Inspiratory muscle fatigue increases sympathetic vasomotor outflow and blood pressure during submaximal exercise

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Katayama K, Iwamoto E, Ishida K, Koike T, Saito M. Inspiratory muscle fatigue increases sympathetic vasomotor outflow and blood pressure during submaximal exercise. Am J Physiol Regul Integr Comp Physiol 302: R1167–R1175, 2012.—The purpose of this study was to elucidate the influence of inspiratory muscle fatigue on muscle sympathetic nerve activity (MSNA) and blood pressure (BP) response during submaximal exercise. We hypothesized that inspiratory muscle fatigue would elicit increases in sympathetic vasomotor outflow and BP during dynamic leg exercise. The subjects carried out four submaximal exercise tests: two were maximal inspiratory pressure (Plmax) tests and two were MSNA tests. In the Plmax tests, the subjects performed two 10-min exercises at 40% peak oxygen uptake using a cycle ergometer in a semirecumbent position [spontaneous breathing for 5 min and with or without inspiratory resistive breathing for 5 min (breathing frequency: 60 breaths/min, inspiratory and expiratory times were each set at 0.5 s)]. Before and immediately after exercise, Plmax was estimated. In MSNA tests, the subjects performed two 15-min exercises (spontaneous breathing for 5 min, with or without inspiratory resistive breathing for 5 min, and spontaneous breathing for 5 min). MSNA was recorded via microneurography of the right median nerve at the elbow. Plmax decreased following exercise with resistive breathing, whereas no change was found without resistance. The time-dependent increase in MSNA burst frequency (BF) appeared during exercise with inspiratory resistive breathing, accompanied by an augmentation of diastolic BP (DBP) (with resistance: MSNA, BF +83.4%; DBP, +23.8%; without resistance: MSNA BF, +19.2%; DBP, −0.4%, from spontaneous breathing during exercise). These results suggest that inspiratory muscle fatigue induces increases in muscle sympathetic vasomotor outflow and BP during dynamic leg exercise at mild intensity.

respiratory muscle; sympathetic outflow; metaboreflex; dynamic leg exercise

It has been reported that high-intensity whole body exercise elicits respiratory muscle (diaphragm) fatigue (17, 30). This exercise-induced diaphragm fatigue does not limit the hyperventilatory response throughout exercise in healthy humans (6, 7). However, it is thought that the fatiguing diaphragm affects cardiovascular regulation and blood flow distribution during exercise (6, 7, 13, 14, 30).

High-intensity voluntary contraction of the inspiratory muscle against resistive loads causes a time-dependent increase in muscle sympathetic nerve activity (MSNA) with a corresponding increase in arterial blood pressure (BP) (36, 39). This sympathoexcitation occurs through a diaphragm fatigue-induced metaboreflex (15). The gradual increase in MSNA is accompanied by a significant decrease in limb vascular conductance and limb blood flow (36, 37). However, in these studies, inspiratory resistive breathing was performed during resting conditions, and breathing frequencies were lower compared with spontaneous breathing during exercise (36, 37, 39). In addition, during whole body exercise, the situation is more complicated because an increase in blood flow in the muscles depends on the cardiac output limitation and the opposing effects of local vasodilators and vasoconstrictors (30). The influence of resistive breathing on sympathetic nerve activity and cardiovascular response during submaximal exercise has not been previously studied except by Wetter et al. (47), who showed no increase in norepinephrine spillover and BP and no change in leg blood flow during submaximal cycling with inspiratory resistance. They supposed that the effects of increasing respiratory work on sympathetic vasoconstrictor outflow and on limb blood flow would not be realized until maximal or at least very near-maximal exercise intensities (47). Indeed, Harms et al. (13) reported that norepinephrine spillover increased during heavy intensity cycling exercise with inspiratory resistance. However, direct recording of sympathetic nerve activity is more accurate than the measurement of norepinephrine concentrations (10, 35).

Clinically, respiratory muscle fatigue could play an important role in limiting oxygen delivery in patients with obstructive sleep apnea (OSA) (1, 24), chronic heart failure (18), and chronic obstructive pulmonary disease (COPD) (2). It is still unclear whether diaphragm fatigue, thereby metaboreflex leads to alternations in sympathetic nerve activity and cardiovascular variables during dynamic leg exercise at submaximal intensity.

Therefore, the purpose of the present study was to clarify the effects of respiratory muscle fatigue on sympathetic vasoconstrictor outflow and cardiovascular parameters during dynamic leg exercise. We recorded MSNA and cardiovascular variables during a leg-cycling exercise at mild intensity with or without inspiratory resistance. We hypothesized that inspiratory muscle fatigue would elicit increases in sympathetic vasoconstrictor outflow and BP during submaximal exercise.

