Title

Age and sex differences in blood pressure responses during hyperpnea

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New findings

What is the central question of this study?

Increased respiratory muscle activation is associated with neural and cardiovascular consequences via respiratory muscle-induced metaboreflex. Does aging and/or sex influence the arterial blood pressure response during voluntary normocapnic incremental hyperpnea?

What is the main finding and its importance?

The increase in blood pressure during hyperpnea was smaller in younger females than in older females, whereas no difference was found between older males and older females. The blunted respiratory muscle-induced metaboreflex in younger females is normalized with advancing age, whereas aging has no such effect in males.

Abstract

We hypothesized that older females (OF) have a greater arterial blood pressure response to increased respiratory muscle work compared with younger females (YF) and that no such difference exists between older males (OM) and younger males (YM). To test these hypotheses, cardiovascular responses during voluntary normocapnic incremental hyperpnea were evaluated and compared between older and younger subjects. An incremental respiratory endurance test (IRET) was performed as follows: target minute ventilation was initially set at 30% of the maximal voluntary ventilation (MVV_{12}) and was increased by 10%MVV_{12} every 3 min. The test was terminated when the subject could not maintain the target %MVV_{12}. Heart rate and mean arterial blood pressure (MAP) were continuously recorded. The increase in MAP from baseline (ΔMAP) during the IRET in OM (+24.0 ± 14.7 mmHg, mean ± SD) did not differ (P = 0.144) from that in YM (+24.3 ± 13.4 mmHg) but it was greater (P = 0.004) in OF (+31.2 ± 11.6 mmHg) than in YF (+10.3 ± 5.5 mmHg). No significant difference in ΔMAP during the IRET was observed between OM and OF (P = 0.975). These results suggest that the respiratory muscle-induced metaboreflex is blunted in YF, but it could be normalized with advancing age. In males, aging has little effect on the respiratory muscle-induced metaboreflex. These results show no sex difference in the respiratory muscle-induced metaboreflex in older adults.

1. Introduction

It has been reported that high-intensity whole-body exercise leads to increased respiratory muscle work and thus to respiratory (inspiratory and expiratory) muscle fatigue (Johnson *et al.*, 1993; Romer & Polkey, 2008). Respiratory muscle fatigue and the concomitant accumulation of metabolites affect neural and cardiovascular regulation through the respiratory muscle-induced metaboreflex (Hill, 2000; Sheel *et al.*, 2001; Dempsey *et al.*, 2008; Sheel *et al.*, 2018). Indeed, enhanced respiratory muscle work leads to an increase in sympathetic vasomotor outflow, with a corresponding increase in mean arterial blood pressure (MAP) (St Croix *et al.*, 2000; Katayama *et al.*, 2012; Katayama *et al.*, 2015; Katayama *et al.*, 2018). This respiratory muscle-induced metaboreflex reduces blood flow (oxygen transport) to active limbs, thereby exacerbating limb fatigue and compromising whole-body endurance performance (Harms *et al.*, 1997; McConnell & Lomax, 2006; Dempsey *et al.*, 2008; Dominelli *et al.*, 2017; Sheel *et al.*, 2018).

Numerous structural and functional changes in the lung, chest wall, and respiratory muscles occur with age (Johnson & Dempsey, 1991). The changes relate to a decrease in the elastic recoil of lung tissue (Gibson *et al.*, 1976; Knudson *et al.*, 1977), stiffening of the chest wall (Mittman *et al.*, 1965; Johnson *et al.*, 1994), a decrease in expiratory flow rates (Knudson *et al.*, 1977; Smith *et al.*, 2017b), and an apparent reduction in respiratory muscle strength (Black & Hyatt, 1969; Johnson & Dempsey, 1991). Additionally, older subjects reportedly exhibit less respiratory muscle endurance (Chen & Kuo, 1989; Watsford *et al.*, 2007). Consequently, in older individuals, dynamic

