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Sex differences in blood pressure and inactive limb blood flow responses during dynamic leg exercise with increased inspiratory muscle work

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ABSTRACT

We hypothesized that, compared with young males, young females have a smaller decrease in blood flow to the inactive limb, accompanied by a smaller increase in arterial blood pressure, during dynamic exercise with increased inspiratory muscle work. Young males and females performed dynamic knee-extension and -flexion exercises for 10 min (spontaneous breathing for 5 min and voluntary hyperpnoea with or without inspiratory resistance for 5 min). Mean arterial blood pressure (MAP) and mean blood flow (MBF) in the brachial artery were continuously measured by means of finger photoplethysmography and Doppler ultrasound, respectively. No sex differences were found in the ΔMAP and ΔMBF (Δ: from baseline) during exercise without inspiratory resistance. In contrast, the ΔMAP during exercise with inspiratory resistive breathing was greater ($P < 0.05$) in males (+31.3 ± 2.1 mmHg, mean ± SE) than females (+18.9 ± 3.2 mmHg). The MBF during exercise with inspiratory resistance did not change in males (–4.4 ± 10.6 mL/min), whereas it significantly increased in females (+25.2 ± 15.4 mL/min). These results suggest that an attenuated inspiratory muscle-induced metaboreflex in young females affects blood flow distribution during submaximal dynamic leg exercise.

Keywords: exercise, blood flow distribution, respiratory muscle, sex difference, metaboreflex

Abbreviations:
ABP: arterial blood pressure
Ex-SB: exercise with spontaneous breathing
Ex-VH: exercise with voluntary hyperventilation
HR: heart rate
SAP: systolic arterial blood pressure
DAP: diastolic arterial blood pressure
MAP: mean arterial blood pressure
MBF: mean blood flow

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INTRODUCTION

Hyperpnoea during high-intensity whole body exercise leads to a considerable increase in respiratory muscle work. The increased respiratory muscle work and the accompanying accumulation of metabolites are associated with cardiovascular and neural responses that affect the distribution of blood flow during exercise.[1,2] This sympathoexcitation is caused by a respiratory muscle-induced metaboreflex.[3,4] Concerning blood flow regulation, Dominelli et al[5] manipulated the work of breathing during high-intensity leg cycling and found that blood flow in the active limb (quadriceps) during exercise was decreased when inspiratory muscle work was increased: the opposite occurred when inspiratory muscle work was reduced. In our previous study,[6] during mild-intensity dynamic leg exercise, blood flow in the brachial artery (inactive limb) was reduced with inspiratory resistive breathing, while blood flow in the femoral artery (active limb) was maintained. These results show that respiratory muscle work significantly influences the distribution of blood flow during dynamic exercise, although this is modulated by exercise intensity and the magnitude of respiratory work.

Previous studies have shown sex differences in respiratory and cardiovascular responses during exercise,[7,8] whereas a few studies have reported differences in the respiratory muscle metaboreflex between males and females. Guenette et al[9] revealed that the magnitude of diaphragmatic fatigue after high-intensity exercise in females was lower in than in males. Our group found that the magnitude of the increases in arterial blood pressure (ABP) and sympathetic vasomotor outflow in response to enhanced respiratory muscle work were lower in young females than in age-matched males.[10,11] These results indicate that the respiratory muscle metaboreflex is attenuated in young females. Do these sex-based differences in neural and blood pressure responses affect blood flow distribution? Smith et al[12] reported a smaller increase in ABP and a smaller decrease in limb blood flow in females compared with males. However, in their study, subjects performed inspiratory resistive breathing task under resting conditions. During dynamic exercise, the situation is more complicated because the change in blood flow is regulated by multiple neural mechanisms, including the exercise pressor reflex from skeletal muscles.

The aim of this study was to elucidate the sex differences in ABP and inactive limb blood flow during exercise with increased inspiratory muscle work. We hypothesized that young females have a smaller decrease in blood flow to the inactive limb, accompanied by a smaller increase in ABP, during dynamic exercise with increased inspiratory muscle work. To test this hypothesis, we recorded ABP and brachial artery blood flow during mild-intensity dynamic leg exercise with inspiratory resistive breathing in young females and age-matched males.

METHODS

Ethical approval

All procedures conformed to the Declaration of Helsinki and were approved by the Graduate School of Medicine, Nagoya University Institutional Review Board (approval no. 2016-0030). Informed written consent was obtained from all subjects before participation.

Subjects

Fourteen healthy young males and seventeen age-matched females were initially enrolled in this study; we were unable to obtain high-quality blood flow recordings during leg exercise in 4 males and 7 females, so they were excluded from the data analysis. Consequently, 10 males [age,

20 ± 1 yrs (mean ± SE); height, 170 ± 1 cm; body mass, 59 ± 1 kg] and 10 females [age, 20 ± 1 yrs; height, 160 ± 2 cm; body mass, 54 ± 2 kg] completed the study protocol. All subjects were free of any known disease and non-smokers. All females with regular menstrual cycles (25–36 days) were tested randomly throughout the menstrual cycle. We previously found that the menstrual cycle does not affect ABP and limb blood flow responses during hyperpnoea.[13] None of the subjects had taken oral contraceptives or medications that could influence hormone levels during the study.