METHODS

Subjects

Eight healthy males participated in the study (means ± SE: age = 21.5 ± 0.5 yr, height = 175.6 ± 1.8 cm, body mass = 68.7 ± 2.9 kg, forced vital capacity = 4.71 ± 0.14 liters, forced expiratory volume in 1 s = 4.18 ± 0.12 liters, 89.0 ± 2.1%). All were sedentary, nonsmokers, and with no history of cardiorespiratory diseases. 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, Nagoya University.
Experimental Procedure

At the preliminary visit, the 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) (20, 33, 34). Subjects reported to the laboratory on at least four additional occasions, separated by 1 wk.

On day 1, the subjects carried out an incremental exercise test using the ergometer (maximal exercise test). The exercise test began at an initial power output of 90 W, and the workload was increased 15 W every minute until exhaustion (20). The pedaling rate was maintained at 60 rpm with the aid of a metronome. Minute expired ventilation (VE), oxygen uptake (VO2), heart rate (HR), and arterial oxygen saturation (SpO2) were recorded during the test and were averaged every 30 s afterward. The highest VO2 value obtained during the exercise protocol was used as peak VO2 (VO2peak). Then workload at 40% was calculated for submaximal exercise test.

On day 2, subjects practiced submaximal exercise and were again instructed how to hold their right arm during exercise. In addition, the subjects practiced controlling their breath during exercise with or without the inspiratory resistance by means of an oscilloscope. Also, the subjects practiced measuring maximal inspiratory pressure (PImax) before and immediately after exercise.

On day 3, two submaximal exercise tests were performed with PImax measurement (PImax test). The PImax tests were done to evaluate respiratory muscle fatigue using inspiratory resistance during exercise. Subjects arrived at the laboratory and rested for 30 min. Before the submaximal exercise, a PImax measurement was taken. Next, the subjects rested for 5 min (Rest). VE, tidal volume (VT), breathing frequency (fB), VO2, HR, end-tidal O2 fraction (FeT(O2)), and end-tidal CO2 fraction (FeT(CO2)) were measured throughout the experiment. Then submaximal exercise was carried out for 10 min, and exercise intensity was set at 40% VO2peak. 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 breath with or without inspiratory resistance (exercise 2). fB 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. VT was regulated to be twice the resting VT via visual feedback from an oscilloscope marked with target VT levels (36). End-tidal partial pressure of CO2 (PETCO2) was maintained within ±3 mmHg of the spontaneous breathing level during prior exercise, i.e., exercise 1, by adding CO2 to the inspired air. Finally, measurement of PImax was performed immediately after exercise. The procedure was repeated twice, i.e., with or without inspiratory resistance (Resistance and Nonresistance trials), with a 30-min interval between trials. In a preliminary study, we confirmed that the decreased PImax and the changed respiratory and cardiovascular variables following exercise with inspiratory resistance return to preexercise levels within 30 min of the cessation of exercise. The order of exercise with or without inspiratory resistance (Resistance and Nonresistance trials) was randomly assigned and counterbalanced.

On day 4, the subjects performed two submaximal exercise tests with MSNA measurement (MSNA test) to clarify the influence of respiratory muscle fatigue on MSNA and BP during exercise. Subjects arrived at the laboratory and rested for 30 min. First, the variables were measured at rest for 5 min (Rest 1). Then the subjects performed submaximal exercise at 40% VO2peak for 15 min, and the pedaling rate was maintained at 60 rpm. The subjects breathed spontaneously over the first 5 min of exercise (exercise 1). Next, the subjects controlled their breath with or without inspiratory resistance during exercise (exercise 2). The procedures up to this point were identical to those of the PImax test. After 5 min of voluntary hyperpnea, the subjects restored spontaneous breathing and continued to exercise for 5 min (exercise 3). After exercise, subjects rested for 5 min (Recovery). The same procedure was repeated twice, i.e., with or without inspiratory resistance (Resistance and Nonresistance trials), with a 30-min interval between trials. The order of the Resistance and Nonresistance trials for each subject was identical to that in the PImax test.

Initially, a total of 11 subjects entered the study. MSNA recordings were completed for five of these, whereas the remaining six failed to record MSNA during exercise because of displacement of the electrodes from the muscle sympathetic nerve or bursts from electromyographic, effenter, and afferent nerve activities covered MSNA bursts as a result of movement of the arm or body. In these six subjects, MSNA testing was repeated after a break of at least 1 mo, and MSNA recordings were completed for three of these six individuals. Consequently, eight 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) was connected to the inspiratory side via a tube. Inspiratory resistance was set at 40 cmH2O.

