± 1.9</td>
<td>128.2 ± 6.1</td>
<td>74.0 ± 4.6</td>
</tr>
<tr>
<td>Normoxia</td>
<td>64.2 ± 3.6</td>
<td>77.6 ± 4.0</td>
<td>115.2 ± 5.6</td>
<td>133.8 ± 7.5</td>
<td>83.7 ± 5.0</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>89.0 ± 2.7</td>
<td>91.4 ± 3.5</td>
<td>111.6 ± 3.5</td>
<td>123.6 ± 4.5</td>
<td>94.4 ± 4.2</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>91.4 ± 3.1</td>
<td>93.8 ± 3.3</td>
<td>114.8 ± 5.6</td>
<td>134.3 ± 6.6</td>
<td>105.8 ± 3.7</td>
</tr>
</tbody>
</table>

Values are mean ± SE. $V_T$, tidal volume; $f_b$, breathing frequency; $P_{\text{ETO}_2}$, end-tidal partial pressure of O$_2$; $P_{\text{ETO}_2}$, end-tidal partial pressure of CO$_2$; MBP, mean blood pressure. $^aP < 0.05$ versus at rest 1 in the normoxic trial. $^bP < 0.05$ vs. at rest 1 in the hypoxic trial. $^cP < 0.05$ vs. during exercise 1 in the normoxic trial. $^dP < 0.05$ vs. during exercise 1 in the hypoxic trial. $^eP < 0.05$ vs. during exercise 1 in the hypoxic trial. In the comparison with the data during exercise 1, we indicate significance only when values are higher during exercises 2 and recovery.
using supramaximal bilateral phrenic nerve stimulation (BPNS) was concomitant with a decrease in $P_{\text{Imax}}$ by volitional maneuver (22). Thus the decreased $P_{\text{Imax}}$ could be the principle cause of diaphragm fatigue, although we cannot exclude an effect of accessory inspiratory muscle fatigue.

It is reported that respiratory muscle fatigability is exaggerated under hypoxic conditions (13, 21, 44, 45). Thus it is likely that the $P_{\text{Imax}}$ magnitude decreases after exercise with resistive breathing in hypoxia are larger than that in normoxia. The significant decrease in $P_{\text{Imax}}$ appeared following exercise with inspiratory resistive breathing in hypoxia, but the extent of the decreased $P_{\text{Imax}}$ in the hypoxic trial ($-11.3 \pm 4.4\%$) was not different from that in the normoxic trial ($-9.2 \pm 4.1\%$). We need to consider the reasons why the decrease in $P_{\text{Imax}}$ in hypoxia did not differ from that in normoxia. Verges et al. (44) reported that the $P_{\text{di}}$ decreased following voluntary hyperpnea in normoxia and hypoxia; the $P_{\text{di}}$ magnitude of changes were $-22\%$ in normoxia and $-34\%$ in hypoxia. The decrease in $P_{\text{Imax}}$ in their study was greater than that in the present study ($-9\%$ in normoxia and $-11\%$ in hypoxia). The subjects in their study performed 15 min hyperpnea, whereas we used 5 min hyperpnea in this study. The difference in hyperpnea duration may affect the magnitude of $P_{\text{Imax}}$ decrease, and thus it is possible that the significant difference in inspiratory muscle fatigue appears between normoxia and hypoxia if the duration of resistive breathing is prolonged. We must consider the level of inspiratory resistance. Previous studies utilized the same relative resistance [60% maximal inspiratory pressure (MIP)] in each subject (39, 41). In this study, we could not set %MIP for inspiratory resistance in each subject, and alternatively, maximal resistance of the device (40 cmH2O) was used. As a result, the %MIP of each subject differed (range 42–62% MIP), and the %MIP in the present study was lower than that in previous reports (39, 41). Therefore, it is likely that different relative MIP values affect the magnitude of $P_{\text{Imax}}$ in each subject and no difference was observed in $P_{\text{Imax}}$ between normoxia and hypoxia. Another possible reason is the appearance of fatigue. Gudjonsdottir et al. (13) reported that diaphragm fatigue delayed recovery at high altitude compared with at sea level. Babcock et al. (4) found a significant decrease in $P_{\text{di}}$ immediately after exercise in normoxia and hypoxia, but the decrease was of similar magnitude for both. However, the decreased $P_{\text{di}}$ after exercise in normoxia recovered 60 min postexercise, while $P_{\text{di}}$ following hypoxic exercise was still less than the preexercise level after 90 min of recovery. Therefore, it is conceivable that hypoxia-induced diaphragm fatigue may be prolonged compared with that in normoxia.