Experimental procedures

All experiments were performed under controlled temperature conditions (22–24°C). The first visit, the subjects underwent a pulmonary function test and measurement of inspiratory muscle strength. Then, an incremental exercise was carried out using a custom-designed ergometer in semi-recumbent position (150°) with a backrest.[6,14] Before the incremental exercise test, the subjects practiced bilateral dynamic knee-extension and -flexion exercises; they received instructions regarding the maintenance of lateral arm position during exercise. The subjects' feet were putted in an Achilles-bracing heel-cup attached by a steel rod to the pedal arm of a cycle ergometer (Aerobike 75XLIII; Combi), which was placed behind the seated subjects. One complete turn of the pedal arm required knee extension from 90° to 135°.[6,14] Alternating movement of both legs required smoothly coordinated movements, similar to the movements those required when using a standard cycle ergometer. Females began at 10 W and males began at 30 W with power output increasing by 5 W/min. The alternating contraction frequency was kept at 40 per minute per leg. Cardiorespiratory parameters were continuously measured and averaged every 30 seconds thereafter. The highest oxygen uptake ($\dot{V}O_2$) value was recorded as the peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$).

At the second visit, two submaximal exercise tests (with and without inspiratory resistance) were performed during cardiovascular and brachial artery (inactive limb) blood flow measurements (Figure 1). First, the subjects rested with spontaneous breathing for 5 min (rest, baseline, BL).

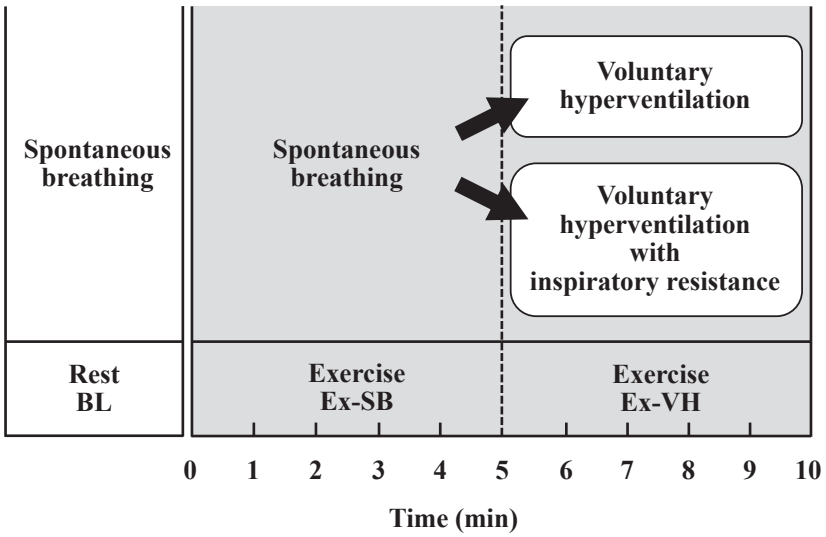

Fig. 1 Time course of the submaximal exercise test

BL: baseline
Ex-SB: exercise with spontaneous breathing
Ex-VH: exercise with voluntary hyperventilation (with or without inspiratory resistive breathing)

Then, submaximal dynamic exercise was started, the intensity was set at 40%$\dot{V}O_{2peak}$. During the first 5 min of exercise (exercise with spontaneous breathing; Ex-SB), subjects breathed spontaneously. During the next 5 min, subjects controlled their breathing with or without inspiratory resistive loading (exercise with voluntary hyperventilation; Ex-VH). Under the Ex-VH, breathing frequency (fb) was kept at 40 breaths/min using auditory feedback from the metronome, the ratio of inspiration and expiration per breath cycle was set at 1:1. Tidal volume (VT) was controlled at twice the baseline VT. The target VT was displayed on a computer screen as a horizontal line for feedback. By adding CO_2 to the inspired air, the end-tidal partial pressure of CO_2 (P_{ETCO2}) was maintained within ± 5 torr during the first minute of Ex-VH; from the second minute to the end of testing, P_{ETCO2} was maintained within ± 4 torr. A threshold resistance instrument, which was set at 40% of the maximal inspiratory pressure (PImax), was attached to the inspiratory side via tubing. The procedure was repeated twice, i.e., with (resistance trial) and without inspiratory resistive loading (nonresistance trial). The order of resistance and nonresistance trials was randomized with a 20-min interval between trials.

Pulmonary function and inspiratory muscle strength

Vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1.0 s ($FEV_{1.0}$, $FEV_{1.0}$/FVC), and maximal voluntary ventilation in 12 s (MVV_{12}) were evaluated by means of a computerized spirometry device (AS-507; Minato Medical Science). Spirometric measurements except for MVV_{12} were repeated five times, and the highest values were selected.[15] The MVV_{12} was measured three times, and the highest value was used in the analysis. The PImax was evaluated using a hand-held mouth pressure meter (AAM337; Minato Medical Science) attached to a computerized spirometry device The PImax was measured from the residual volume, and the maximal value of five maneuvers that varied by < 10% was recorded.[16]

Respiratory variables during exercise test

During the incremental exercise test, $\dot{V}O_2$ was determined using a previously described method.[6,17-19] Subjects breathed through a facemask, which was attached to a Fleisch pneumotachometer (PN-230; Arco Systems). The pneumotachometer was attached to a device with a low-resistance, one-way valve. To record the expired gas fraction, a sampling tube was connected to the pneumotachometer. A mass spectrometer (ARCO-1000; Arco Systems) was used to measure gas fractions. The signals from the pneumotachometer and the mass spectrometer were sampled at 200 Hz and stored on a computer (PC-9821Ra40; NEC).