Inspiratory Muscle Strength

PImax, as the index of inspiratory muscle strength (4, 8, 45), was measured using a hand-held mouth pressure meter (AAM377, Minato Ikagaku) connected to a computerized spirometry system (AS-507, Minato Ikagaku). All measurements were taken from the residual volume (4, 8, 45). For each measurement, five trials were completed, and the highest of three measurements with <5% variability was averaged and used as PImax (45, 46).

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 VT as a horizontal line for visual feedback. Sample gas was drawn through a sampling tube connected to the mouthpiece to measure FeT(O2) and FeT(CO2) by means of a gas analyzer (MG-360, Minato Ikagaku). VE, VT, fB, and VO2 were determined using an on-line system with mixing chamber, as in our previous studies (20–22). Expired gas volume was measured by a Fleisch pneumotachometer (PN-230, Arco Systems), which was connected to the expiratory side of the valve via a tube. Sample gas was drawn through a sampling tube inserted into the pneumotachometer to measure expired gas fractions. The expired gas fractions were analyzed using a mass spectrometer (ARCO-1000, Arco Systems) that was calibrated and confirmed before each test. Breath-by-breath data were analyzed continuously using customized computer software (PC-9821Ra40, NEC). Arterial oxygen saturation (SpO2) was measured using a finger pulse oximeter (Biox 3740, Ohmeda) 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) and were stored in a computer (CF-F8, Panasonic). The signals were analyzed afterward by means of our own computer software.

MSNA

Multiunit muscle sympathetic nerve discharges were recorded using means of the microneurographic technique. A recording system similar to that in our previous study (20) was utilized. 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 (20, 34). 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 heartbeat and enhanced by Valsalva maneuver or breath holding, but showing no change in response to sensory stimuli, such as a loud noise or cutaneous touch (5, 9, 34, 40). Additionally, we asked the subjects to hold their breath to identify MSNA at the middle phase during the Rest, exercise 3, 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 sampling frequency of 200 Hz for storage on a computer. MSNA bursts were identified from the mean voltage neurogram using a customized computer program-assisted inspection (20), which accounted for the latency from the ECG-R wave to the sympathetic burst (9). MSNA recordings at rest were accepted when signal-to-noise ratio was >3:1. MSNA was quantified as burst frequency (BF, bursts/min) and burst incidence (BI, bursts/100 heart beats) (20, 32, 34). We could not calculate MSNA burst amplitude and total activity, because electromyographic, efferent, and afferent nerve activities altered the baseline of the integrated neurogram during dynamic leg cycling in most recordings (20, 34).