Effect of Inspiratory Resistance on MSNA and BP Response During Dynamic Exercise

The diaphragm has an abundance of type IV metaboreceptors, and fatiguing the diaphragm via phrenic nerve stimulation leads to an increase in type IV afferent discharge (18). In addition, when metaboreceptors in the diaphragm were stimulated electrically, pharmacologically, or with local lactic acid
infusions, efferent sympathetic nerve activity increased and vascular conductance decreased in selected vascular beds (20, 31, 34). In the present study, inspiratory resistive breathing during dynamic exercise in the normoxic trial caused a progressive increase in MSNA BF with a progressive increase in BP, as shown in Fig. 3. These results are in agreement with previous reports of an increase in MSNA and BP during voluntary hyperpnea with resistance at rest (39, 41) and during exercise (26). Collectively, it is conceivable that fatiguing diaphragm, and thereby the metaboreflex, has an influence on sympathetic vasoconstrictor outflow and BP during exercise.

Under hypoxic conditions, larger increases in MSNA BF and BP appeared during exercise with inspiratory resistance compared with the normoxic trial (Fig. 3). These findings imply that enhancement of inspiratory muscle activity under hypoxic conditions induces larger increases in sympathetic vasomotor outflow and BP during dynamic leg exercise. However, the magnitude of the changes in MSNA (ΔMSNA BF) during exercise with resistive breathing did not differ between the normoxic and hypoxic trials, as shown in Fig. 4A. These data may indicate that an increase in MSNA does not potentiate during resistive breathing under hypoxic condition. In the present study, MSNA was represented as BF, which have been used in previous studies to record MSNA during dynamic leg cycling (25, 26, 37, 38). Burst amplitude was not estimated because electromyographic and efferent and afferent nerve activities altered the baseline of the integrated neurogram during dynamic leg cycling irrespective of normoxic or hypoxic conditions (25, 26, 38). Thus it is possible that the change in MSNA underestimates during exercise with inspiratory resistance in hypoxia, and this is the technical limitation of the present study. Concerning BP response to inspiratory resistance,
the magnitude of changes in ΔSBP and ΔDBP during exercise 2 in the hypoxic trial was larger than those in the normoxic trial, as shown in Fig. 4, B and C. These results suggest that an enhancement of inspiratory muscle activity under hypoxic conditions has a large influence on BP during exercise.

It has been reported that respiratory muscle fatigability is exaggerated during hypoxia (13, 21, 36, 44, 45). From these findings, we hypothesized that an inspiratory resistive breathing during hypoxic exercise would potentiate MSNA and BP. However, the extent of decreases in PImax in the hypoxic trial was not different from that in the normoxic trial. This result does not support our supposition that large inspiratory muscle fatigue may lead to increases in MSNA BF and BP during submaximal exercise. Although greater inspiratory muscle fatigue did not appear after exercise with inspiratory resistance in hypoxia compared with normoxia, it is likely that inspiratory muscle activity was greater in hypoxia than in normoxia. This speculation may be supported by the results of an animal study, in which electromyographic (EMG) activity of the diaphragm was larger in hypoxia than normoxia (29). EMG provides a measure of the motor unit recruitment and firing rate due to changes in intracellular metabolism in response to muscle fatigue development. It is assumed that greater motor unit recruitment and/or firing rate of motor units may occur during resistive breathing in hypoxia, and these changes are associated with an increased rate of metabolic accumulation. In fact, the rate of lactate production during inspiratory resistive breathing under hypoxia at rest was greater than that under normoxia (21, 44). Recent in vitro studies have questioned the deleterious role of [H+] to limb muscle fatigue (32, 33) and suggested inorganic phosphate (P"