During the submaximal test, the subjects breathed through a mouthpiece connected to the Fleisch pneumotachometer (PN-230; Arco Systems). The sample gas was drawn through a tube inserted into the mouthpiece to record the fraction of end-tidal CO_2 (F_{ETCO2}) using a mass spectrometer (ARCO-2000; Arco Systems): the P_{ETCO2} was calculated. Arterial oxygen saturation (SpO_2) was monitored by means of a finger pulse oximeter (Radical 7; Masimo). The signals from the apparatuses were sampled at a frequency of 200 Hz through an analog-to-digital converter (CSI-320416; Interface) and stored in a computer (CF-F8; Panasonic).

Cardiovascular variables during exercise test

An electrocardiogram (ECG) during the exercise tests was recorded by means of a three-lead electrocardiograph (AB-621; Nihon Koden); heart rate (HR) was calculated from each R-R interval. Beat-by-beat ABP during the submaximal dynamic exercise test was measured using photoplethysmography from the middle finger of the left hand (Finometer; Finapress Medical Systems BV). Signals of ECG and ABP were sampled and stored using a method similar to the method employed for respiratory variables. Systolic and diastolic artelial blood pressure (SAP and

DAP) were determined from the blood pressure waveform signal. Mean arterial blood pressure (MAP) was calculated as follows: [MAP = (SAP – DAP)/3 + DAP].

Brachial artery blood flow and forearm vascular conductance during exercise test

Brachial artery blood flow was measured using an ultrasound device (Vivid i; GE Yokokawa Medical Systems) (8.8 MHz multifrequency linear probe). Ultrasound measures were acquired at the distal one-third of the upper arm to assess the brachial artery.[6] Ultrasound settings were set to optimize longitudinal B-mode images of the lumen-arterial wall interface. Pulsed Doppler signals were recorded at an insonation angle of 60° with the sample volume encompassing the arterial diameter. Images of the brachial artery and velocity waveform were stored on a computer at a frequency of 30 Hz using a frame grabber (DVI2USB3.0; Epiphan Video). Brachial diameter and mean blood velocity were analyzed using automated, custom-designed wall-tracking and edge-detecting software (S-13037 version 2.0.1.; Takei Scientific Instruments Co, Ltd).[6] From the synchronized arterial diameter and mean blood velocity data, mean blood flow (MBF) was computed as 60 × cross-sectional area × mean blood velocity.[6] Forearm vascular conductance was calculated as MBF/MAP.

Statistical analysis

Variables are expressed as mean ± SE. The assumption of normality was verified using the Shapiro-Wilk test. If the distribution was normal, comparisons of parameters between males and females were performed using Student's unpaired *t* test. When the distribution was abnormal, the Mann-Whitney *U* test was used. We performed a three-way analysis of variance with repeated measures (three-way ANOVA RM) to analyze the effects of time (baseline, 0–10 min), sex (males vs females), and trial (nonresistance vs resistance). Changes in variables during the nonresistance or resistance trials were analyzed using the Bonferroni test (from baseline and from 5 min of Ex-SB). Comparisons of parameters between males and females during each trial were conducted by two-way ANOVA RM. Because of the baseline differences in HR, MAP, MBF, and forearm vascular conductance between males and females, the absolute changes from baseline (ΔHR, ΔMAP, ΔMBF, and Δ forearm vascular conductance) were compared as reported previously.[10,12,20-22] The SPSS statistical package (v.22.0; IBM.) was used only to execute the Shapiro-Wilk test. StatView software (5.0; SAS Institute) was used for the other statistical analyses. Significance was set at $P < 0.05$ for all statistical comparisons.

RESULTS

Pulmonary function and respiratory muscle strength

The pulmonary function and inspiratory muscle strength data are shown in Table 1. Significantly lower values of VC, FVC, $FEV_{1.0}$, $FEV_{1.0}$/FVC, and MVV_{12} were found in females compared with males, whereas there was no difference in PImax between the two groups.

Table 1 Pulmonary function and respiratory muscle strength

	Males (n = 10)	Females (n = 10)
VC (L)	4.8 ± 0.1	3.3 ± 0.1*
FVC (L)	4.6 ± 0.1	3.3 ± 0.1*
$FEV_{1.0}$ (L)	4.3 ± 0.1	2.9 ± 0.1*
$FEV_{1.0}$/FVC (%)	93.3 ± 1.3	88.2 ± 1.2*
MVV_{12} (L/min)	176.1 ± 8.4	127.1 ± 5.7*
PImax (cmH_2O)	114.7 ± 4.2	98.1 ± 11.5

Values are mean ± SE.
VC: vital capacity
FVC: forced vital capacity
$FEV_{1.0}$: forced expiratory volume in 1.0 s
MVV_{12}: maximal voluntary ventilation for 12 s
PImax: maximal inspiratory pressure
SE: standard error
*$P < 0.05$ vs Males.

Incremental exercise test

Cardiopulmonary parameters at exhaustion during the incremental exercise test were as follows: $\dot{V}O_{2peak}$ was lower ($P < 0.05$) in females (1.3 ± 0.1 L/min, 23.1 ± 1.3 mL/kg/min) than in males (1.8 ± 0.1 L/min, 29.8 ± 1.8 mL/kg/min), whereas there was no difference in HR at exhaustion between the two groups (males: HR = 163.4 ± 2.0 beats/min, females: HR = 159.4 ± 4.3 beats/min). The workload at exhaustion was lower ($P < 0.05$) in females (62.0 ± 2.7 W) than males (83.5 ± 2.9 W).