compliance of the lung declines, resulting in a higher work of breathing for a given ventilation compared with younger individuals (Johnson & Dempsey, 1991; Johnson *et al.*, 1994; Molgat-Seon *et al.*, 2018). This process contributes to increased dyspnea during everyday tasks, limits exercise performance, and leads to reduced physical activity levels and quality of life (Ho *et al.*, 2001; Jensen *et al.*, 2009; Mills *et al.*, 2015). Based on these previous reports, older individuals are expected to exhibit an excessive cardiovascular response to increased respiratory muscle work (Smith *et al.*, 2017a). The influence of age on the respiratory muscle-induced metaboreflex has not been previously studied except by Smith et al. (Smith *et al.*, 2017a). They showed that the magnitude of the increase in MAP in response to increased inspiratory muscle work was greater in older females (OF) than younger females (YF). Furthermore, they found no significant difference in the degree of increase in MAP between older males (OM) and younger males (YM) or between OM and OF. These results suggest that YF exhibit a blunted inspiratory muscle-metaboreflex compared with YM, but the metaboreflex exaggerates with advancing age in females. These results also revealed no sex differences in the inspiratory muscle metaboreflex in older adults. However, in their study (Smith *et al.*, 2017a), breathing against an artificial inspiratory load (i.e., high-resistance, low-speed inspiratory muscle contractions) was utilized to increase inspiratory muscle work. Another way to assess the respiratory muscle-induced metaboreflex is to measure the cardiovascular response to exercise-mimicking hyperpnea (i.e., low-resistance, high-speed inspiratory and expiratory muscle contractions) (Itoh *et al.*, 2016; Shimizu *et al.*, 2018; Katayama *et al.*, 2019; Shimizu *et*

al., 2020). This approach is more ecologically valid than inspiratory loading, as it provides a more physiologically relevant approximation of the pattern and magnitude of respiratory muscle work during whole-body exercise. Recently, we investigated sex differences in cardiovascular responses during voluntary normocapnic incremental hyperpnea between YM and YF (Shimizu *et al.*, 2018). Consequently, the increase in MAP during the hyperpnea was lower in YF than in YM. However, the influence of age on the MAP response during the incremental hyperpnea was not evaluated in our previous study.

We hypothesized that OF have a greater MAP response to increased inspiratory and expiratory muscle work compared to YF and that the MAP response does not differ between OM and YM. To test these hypotheses, cardiovascular responses during voluntary normocapnic incremental hyperpnea were evaluated in older individuals and compared with the responses in younger subjects.

2. Methods

2.1. Ethical approval

This study was approved by the Ethics Board of the Nagoya University Graduate School of Medicine (approval no. 2016–0030), and it conformed to the standards set by the Declaration of Helsinki except for registration in a database. All subjects were informed of the experimental procedures and potential risks involved, and written consent was obtained.

2.2. Subjects

Younger (n = 27, age range 18–25 years; 14 males and 13 premenopausal females) and older subjects (n = 27, age range 66–75 years; 13 males and 14 postmenopausal females) participated in this study. Physical characteristics are shown in Table 1. All subjects were non-smokers and none engaged in endurance or resistance exercise training. Exclusion criteria included the presence of acute and/or chronic respiratory, cardiovascular, and metabolic diseases. YF were menstruating normally for at least the past 6 months and were not taking medications or oral contraceptives that might influence hormone levels.

2.3. Experimental procedure

All experiments were conducted at temperatures of 22–24°C. Subjects visited the laboratory on two different occasions. On day 1, the subjects performed a pulmonary function test and respiratory muscle strength measurement. They were familiarized with the equipment and practiced the incremental respiratory endurance test (IRET). On day 2, the IRET was performed. In YF, IRET was conducted during the early follicular phase (days 1–4) of their menstrual cycle.