Clinical Implications

Clinically, respiratory muscle work could play a particularly important role in determining oxygen transport, limb muscle fatigue, and exercise tolerance in patients with obstructive sleep apnea (1, 28), heart failure (23), and COPD (2). It is supposed that poor leg exercise tolerance in COPD patients is caused by an enhanced ventilatory requirements, dynamic hyperinflation, and dyspnea via the stimulation of group III/IV lower limb sensory muscle afferents (11). People perform exercise under hypoxic conditions in many situations, e.g., at altitude or under pathological conditions. Simon et al. (40) investigated leg blood flow during exercise in COPD patients: blood flow to the exercising legs appeared to plateau during submaximal exercise in several COPD patients with arterial hypoxemia, despite the fact that total VO2, i.e., cardiac output, continued to increase. They speculated that redistribution of cardiac output and oxygen transport occurred from the exercising muscles of the lower limb to the respiratory muscle. Amann et al. (2) examined the effects of respiratory muscle work and arterial oxygenation on exercise-induced locomotor muscle fatigue in COPD patients. The patients carried out leg cycle exercise under arterial hypoxemia, while control subjects performed the same exercise with normal arterial oxygenation. Consequently, the reduction in quadriceps force in the patients was larger than that in control subjects, and they concluded that exercise-induced quadriceps muscle fatigability was exaggerated in COPD patients. In this study, the increased MSNA and BP during exercise with inspiratory resistance in hypoxia were larger than those in normoxia. Therefore, we suppose that vasoconstriction, which is elicited by sympathoexcitation via large respiratory muscle activity during exercise under hypoxic condition, affects oxygen transport and consequent locomotor muscle fatigue and exercise tolerance in patients.

Technical Considerations and Limitations

Some technical considerations and limitations to this study should be noted. As in our previous study (26), we utilized 40% VO2peak exercise intensity for several reasons. First, the percentage of successful MSNA recordings is high when the exercise intensity is mild (26), and movements of the arm and the body become greater during leg cycling with inspiratory resistance. Second, previous reports indicate that MSNA BF is not altered during leg cycling at 40% VO2peak exercise under normoxic condition compared with when at rest (25, 38). Therefore, we supposed that an increase in MSNA BF with inspiratory resistance would become apparent under these conditions.

We utilized PImax as an index of inspiratory muscle fatigue. There is controversy as to whether PImax is a valid measure of inspiratory muscle strength. To address this, five measurements were performed, and the highest of three measurements with less than 5% variability was used as PImax (46, 47). Accordingly, coefficient of variation for the three highest PImax measurements was 3.0%, and the within-day coefficient of variation for PImax was 2.9%. Thus PImax data presented in this study are valid and that PImax levels were comparable before and after exercise.

Another limitation of this study is that a limited number of subjects and men only were included. It has been reported a comparison of inspiratory muscle fatigue during inspiratory resistive breathing at rest (12) and during sustained high-intensity exercise (14) between males and females; the rate of inspiratory muscle fatigue during resistive breathing in females was slower than in males. Thus it is assumed that MSNA and
BP responses to dynamic leg exercise with inspiratory resistive breathing in females differ from that in males.

Perspectives and Significance

In the present study, a progressive increase in MSNA BF occurred during exercise with inspiratory resistance in normoxia and hypoxia and was accompanied by an augmentation of BP. Additionally, the increased MSNA BF and BP during exercise with inspiratory resistive breathing in hypoxia were larger than those in normoxia. These results suggest that an enhancement of inspiratory muscle activity under hypoxic condition leads to large increases in muscle sympathetic vasomotor outflow and BP during dynamic leg exercise.

It is possible that this large vasoconstrictor activity under hypoxic condition reduces blood flow and oxygen transport to the working limb, thereby exacerbating limb fatigue and compromising exercise performance (7, 15, 24). Downey et al. (9) reported that inspiratory muscle training reduced ventilatory response to submaximal exercise under hypoxic condition. Therefore, it is assumed that respiratory muscle activity during hypoxic exercise may decline following inspiratory muscle training and increase blood flow and oxygen transport to the working muscle. Further research is needed to confirm this assumption.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: K.K., K.I., and M.S. conception and design of experiment; K.K., K.I., E.I., and M.S. performed experiments; K.K., S.Y., and K.I. analyzed data; K.K., K.I., E.I., T.K., and M.S. interpreted results of experiments; K.K. prepared figures; K.K. and S.Y. drafted manuscript; K.K., K.I., E.I., T.K., and M.S. edited and revised manuscript; K.K., S.Y., K.I., E.I., T.K., and M.S. approved final version of manuscript.

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