Submaximal exercise test

Baseline descriptive data. The respiratory and cardiovascular variables at baseline are shown in Tables 2–4. The SAP, MAP, and MBF at BL were lower ($P < 0.05$) in females than in males. The workload during submaximal exercise was 32.4 ± 2.3 and 20.2 ± 1.7 W for males and females, respectively.

Respiratory variables. Changes in respiratory variables during the experiment are shown in Table 2. The $\dot{V}E$, VT, and fb increased during Ex-SB, and $\dot{V}E$ and fb increased further during Ex-VH with or without inspiratory resistance, as expected. P_{ETCO2} was unchanged during submaximal exercise in the nonresistance or resistance trials. There were no significant differences in the changes in any respiratory variables during the submaximal exercise test between males and females.

Cardiovascular variables. Table 3 shows the changes in cardiovascular parameters during the submaximal exercise test. In each trial, HR increased significantly during Ex-SB, and further

Table 2 Respiratory variables during the submaximal exercise test

	Trial	Group	Baseline	Ex-SB	Ex-VH	Statistics
$\dot{V}E$ (L/min)	Nonresistance	Males	10.2 ± 0.6	28.8 ± 2.5	60.5 ± 3.3	**Three-way ANOVA RM** F = 0.1 P = 0.999
		Females	7.7 ± 0.5	20.1 ± 0.8	50.7 ± 2.9	**Two-way ANOVA RM** Males vs Females
	Resistance	Males	10.2 ± 0.7	30.0 ± 3.1	62.2 ± 4.6	Nonresistance trial F = 1.2 P = 0.301
		Females	7.9 ± 0.6	20.3 ± 0.8	51.0 ± 3.0	Resistance trial F = 1.1 P = 0.397
VT (L)	Nonresistance	Males	0.8 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	**Three-way ANOVA RM** F = 0.2 P = 0.995
		Females	0.7 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	**Two-way ANOVA RM** Males vs Females
	Resistance	Males	0.8 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	Nonresistance trial F = 0.7 P = 0.733
		Females	0.7 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	Resistance trial F = 0.7 P = 0.713
fb (breaths/min)	Nonresistance	Males	14.0 ± 1.7	22.4 ± 2.0	40.0 ± 0.1	**Three-way ANOVA RM** F = 0.1 P = 0.999
		Females	12.5 ± 1.6	22.2 ± 1.2	40.0 ± 0.1	**Two-way ANOVA RM** Males vs Females
	Resistance	Males	14.0 ± 1.4	23.7 ± 3.0	39.8 ± 0.2	Nonresistance trial F = 0.2 P = 0.998
		Females	12.7 ± 1.6	22.2 ± 1.6	40.0 ± 0.1	Resistance trial F = 0.2 P = 0.997
P_{ETCO2} (torr)	Nonresistance	Males	42.2 ± 0.5	42.4 ± 0.7	42.1 ± 0.3	**Three-way ANOVA RM** F = 0.3 P = 0.988
		Females	40.2 ± 0.6	39.5 ± 0.8	39.0 ± 0.9	**Two-way ANOVA RM** Males vs Females
	Resistance	Males	42.3 ± 0.3	42.8 ± 0.5	42.4 ± 0.5	Nonresistance trial F = 0.5 P = 0.915
		Females	40.3 ± 0.6	39.5 ± 0.7	39.5 ± 0.6	Resistance trial F = 1.2 P = 0.266
SpO_2 (%)	Nonresistance	Males	97.0 ± 0.4	96.7 ± 0.4	97.1 ± 0.4	**Three-way ANOVA RM** F = 0.7 P = 0.711
		Females	97.6 ± 0.3	97.5 ± 0.2	98.1 ± 0.2	**Two-way ANOVA RM** Males vs Females
	Resistance	Males	96.7 ± 0.2	97.0 ± 0.4	97.0 ± 0.3	Nonresistance trial F = 0.2 P = 0.995
		Females	97.3 ± 0.2	97.4 ± 0.2	98.0 ± 0.4	Resistance trial F = 1.1 P = 0.401

Values are mean ± SE.
Baseline: spontaneous breathing at rest
Ex-SB: exercise with spontaneous breathing
Ex-VH: exercise with voluntary hyperventilation
$\dot{V}E$: expired minute ventilation
VT: tidal volume
fb: breathing frequency
P_{ETCO2}: end-tidal partial pressure of CO_2
SpO_2: arterial oxygen saturation
SE: standard error