Cardiovascular Variables

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

### Table 1. Respiratory variables, MBP, and MSNA

<table>
<thead>
<tr>
<th>Trials</th>
<th>Rest</th>
<th>Exercise 1</th>
<th>Exercise 2</th>
<th>Exercise 3</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>V̇E, l/min</td>
<td>Nonresistance</td>
<td>12.4 ± 2.3</td>
<td>33.2 ± 1.4*</td>
<td>73.3 ± 2.8#</td>
<td>36.2 ± 1.4*</td>
</tr>
<tr>
<td></td>
<td>Resistance</td>
<td>10.2 ± 0.6</td>
<td>33.5 ± 1.4†</td>
<td>71.1 ± 2.9‡</td>
<td>37.1 ± 1.9‡</td>
</tr>
<tr>
<td>V̇T, liter</td>
<td>Nonresistance</td>
<td>0.71 ± 0.09</td>
<td>1.38 ± 0.07†</td>
<td>1.22 ± 0.05*</td>
<td>1.36 ± 0.05*</td>
</tr>
<tr>
<td></td>
<td>Resistance</td>
<td>0.68 ± 0.05</td>
<td>1.38 ± 0.07†</td>
<td>1.18 ± 0.05†</td>
<td>1.35 ± 0.06†</td>
</tr>
<tr>
<td>Fb, breaths/min</td>
<td>Nonresistance</td>
<td>15.8 ± 1.9</td>
<td>24.2 ± 0.8*</td>
<td>59.8 ± 0.2‡‡</td>
<td>27.1 ± 1.1*</td>
</tr>
<tr>
<td></td>
<td>Resistance</td>
<td>15.4 ± 1.3</td>
<td>24.6 ± 1.3‡</td>
<td>59.6 ± 0.3‡‡</td>
<td>28.3 ± 1.4‡</td>
</tr>
<tr>
<td>V̇O₂, l/min</td>
<td>Nonresistance</td>
<td>0.33 ± 0.02</td>
<td>1.37 ± 0.04*</td>
<td>1.38 ± 0.05*</td>
<td>1.38 ± 0.05*</td>
</tr>
<tr>
<td></td>
<td>Resistance</td>
<td>0.34 ± 0.03</td>
<td>1.37 ± 0.05†</td>
<td>1.47 ± 0.06‡‡</td>
<td>1.39 ± 0.06†</td>
</tr>
<tr>
<td>SpO₂, %</td>
<td>Nonresistance</td>
<td>97.7 ± 2.5</td>
<td>98.0 ± 2.5</td>
<td>98.7 ± 0.3‡#</td>
<td>97.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Resistance</td>
<td>97.9 ± 0.3</td>
<td>98.0 ± 0.3</td>
<td>98.3 ± 0.3‡</td>
<td>97.8 ± 0.3</td>
</tr>
<tr>
<td>P̄ETO₂, mmHg</td>
<td>Nonresistance</td>
<td>107.9 ± 1.6</td>
<td>115.1 ± 3.4‡</td>
<td>126.3 ± 3.6‡‡</td>
<td>115.1 ± 2.3‡</td>
</tr>
<tr>
<td></td>
<td>Resistance</td>
<td>108.5 ± 0.3</td>
<td>116.8 ± 4.2‡</td>
<td>127.9 ± 2.2‡‡</td>
<td>118.2 ± 3.3‡</td>
</tr>
<tr>
<td>P̄ETCO₂, mmHg</td>
<td>Nonresistance</td>
<td>43.1 ± 1.1</td>
<td>44.8 ± 0.7*</td>
<td>44.9 ± 0.8*</td>
<td>43.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Resistance</td>
<td>43.6 ± 1.0</td>
<td>44.7 ± 0.8†</td>
<td>44.6 ± 0.8‡</td>
<td>44.3 ± 0.9†</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>Nonresistance</td>
<td>95.4 ± 4.8</td>
<td>118.7 ± 7.0*</td>
<td>117.2 ± 3.7‡</td>
<td>115.3 ± 6.4³</td>
</tr>
<tr>
<td></td>
<td>Resistance</td>
<td>98.6 ± 8.0</td>
<td>121.6 ± 10.0†</td>
<td>135.7 ± 10.6‡‡</td>
<td>125.4 ± 10.6‡</td>
</tr>
<tr>
<td>MSNA BF, bursts/min</td>
<td>Nonresistance</td>
<td>22.6 ± 1.7</td>
<td>22.9 ± 2.8</td>
<td>27.6 ± 3.0§</td>
<td>25.5 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Resistance</td>
<td>22.0 ± 1.7</td>
<td>24.6 ± 2.4</td>
<td>43.3 ± 2.1‡‡§</td>
<td>35.6 ± 3.0§§</td>
</tr>
<tr>
<td>MSNA BI, bursts/100 heart rate</td>
<td>Nonresistance</td>
<td>32.4 ± 3.2</td>
<td>20.8 ± 2.2*</td>
<td>24.0 ± 2.1*</td>
<td>24.2 ± 2.5*</td>
</tr>
<tr>
<td></td>
<td>Resistance</td>
<td>33.1 ± 2.7</td>
<td>22.1 ± 2.1†</td>
<td>33.5 ± 2.0§</td>
<td>31.9 ± 3.0§</td>
</tr>
</tbody>
</table>

Values are means ± SE. V̇E, expired minute ventilation; V̇T, tidal volume; Fb, breathing frequency; V̇O₂, oxygen uptake; SpO₂, arterial oxygen saturation; P̄ETCO₂, end-tidal partial pressure of CO₂; P̄ETO₂, end-tidal partial pressure of O₂; PETCO₂, end-tidal partial pressure of CO₂; MBP, mean blood pressure; MSNA BF, muscle sympathetic activity burst frequency; MSNA BI, muscle sympathetic activity burst incidence. *Significant from Rest in the Nonresistance trial, P < 0.05. †Significant from exercise 1 in the Nonresistance trial, P < 0.05. ‡Significant from Rest in the Resistance trial, P < 0.05. §Significant from exercise 1 in the Resistance trial, P < 0.05. $Significant from the Nonresistance trial, P < 0.05.