2.4. Pulmonary function and respiratory muscle strength

Pulmonary function (vital capacity [VC], forced vital capacity [FVC], forced expiratory volume in 1 s [$FEV_{1.0}$, $FEV_{1.0}/FVC$], and maximal voluntary ventilation for 12 s [MVV_{12}]) was determined using a computerized spirometry system (AS–507, Minato Ikagaku, Osaka, Japan) according to American Thoracic Society/European Respiratory Society guidelines (ATS/ERS, 2002). VC and FVC measurements were

repeated five times, and the three highest values were averaged for all variables. MVV_{12} was measured three times, and the highest value was accepted. The accepted values for each measurement agreed within 3% for VC, FVC, $FEV_{1.0}$, and MVV_{12} (Miller *et al.*, 2005). The maximal inspiratory and expiratory pressures (PI_{max} and PE_{max}) were determined using a handheld mouth pressure meter (AAM377, Minato Ikagaku, Osaka, Japan) connected to a computerized spirometry system. The PI_{max} was taken from residual volume, and the PE_{max} was taken from total lung capacity. The accepted values of each PI_{max} and PE_{max} measurement agreed within 10% (Miller *et al.*, 2005).

2.5. Incremental respiratory endurance test (IRET)

The IRET protocol was performed in a sitting position according to 2002 American Thoracic Society/European Respiratory Society recommendations, as utilized in our previous studies (Itoh *et al.*, 2016; Shimizu *et al.*, 2018; Katayama *et al.*, 2019; Shimizu *et al.*, 2020). First, baseline respiratory and cardiovascular variables were measured for 5 min during spontaneous breathing. Then the IRET was started. The target minute ventilation ($\dot{V}_E$) was set at 30%MVV_{12} for the first 3 min, and was increased by 10%MVV_{12} every 3 min. The tidal volume (V_T) was fixed at 60%VC, and breathing frequency (fb) was increased every 3 min to set target $\dot{V}_E$. The ratio of inspiratory to expiratory time per breath cycle was set to 1:1 based on auditory feedback from a metronome. End-tidal partial pressure of CO_2 (P_{ETCO_2}) was maintained at ± 5 mmHg of the spontaneous breathing level for the first minute, and P_{ETCO_2} was maintained at ± 4 mmHg by adding CO_2 to the inspired air from the second minute to the end of testing.

The IRET ended when the subjects no longer maintained the target V_T (60%VC) or fb despite "warnings" for three consecutive breaths. Respiratory endurance is expressed in minutes rounded to two decimal places (e.g., 10 min 30 s is expressed as 10.5 min). The subjects were asked to report their level of dyspnea (scale: 0–10) immediately after the end of IRET.

During the IRET test, the subjects breathed through a mouthpiece attached to a Fleisch pneumotachometer (PN–230, Arco Systems, Chiba, Japan), and their noses were occluded. The pneumotachometer was connected to a rebreathing bag set to approximately 10 liters. A three-lead electrocardiogram (ECG) was monitored (AB–621, Nihon Koden, Tokyo, Japan), and heart rate (HR) was calculated based on R-R intervals recorded from the ECG. Beat-to-beat arterial blood pressure was obtained using finger photoplethysmography from the middle finger of the left hand (Finometer, Finapres Medical Systems BV, Amsterdam, Netherlands). We performed the "Return to Flow (RTF)" function on the Finometer to improve the accuracy of the blood pressure. Systolic and diastolic blood pressure (SAP and DAP) values were determined from the blood pressure waveform signal, and MAP was calculated as MAP = (SAP – DAP)/3 + DAP. To validate absolute arterial blood pressure values from the Finometer, an automated sphygmomanometer (STBP–780, Colin Medical Instruments, San Antonio, TX, USA) was used to record arterial blood pressure in the brachial artery of the left arm before baseline measurements. The flow, CO_2, ECG, and blood pressure signals were sampled at a frequency of 200 Hz through an analog-to-digital converter (CSI–

3204, Interface, Hiroshima, Japan) and saved to a computer (CF–F8, Panasonic, Osaka, Japan).