Table 3 Cardiovascular variables during the submaximal exercise test

	Trial	Group	Baseline	Ex-SB	Ex-VH	Statistics
HR (beats/min)	Nonresistance	Males	73.3 ± 3.4	104.2 ± 5.1	109.2 ± 5.0	**Three-way ANOVA RM** F = 0.5 P = 0.902
		Females	67.6 ± 2.0	96.6 ± 2.5	102.2 ± 2.6	**Two-way ANOVA RM** Males vs Females
	Resistance	Males	73.5 ± 3.5	105.0 ± 5.5	125.9 ± 7.0	Nonresistance trial F = 0.5 P = 0.902
		Females	67.4 ± 2.2	97.3 ± 3.0	119.1 ± 3.6	Resistance trial F = 0.5 P = 0.886
SAP (mmHg)	Nonresistance	Males	123.8 ± 1.4	153.2 ± 3.4	150.6 ± 2.0	**Three-way ANOVA RM** F = 1.2 P = 0.322
		Females	107.0 ± 3.7	131.1 ± 6.8	129.9 ± 7.1	**Two-way ANOVA RM** Males vs Females
	Resistance	Males	123.5 ± 2.0	152.5 ± 1.9	180.9 ± 4.2	Nonresistance trial F = 0.7 P = 0.760
		Females	106.7 ± 3.9	134.7 ± 6.6	147.4 ± 7.7	Resistance trial F = 1.8 P = 0.060
DAP (mmHg)	Nonresistance	Males	71.9 ± 1.5	74.7 ± 1.6	73.3 ± 1.6	**Three-way ANOVA RM** F = 3.0 P = 0.001
		Females	70.8 ± 2.9	75.3 ± 4.3	72.8 ± 4.9	**Two-way ANOVA RM** Males vs Females
	Resistance	Males	72.4 ± 1.2	77.1 ± 1.8	90.6 ± 1.7*	Nonresistance trial F = 0.9 P = 0.494
		Females	69.0 ± 3.1	73.3 ± 3.6	80.2 ± 4.8†	Resistance trial F = 3.3 P = 0.001
MAP (mmHg)	Nonresistance	Males	89.2 ± 1.3	100.9 ± 1.9	99.1 ± 1.5	**Three-way ANOVA RM** F = 2.0 P = 0.028
		Females	82.7 ± 3.1	93.9 ± 4.8	91.2 ± 5.4	**Two-way ANOVA RM** Males vs Females
	Resistance	Males	89.4 ± 1.2	102.3 ± 1.4	120.7 ± 2.1*§	Nonresistance trial F = 0.7 P = 0.750
		Females	81.6 ± 3.2	92.0 ± 4.6	100.5 ± 5.2†	Resistance trial F = 3.6 P < 0.001

Values are mean ± SE.
Baseline: spontaneous breathing at rest
Ex-SB: exercise with spontaneous breathing
Ex-VH: exercise with voluntary hyperventilation
HR: heart rate
SAP: systolic arterial blood pressure
DAP: diastolic arterial blood pressure
MAP: mean blood pressure
SE: standard error
*P < 0.05 vs Ex-SB (Males).
†P < 0.05 vs Ex-SB (Females).
§P < 0.05 vs Females.

increases in HR appeared during Ex-VH with and without inspiratory resistance. Regarding ΔHR during the submaximal exercise test, there was no significant three-way interaction (time × sex × trial) (F = 0.5 P = 0.902). No significant difference in ΔHR appeared was evident between males and females in the nonresistance or resistance trials (Figure 2).

Changes in SAP, DAP and MAP are shown in Table 3 and ΔMAP is indicated in Figure 3. Significant three-way interactions (time × sex × trial) for DAP, MAP, and ΔMAP (F = 2.0 P = 0.028) were found during the submaximal exercise test. In the nonresistance trial, MAP

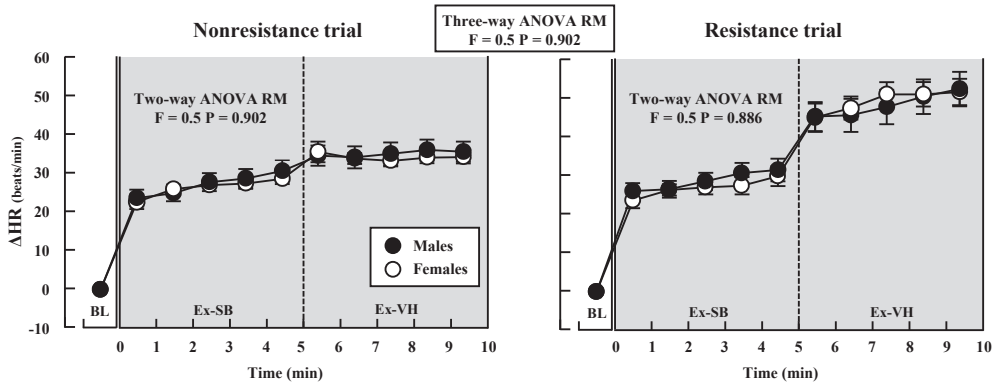

Fig. 2 Changes in HR during the submaximal exercise test
Values are mean absolute changes from BL (Δ).
HR: heart rate
BL: baseline
Ex-SB: exercise with spontaneous breathing
Ex-VH: exercise with voluntary hyperventilation

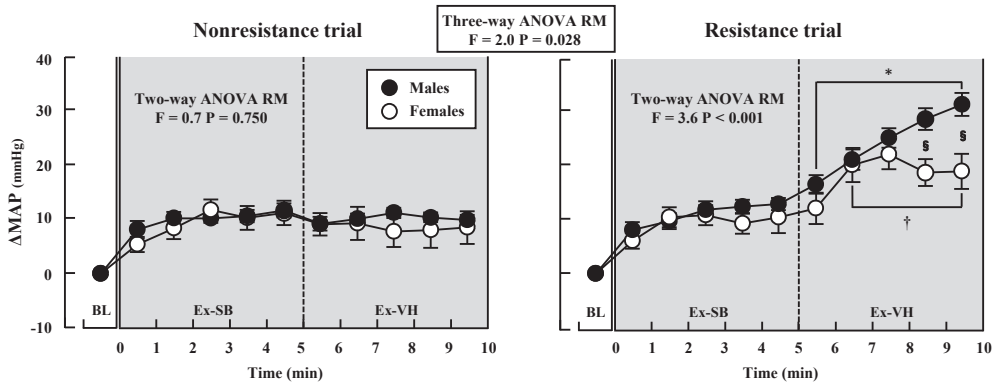

Fig. 3 Changes in MAP during the submaximal exercise test
Values are mean absolute changes from BL (Δ).
MAP: mean arterial blood pressure
BL: baseline
Ex-SB: exercise with spontaneous breathing
Ex-VH: exercise with voluntary hyperventilation
*P < 0.05 vs at 5 min (males).
†P < 0.05 vs at 5 min (females).
§P < 0.05 between males and females.