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, i.e., vs. at 5 min at Rest or vs. at 5 min during exercise 1. In comparisons with the data at 5 min during exercise 1, we indicate significance only when values were higher during exercises 2 and 3 and Recovery. Comparisons of parameters between the Resistance and Nonresistance trials were performed using paired t-test (parametric test) if the distribution was regular. When the distribution was not regular, Wilcoxon test (nonparametric test) was used. The StatView (5.0, SAS Institute) and the SPSS (11.5, SPSS) statistical packages were used for the analyses. A P < 0.05 was considered significant.

**RESULTS**

**Maximal Exercise Test**

Cardiorespiratory parameters at exhaustion during maximal exercise test are as follows: V̇O₂ = 3.17 ± 0.14 l/min, 46.4 ± 2.1 ml·kg⁻¹·min⁻¹, V̇CO₂ = 3.76 ± 0.15 l/min, V̇E = 131.1 ± 7.5 l/min, HR = 187.3 ± 24 beats/min, and SpO₂ = 96.3 ± 0.4%.

**Submaximal Exercise Test**

**Baseline descriptive data.** There were no significant differences in any of the respiratory variables and HR at rest (Rest) and during exercise (exercises 1 and 2) between the PImax and MSNA tests (Table 1). No differences in these variables were found at Rest and exercise 1 between the Resistance and Nonresistance trials. Workload during submaximal exercise was 93.8 ± 5.2 Watts.

**Inspiratory muscle strength.** In the Nonresistance trial, PImax was unchanged after exercise (124.1 ± 4.8 to 126.4 ± 5.2
In contrast, $P_{\text{Imax}}$ was decreased significantly following exercise in the Resistance trial (124.3 ± 5.3 to 110.5 ± 5.9 cmH$_2$O, $P < 0.05$).

**Respiratory variables.** Representative flow and partial pressure of CO$_2$ ($PCO_2$) during the Resistance trial are presented in Fig. 1. Respiratory variables are shown in Table 1. $\dot{V}E$, $VT$, $fb$, $VO_2$, and the end-tidal partial pressure of O$_2$ ($P_{ETO_2}$) increased significantly ($P < 0.05$) during exercise 1 in each trial, whereas $SpO_2$ was unchanged. Additionally, $\dot{V}E$, $fb$, and $P_{ETO_2}$ showed further increases ($P < 0.05$) during exercise 2 in each trial. $SpO_2$ during exercise 2 demonstrated a small but significant ($P < 0.05$) increase in both trials. $VO_2$ during exercise 2 increased significantly ($P < 0.05$) in the Resistance trial, compared with prior exercise, but not in the Nonresistance trial. Variables increased by voluntary hyperpneation returned to prehyperpnea levels during exercise 3 and to preexercise levels during Recovery. $P_{ETCO_2}$ increased slightly but significantly during exercise 1 in each trial, but no further changes appeared during exercises 2 and 3. There were no significant differences in all respiratory variables between the Nonresistance and Resistance trials throughout experiment, with the exception of $VO_2$ during exercise 2.

MSNA. Typical MSNA recordings are described in Figs. 1 and 2, and mean MSNA BF and BI values are indicated in Table 1 and Fig. 3. MSNA BF was unchanged during exercise 1 in each trial. In the Resistance trial, a progressive increase ($P < 0.05$) in MSNA BF occurred during exercise 2. In the Nonresistance trial, MSNA BF during exercise 2 exhibited a small but significant ($P < 0.05$) increase by voluntary hyperpnea without inspiratory resistance. Significant losses of MSNA BF occurred during exercise 3 and Recovery in the Nonresistance trial, whereas the increased MSNA BF was remained during exercise 3 and Recovery in the Resistance trial ($P < 0.05$). There was a significant ($P < 0.05$) difference in MSNA BF during exercises 2 and 3 and Recovery between the two trials.

MSNA BI is shown in Table 1 and Fig. 3. In the Nonresistance trial, MSNA BI decreased significantly ($P < 0.05$) during exercises 1, 2, and 3, and returned to preexercise level during Recovery. In the Resistance trial, a significant decrease in MSNA BI occurred during exercise 1 but returned to preexercise level during exercises 2 and 3. In addition, MSNA BI was increased ($P < 0.05$) during Recovery in the Resistance trial. MSNA BI during exercises 2 and 3 and Recovery in the

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**Fig. 1.** Representative flow, $P_{CO_2}$, electrocardiogram (ECG), blood pressure (BP), and muscle sympathetic nerve activity (MSNA) recordings in the Resistance trial.

**Fig. 2.** Typical recording of BP and MSNA during exercise with resistive breathing (exercise 2) in the Resistance trial.
Resistance trial was higher \((P < 0.05)\) than those in the Nonresistance trial.

**Cardiovascular variables.** Mean changes in HR values are shown in Fig. 4. In both trials, HR increased significantly \((P < 0.05)\) during exercise 1 compared with Rest. During voluntary hyperventilation (exercise 2), HR increased significantly \((P < 0.05)\) in the Resistance trial but not in the Nonresistance trial. The increased HR during exercise 2 returned to prehyperpnea level during exercise 3 in the Resistance trial. There was a significant difference in HR during exercise 2 between the two trials. HR remained higher \((P < 0.05)\) during Recovery compared with Rest in each trial.

Representative BP recording is indicated in Figs. 1 and 2, and SBP, DBP, and MBP values are shown in Fig. 4 and Table 1. SBP, DBP, and MBP increased significantly \((P < 0.05)\) during exercise 1 in both trials. In the Resistance trial, SBP, DBP, and MBP tended to increase during exercise 2, and a significant \((P < 0.05)\) increase in DBP occurred at 4 and 5 min during exercise 2 compared with exercise 1. In contrast, there were no changes in SBP, DBP, and MBP during exercise 2 in the Nonresistance trial. SBP, DBP, and MBP during the latter part of exercise 2 in the Resistance trial were higher \((P < 0.05)\) than those in the Nonresistance trial. The increased SBP, DBP, and MBP in the Resistance trial returned to prehyperpnea levels during exercise 3, but DBP and MBP remained higher \((P < 0.05)\) than those in the Nonresistance trial. During Recovery, SBP in both trials tended to return to preexercise levels but remained higher \((P < 0.05)\) than during Rest. Significant losses in DBP and MBP occurred during Recovery in the Nonresistance trial, while the increased DBP and MBP in the Resistance trial remained during Recovery. DBP and MBP during Recovery in the Resistance trial were significantly \((P < 0.05)\) higher than in the Nonresistance trial.

**DISCUSSION**

The major findings of this study were as follows: 1) the time-dependent increase in MSNA BF during exercise with inspiratory resistance and the increased MSNA BF remained after exercise, and 2) SBP, DBP, and HR increased during exercise with inspiratory resistance, and DBP remained higher following exercise. These findings support our hypothesis that inspiratory muscle fatigue may lead to increases in sympathetic vasomotor outflow and BP during submaximal exercise. To our
knowledge, this is the first study to clarify MSNA and cardiovascular responses during dynamic leg exercise with inspiratory resistance.

**Effect of Respiratory Muscle Fatigue on MSNA and BP Response During Exercise**

$P_{Imax}$, as an index of inspiratory muscle strength, decreased significantly following exercise with inspiratory resistance. Although we have no evidence of this, the decreased inspiratory muscle strength may be the principle cause of diaphragm fatigue. The diaphragm has an abundance of type IV metaboreceptors, and fatiguing the diaphragm via phrenic nerve stimulation caused an increase in type IV afferent discharge in anesthetized rats (15). Furthermore, 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 (16, 28, 29). As shown in Figs. 2 and 3, inspiratory resistive breathing during leg cycle exercise did cause a time-dependent increase in MSNA BF. Consequently, these results suggest that the fatiguing diaphragm, and thereby the metaboreflex, has a powerful influence on sympathetic vasoconstrictor outflow during exercise at mild intensity.

Several mechanisms other than a metaboreflex could relate to the increased MSNA during exercise with inspiratory resistive breathing. First, we need to consider arterial baroreflex. It has been reported that heightened inspiratory effort during Muller maneuver evokes a negative intrathoracic pressure, an elevation of aortic transmural pressure, and a reduction of BP (3, 27). Thus increased aortic baroreceptors should elicit a reflex increase in MSNA. However, this is unlikely, because BP increased during exercise 2 with inspiratory resistive breathing (Fig. 4) (36). Second, increased central respiratory motor output may be a cause of the observed sympathoexcitation. It is likely that central respiratory motor output (central command) increased progressively as the diaphragm became more fatigued and additional motor units were recruited. Several lines of evidence argue against an important role for central command in causing the sympathetic activation associated with inspiratory resistive breathing. St. Croix et al. (39) reported that an increase in central respiratory motor output produced immediate and sustained increases in HR without significant effect on sympathetic outflow. However, the exceptional subject showed a rapid increase in MSNA in response to nonfatiguing, resistive breathing. From this, they suppose that extreme levels of central respiratory motor output per se may influence MSNA during heavy respiratory muscle work. In the present study, a small but significant increase in MSNA BF was noted in the Nonresistance trial (Fig. 3), and this may support, in part, the supposition. Finally, it is possible that pain and/or mental stress during resistive breathing may be associated with the increased MSNA (36, 39). Vissing et al. (44) observed that exercise-induced increases in MSNA disappeared following exercise involving persistent muscle pain. Thus it is unlikely that such distress is related to the increased MSNA during hyperpnea against inspiratory resistance.