increased during Ex-SB, and no further increase in MAP appeared during Ex-VH without inspiratory resistance both males and females. No significant difference in ΔMAP was detected between males and females in the nonresistance trial. In the resistance trial, MAP increased during Ex-SB and further increased during Ex-VH with inspiratory resistance in both males and females. However, ΔMAP during the latter half of the submaximal exercise test with inspiratory resistance was smaller in females than in males (Figure 3). There was a significant difference in ΔMAP between the two groups in the resistance trial.

Blood flow and forearm vascular conductance. Table 4 and Figure 4 indicate changes in brachial artery blood flow. There was a significant three-way interaction (time × sex × trial) for MBF and ΔMBF ($F = 3.2$ $P = 0.001$) during the submaximal exercise test. MBF decreased at the onset of Ex-SB, compared with baseline, and returned to the baseline level in each group. A further increase in MBF occurred during Ex-VH without inspiratory resistance in both males and females. There was no significant difference in ΔMBF between the two groups in the nonresistance trial (Figure 4). During Ex-VH with inspiratory resistance, MBF did not change in males, whereas it increased in the latter half of Ex-VH with inspiratory resistance in females (Figure 4). A significant difference in ΔMBF in the resistance trial was observed between males and females.

Changes in forearm vascular conductance are indicated in Table 4 and Figure 5. There were significant three-way interactions (time × sex × trial) for forearm vascular conductance and Δ forearm vascular conductance ($F = 3.2$ $P = 0.001$) during the submaximal exercise test. Forearm vascular conductance decreased at the onset of Ex-SB, compared with baseline, and returned to

Table 4 Brachial artery blood flow variables and forearm vascular conductance during the submaximal exercise test

	Trial	Group	Baseline	Ex-SB	Ex-VH	Statistics
Mean blood flow (mL/min)	Nonresistance	Males	75.0 ± 8.8	77.3 ± 8.7	116.2 ± 10.0	**Three-way ANOVA RM** F = 3.2 P = 0.001
		Females	47.4 ± 6.7	54.9 ± 7.9	73.6 ± 6.7	**Two-way ANOVA RM** Males vs Females
	Resistance	Males	72.4 ± 9.0	69.7 ± 10.4	68.0 ± 12.9	Nonresistance trial F = 1.2 P = 0.304
		Females	46.9 ± 6.7	46.2 ± 5.3	72.1 ± 15.3†	Resistance trial F = 2.3 P = 0.016
Forearm vascular conductance (mL/min/mmHg)	Nonresistance	Males	0.8 ± 0.1	0.8 ± 0.1	1.2 ± 0.1	**Three-way ANOVA RM** F = 3.2 P = 0.001
		Females	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	**Two-way ANOVA RM** Males vs Females
	Resistance	Males	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	Nonresistance trial F = 0.7 P = 0.691
		Females	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	Resistance trial F = 3.1 P = 0.001

Values are mean ± SE.
Baseline: spontaneous breathing at rest
Ex-SB: exercise with spontaneous breathing
Ex-VH: exercise with voluntary hyperventilation
SE: standard error
†$P < 0.05$ vs Ex-SB (Females).

the baseline level. Forearm vascular conductance showed a further increase during Ex-VH without inspiratory resistance in each group. During Ex-VH with inspiratory resistance, forearm vascular conductance in males decreased as compared with the end of Ex-SB, whereas it increased in females in the latter half of Ex-VH with inspiratory resistance (Figure 5). There was a significant difference in Δ forearm vascular conductance between the two groups in the resistance trial.

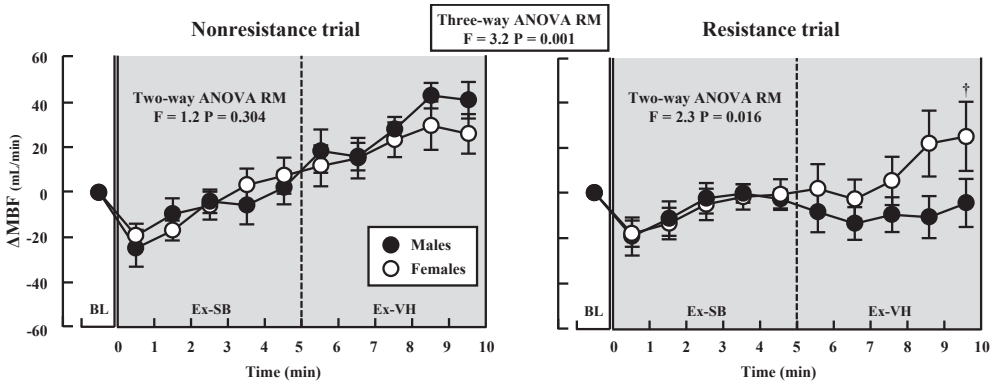

Fig. 4 Changes in MBF in brachial artery during the submaximal exercise test
Values are mean absolute changes from BL (Δ).
MBF: mean blood flow
BL: baseline
Ex-SB: exercise with spontaneous breathing
Ex-VH: exercise with voluntary hyperventilation
†$P < 0.05$ vs at 5 min (females).