A progressive increase in DBP appeared during exercise with resistive breathing, as shown in Fig. 4. SBP and MBP also showed nonsignificant increases during exercise 2 in the Resistance trial (Fig. 4 and Table 1). These results are in agreement with previous studies that revealed an increase in MBP during voluntary hyperpnea with resistance at rest (36, 39). In contrast, Wetter et al. (47) reported no changes in MBP during submaximal exercise with resistive breathing. The discrepancy between their study and this study may be related to several factors. For one thing, the inspiratory resistive load placed on the respiratory muscles during submaximal exercise in the study of Wetter et al. (47) could simply be insufficient to cause such a change. They added inspiratory resistance to the subjects, but breathing frequency was maintained at the level of spontaneous breathing (~25 breaths/min). In contrast, in this study, the subjects experienced an increased breathing frequency (60 breaths/min) with resistive load. Another possibility is that of the subjects’ characteristics. The subjects in the report by Wetter et al. (47) were competitive cyclists, whereas those in the present study were untrained. Sedentary subjects are more susceptible to respiratory muscle fatigue compared with trained athletes (4). Although further investigation is needed to clarify the reasons for these contradictory data, the results in this study suggest that a fatiguing diaphragm causes vasoconstriction, which is induced by sympathoexcitation via the metaboreflex.

**Sustained Increases in MSNA and DBP After Exercise With Resistive Breathing**

It is interesting to note that enhanced MSNA BF and DBP, which were induced by resistive breathing, remained during later exercise with spontaneous breathing (exercise 3) and during Recovery (Figs. 4 and 5B). These results are in agreement with several previous reports (42, 43), which indicated that augmented MSNA and/or BP remained for several minutes following arm cycling. As mentioned in the METHODS section, we confirmed that the changed respiratory and circulatory parameters induced by exercise with inspiratory resistance returned to preexercise level within 30 min of cessation of exercise in the preliminary study. Additionally, when the Resistance trial was performed before the Nonresistance trial, the increased MSNA BF and BI and DBP after Resistance trial went back to preexercise levels at the beginning of the Nonresistance trial. Therefore, enhancement of MSNA and DBP was not prolonged over 30 min.

The augmented MSNA BF and BP after exercise could result from respiratory muscle metaboreflex, which may be supposed by limb muscle metaboreflex by using vascular occlusion following exercise (25, 31, 43). Another possible mechanism of the sustained increase in MSNA BF and BP following inspiratory resistance during exercise is chemoreflex from peripheral and/or central chemoreceptors (26). However, $P_{ETCO_2}$, $P_{ETO_2}$, and $S_{PO_2}$ returned quickly to preexercise levels after exercise, as shown in Table 1. Therefore, we consider it unlikely that sympathetic outflow remained elevated following exercise with inspiratory resistance because of persistent chemical stimuli, although we cannot exclude the possibility of persistent changes in the environment of peripheral and central chemoreceptive tissues.

**Clinical Implications**

The fatiguing diaphragm affects cardiovascular regulation and blood flow distribution during maximal exercise in trained endurance athletes (6, 7, 13, 14, 30). However, there are many
situations where the work of breathing increases during submaximal exercise in healthy subjects, e.g., elderly people. Furthermore, clinically, respiratory muscle work could play a particularly important role in determining oxygen transport, limb muscle fatigue and, hence, exercise tolerance in patients with OSA (1, 24), chronic heart failure (18), and COPD (2). Simon et al. (38) researched leg blood flow during exercise in COPD patients. They found that blood flow to the exercising legs appeared to plateau during submaximal exercise in some patients with COPD, despite the fact that total VO2, i.e., cardiac output, continued to increase. The authors speculated redistribution of cardiac output and oxygen transport from the exercising muscles of the lower limb to the respiratory muscle. In the present study, respiratory muscle fatigue enhanced MSNA BF and BI and BP during exercise. Therefore, in addition to redistribution of blood flow, we assume that vasoconstriction, which is induced by sympathoexcitation via the fatiguing diaphragm during submaximal exercise, affects locomotor oxygen transport and consequent locomotor muscle fatigue and exercise tolerance in patients.