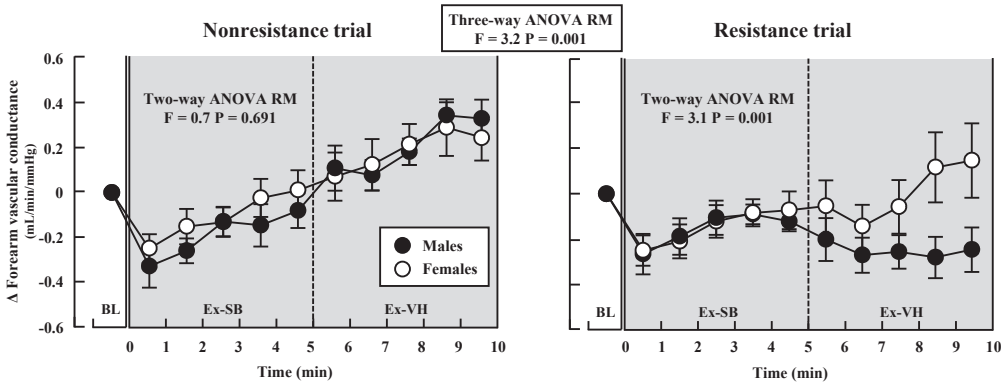

Fig. 5 Changes in forearm vascular conductance responses during the submaximal exercise test
Values are mean absolute changes from BL (Δ).
BL: baseline
Ex-SB: exercise with spontaneous breathing
Ex-VH: exercise with voluntary hyperventilation
*$P < 0.05$ vs at 5 min (males).
†$P < 0.05$ vs at 5 min (females).

DISCUSSION

The major findings of the present study were as follows: 1) MAP increased during dynamic leg exercise with inspiratory resistance in both males and females, however, the magnitude of the increase in MAP was smaller in females compared with males; 2) brachial artery blood flow was unchanged during submaximal dynamic leg exercise with inspiratory resistance in males, whereas it increased in females; 3) forearm vascular conductance was decreased during dynamic leg exercise with inspiratory resistance in males, whereas it increased in females. These findings provide novel insights into the effects of sex on cardiovascular and blood flow regulation during dynamic submaximal exercise with increased work of breathing.

Respiratory muscle metaboreflex and limb blood flow

In animal studies, when lactic acid was injected into the phrenic artery to stimulate metaboreceptors in the diaphragm, efferent sympathetic nerve activity was increased whereas blood flow in the active limb and limb vascular conductance were decreased.[23-25] Under resting conditions, in humans, increased respiratory muscle work with inspiratory or expiratory resistance leads to increased sympathetic vasomotor outflow and MAP, as well as decreased limb blood flow.[12,26-28] Additionally, increased work of breathing during dynamic exercise has been shown to increase sympathetic vasomotor outflow and MAP.[6,10,17-19] As shown in Figure 3 and Table 3, in both males and females, dynamic exercise with inspiratory resistance led to an increase in MAP. These data confirmed that inspiratory resistance during exercise in the present study induced a respiratory muscle-induced metaboreflex. Several mechanisms other than a metaboreflex could increase the MAP during Ex-VH with inspiratory resistance. One of the possible mechanisms is an increased central respiratory motor output (i.e., central command). Sympathetic vasomotor outflow was increased during active zero-load cycling, compared to passive cycling, indicating that the central command ordered a sympathoexcitatory drive.[29] Another possible mechanism is a discharge in response to mechanical stimulation (i.e., mechanoreflex). A passive muscle stretch of muscle elicits a transient increase in sympathetic vasomotor outflow.[30] These factors might have contributed to the increase in MAP, especially during the early stages of Ex-VH with inspiratory resistance.

Sex differences in the respiratory muscle metaboreflex and limb blood flow

Females have lower lung volume and smaller airways compared with males; these anatomical and mechanical differences increase respiratory muscle work during exercise in females.[31-33] Accordingly, during high-intensity dynamic exercise, the work of breathing is greater in females than in males. Thus, females are presumed to exhibit a greater respiratory muscle fatigue and a stronger respiratory muscle metaboreflex.[31,34] In contrast to this presumption, young females exhibited less respiratory muscle fatigue than did males during whole-body exercise.[9,34,35] Additionally, the magnitude of the increase in sympathetic vasomotor outflow and MAP during increased respiratory muscle work was smaller in young females than in age-matched males.[10,11,36,37] Similar to these previous studies, the increase in ΔMAP during dynamic exercise with inspiratory resistance was smaller in females than in males (Figure 3). It is important to understand this sex difference in how the respiratory muscle metaboreflex affects blood flow distribution. Under resting conditions, Smith et al[12] assessed limb blood flow during inspiratory resistive breathing in males and females. Although a decrease in limb blood flow during inspiratory loaded breathing was observed in both groups, the magnitude of the decrease in blood flow was smaller in females than males. To our knowledge, no study has compared the effects of increased work of breathing during exercise on inactive limb blood flow between males and females. In this study, MBF in the

brachial artery did not change during dynamic leg exercise with inspiratory resistance in males, whereas it increased in females (Figure 4). These results suggest that sex-based differences in the respiratory muscle-induced metaboreflex affect blood flow distribution during dynamic exercise.