**Technical Considerations and Limitations**

PImax was used as an index of inspiratory muscle fatigue in the present study. There is controversy as to whether or not PImax is a valid measure of inspiratory muscle strength (17). To address this, five measurements were performed, and the highest of three measurements with less than 5% variability was utilized as PImax (45, 46). Consequently, we ensured good reliability in our measurements: coefficient of variation for three highest PImax measurements was 2.5%, and the within-day coefficient of variation for PImax was 2.1%. Therefore, PImax data presented in this study are valid and that PImax levels were comparable before and after exercise with or without inspiratory resistance.

In previous studies, inspiratory resistance was set at 3 to 10 cmH2O L−1 s−1 (23, 47) or 60% of maximal inspiratory pressure (MIP) (17, 36). Additionally, mouth and esophageal pressures were recorded during voluntary hyperpea against resistance (36, 37, 47). We could not set %MIP for inspiratory resistance in each subject, and alternatively, maximal resistance of the device, i.e., 40 cmH2O, was used. As the result, %MIP in each subject differed (range 29–40% MIP). Thus it is likely that different relative MIP values would affect the magnitude of inspiratory muscle fatigue and therefore the metaboreflex in each subject.

Exercise intensity was set at 40% VO2peak for the following reasons. First, the percentage of successful MSNA recording is high when the exercise intensity is mild. Movements of the arm and the body became large during leg cycling with inspiratory resistive breathing. Second, this study as well as others reported that MSNA BF did not change during leg cycling at 40% VO2peak exercise compared with when at rest (20, 34). Thus we speculated that an increase in MSNA BF with inspiratory resistive breathing would become apparent under these conditions.

PETO2 showed a small but significant increase during exercise (exercise 1), although the dead space was not large (~130 ml). Therefore, during voluntary, e.g., exercise 2, PETO2 was maintained at a spontaneous breathing level during exercise 1, but not at Rest, by adding CO2 to the inspired air. Consequently, there were no differences in PETO2 between exercises 1 and 2 in each trial and between the Nonresistance and Resistance trials throughout the experimental period. Therefore, the changes in PETO2 during exercise are unlikely to be responsible for the differences in circulatory variables in the Nonresistance and Resistance trials.

MSNA was represented as BF and BI, which has been used in previous studies to record MSNA during dynamic leg cycling (20, 33, 34). Burst amplitude was not estimated because electromyographic and efferent and afferent nerve activities altered the baseline of the integrated neurogram during dynamic leg cycling (20, 34). However, previous studies have demonstrated that there is a positive correlation between BF and burst amplitude (25) and parallel increases in BF and burst amplitude during exercise (35). In some cases, we could not preserve signal-to-noise ratio >3:1 during leg cycling because of changes in the baseline. To identify MSNA, we asked the subjects to hold their breath at the middle phase, not only at rest, but also during exercise (exercise 3). Thus it seems reasonable to suppose that our MSNA BF values are valid and that MSNA levels were comparable during exercise with or without resistive breathing in this study.

In this study, the subjects were men only. Gonzales and Sheuermann (11) compared a reduction in PImax during inspiratory resistive breathing at rest between males and females. They found that the rate of inspiratory muscle fatigue during resistive breathing in females was slower than in males. Therefore, it is conceivable that MSNA and BP responses to dynamic leg exercise with inspiratory resistive breathing in females differ from that in males.

**Perspectives and Significance**

PImax decreased significantly after moderate leg cycling exercise with inspiratory resistance but not without resistance. Time-dependent increases in MSNA BF occurred during exercise with inspiratory resistive breathing, accompanied by an augmentation of BP. These results suggest that inspiratory muscle fatigue induces increased muscle sympathetic vasomotor outflow and BP during dynamic leg exercise at mild intensity.

Inspiratory muscle fatigue reflexively induces sympathetically mediated vasoconstrictor activity. In turn, it is likely that blood flow and oxygen transport to the working limb are reduced, thereby exacerbating limb fatigue and compromising exercise performance (6, 13, 19). In contrast, some investigators have demonstrated that inspiratory muscle training increases respiratory muscle endurance and/or improvements in exercise performance in healthy subjects (8) and patients (12). It is assumed that respiratory muscle fatigue during exercise may alleviate following inspiratory muscle training and increase blood flow and oxygen transport to the working muscle. In addition, there are many situations in which people perform exercise under hypoxic conditions, e.g., at altitude or under pathophysiological conditions. Respiratory muscle fatigue is exaggerated during hypoxic exercise (41). Thus it is hypothesized that a large inspiratory muscle fatigue during exercise in hypoxia induces sympathetically mediated vasoconstrictor activity, thereby compromising blood flow to the active limb. Further research is necessary to confirm these speculations.