Several factors contribute to sex differences in MAP and blood flow responses to the increased work of breathing during exercise. First, our research group recently reported that young females exhibit less sympathetic vasomotor outflow during mild-intensity leg cycling with increased work of breathing.[10] This report is supported by previous studies [20,21] showing a lower sympathetic vasomotor outflow during the static handgrip in premenopausal females than in males. Moreover, premenopausal females have lower metabolite accumulation (H^+ and $H_2PO_4^-$) during handgrip exercise compared with age-matched males.[20] Therefore, the attenuated sympathetic vasomotor outflow might be due to lower accumulation of metabolites the respiratory muscles in females. Similarly, the activation of accessory respiratory muscles was greater in young females than in age-matched males in response to increased ventilation during dynamic exercise.[38] Thus, greater reliance on the inspiratory accessory muscles in females relative to males, could explain the lower metabolite accumulation in females. Second, the blunted MAP and MBF responses in females may be related to sex differences in the peripheral vascular transduction of sympathetic vasomotor outflow.[39-41] Females exhibit a relative inability of sympathetic nerves to induce vasoconstriction.[39,42] This insensitivity results from the offset of α-adrenergically mediated vasoconstriction by an augmented β-adrenergic vasodilatory response.[39,42] Forearm vascular conductance was higher in females than in males during submaximal exercise with inspiratory resistive breathing (Figure 5). Thus, the absence of MBF suppression in females could be attributed to reduced peripheral vasoconstriction by the lower respiratory muscle-induced metaboreflex.

Limitations

The first limitation of this study was that we did not control for the menstrual cycle in female subjects. Previous studies have attempted to clarify the effects of the menstrual cycle on neural and cardiovascular responses, but findings have been inconsistent.[7,8,21] Our group recently reported no phase effect (early follicular vs midluteal phases) on the MAP and MBF responses during voluntary hyperpnoea under resting conditions.[13] Second, we did not measure blood flow to the active limb (ie, femoral artery blood flow). In our previous study,[6] in young males, blood flow in the femoral artery (active limb) during mild-intensity (40%$\dot{V}O_{2peak}$) dynamic leg exercise was maintained when inspiratory resistance was added, whereas a significant increase in MAP occurred. Accordingly, it is likely that blood flow in the active limb in young females is also maintained with increased work of breathing when exercise intensity is mild. However, to our knowledge, no study has reported a sex difference in this redistribution of blood flow to the active limb and inactive parts with respiratory muscle-induced metaboreflex; thus, further research is warranted. Third, the present study included only young subjects. Smith et al[22] assessed MAP and limb blood flow during inspiratory resistive breathing at rest in young and elderly males and females. They found that older females exhibited a large increase in MAP and a greater decrease in MBF, resulting in a greater inspiratory muscle-induced metaboreflex than observed in young females. We also reported that the magnitude of increase in MAP during hyperpnoea was higher in older females than in young females.[43] Blood flow distribution during exercise with increased work of breathing is also expected to differ between elderly and young females. Further studies are needed to confirm this assumption.

Perspective

The present study included only young subjects. With advancing age, many structural and functional changes in the chest wall and respiratory muscles develop.[44] In older individuals, the

work required to breathe at a given ventilation rate is greater than in younger individuals.[44,45] Therefore, older individuals may exhibit a higher cardiovascular response when respiratory muscle work increases. Indeed, we[43] and Smith et al[22] revealed that the increase in MAP response to enhanced inspiratory muscle work was greater in older than younger females, but not in older males compared to younger males or older males compared to older females. Thus, in females, the respiratory muscle metaboreflex exaggerates with advancing age.

An important clinical consideration is that respiratory muscle work can limit the oxygen supply in chronic obstructive pulmonary disease (COPD) patients. Respiratory muscle activity may affect cardiovascular regulation and blood flow distribution during dynamic exercise in COPD patients.[46,47] Indeed, when COPD patients perform submaximal exercise while breathing heliox (to reduce the work of breathing), vascular conductance is greater than when breathing room air.[48] Thus, increased breathing work in COPD patients affects cardiovascular and blood flow redistribution during exercise, thereby exacerbating the development of locomotor muscle fatigue and compromising exercise performance. COPD primarily affects males; however, the epidemiology is changing because more females have become smokers.[49] To the best of our knowledge, sex differences in the MAP and limb blood flow responses when breathing work increases have not been studied in COPD patients. It is important to clarify any sex differences in the respiratory muscle-induced metaboreflex and blood flow distribution during exercise in COPD patients.

CONCLUSION

The present study found that MAP increased during mild-intensity leg exercise with inspiratory resistance in both young females and young males. However, the magnitude of the increased MAP was smaller in young females compared with age-matched males. Brachial artery blood flow increased during dynamic leg exercise with inspiratory resistance in young females, but not in young males, and forearm vascular conductance decreased during dynamic exercise with inspiratory resistance in males, but not in females. These results suggest that the attenuated inspiratory muscle-induced metaboreflex in females affects blood flow distribution during submaximal dynamic leg exercise.

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CONFLICT OF STATEMENT

The authors declare no competing interest.

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