2.6. Statistical analysis

Values are expressed as the mean ± SD. For all data, the assumption of normal distribution was verified using the Shapiro–Wilk test. Comparisons of physical characteristics, pulmonary function, respiratory muscle strength, and baseline variables and respiratory endurance among the four groups (i.e., YM, YF, OM, and OF) were performed using one-way analysis of variance (ANOVA) and pairwise comparisons were performed using a Scheffe test where appropriate. Absolute and percent changes from baseline (Δ) in HR and MAP during the IRET were calculated. Differences in the changes in variables during the IRET among the four groups were analyzed using three-way ANOVA with repeated measures (RM) (age × sex × time). If the three-way interaction was significant, pairwise comparisons at each time point were performed with using a Scheffe test where appropriate. Additionally, difference in the changes in variables during the IRET according to age (YM vs. OM, YF vs. OF) and sex (YM vs. YF, OM vs. OF) were performed using two-way ANOVA with RM (age × time and sex × time). The SPSS statistical package (22.0, IBM, Tokyo, Japan) and StatView software (5.0; SAS Institute, Cary, NC, USA) were used, and values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Physical characteristics

Concerning age differences, there was no difference in height or body mass between YM and OM or between YF and OF (Table 1). Regarding sex differences, height and body mass were higher in males than in females regardless of age.

3.2. Pulmonary function and respiratory muscle strength

Table 1 shows pulmonary function and respiratory muscle strength. In terms of age differences, pulmonary function and PI_{max} were significantly lower in OM than in YM. FVC, $FEV_{1.0}$, $FEV_{1.0}$/FVC, and MVV_{12} were significantly lower in OF than in YF. Concerning sex differences, significantly lower pulmonary function, except for $FEV_{1.0}$/FVC, and respiratory muscle strength were found in YF compared to YM. Similarly, pulmonary function, except for the $FEV_{1.0}$/FVC, and PE_{max} were significantly lower in OF than in OM.

3.3. Incremental respiratory endurance test

3.3.1. Baseline measurements

Data on baseline respiratory and cardiovascular parameters are shown in Tables 2–3. Regarding age differences, there were no significant differences in respiratory variables at baseline between YM and OM or YF and OF (Table 2). Baseline SAP was significantly higher in OM than in YM ($P = 0.009$), and baseline DAP and MAP were higher in OF than in YF (DAP: $P = 0.006$, MAP: $P = 0.004$) (Table 3). Regarding sex differences, there were no significant differences in respiratory variables, except $\dot{V}_E$, between YM and YF or OM and OF. DAP and MAP were lower in YF than in YM (DAP: $P = 0.015$, MAP: $P = 0.032$), whereas no differences were observed between OM

and OF (DAP: P = 0.823, MAP: P = 0.415).

3.3.2. Respiratory endurance

There were no significant differences in time to end during the IRET among the four groups (YM: 11.8 ± 0.6 min, OM: 10.6 ± 0.7 min, YF: 11.7 ± 0.5 min, and OF: 10.0 ± 0.4 min, P = 0.118).

3.3.3. Respiratory variables

Statistical analysis of variables measured during the IRET was limited to the first 7 min and at the end of the test, because one OF could not maintain the target after 8 min of hyperpnea. Representative recordings of flow during the IRET are shown in Figure 1, and mean values of the respiratory parameters are shown in Table 2. The $\dot{V}_E$ and fb increased progressively as expected, whereas V_T did not change throughout the IRET in any group. Similarly, the P_{ETCO_2} did not change during the IRET in any group.

3.3.4. Cardiovascular variables

HR increased during the IRET in all groups (Table 3). Absolute and percent changes in HR (ΔHR) from baseline during the IRET are shown in Figure 2. Three-way ANOVA RM revealed that there was no significant three-way interaction (age × sex × time) for ΔHR. In terms of age differences, no significant difference in ΔHR appeared between YM and OM or between YF and OF. Concerning the sex differences, no significant difference in ΔHR was found between YM and YF or between OM and OF.

Representative recordings of arterial blood pressure during the IRET are shown in Figure 1. SAP, DAP, and MAP increased gradually during the IRET in all groups (Table 3). Absolute and percent changes in MAP (ΔMAP) from baseline during the IRET are

shown in Figure 3. There were significant three-way interactions for SAP, DAP, MAP, and ΔMAP. In terms of age difference, ΔMAP during the IRET did not differ between YM and OM (Figure 3). By contrast, ΔMAP during the IRET was greater in OF than in YF. Concerning the sex differences, ΔMAP during the IRET was lower in YF than in YM (Figure 3), whereas no difference was observed between OM and OF.

3.3.5. Dyspnea

There were no significant differences in the rate of dyspnea among the four groups (YM: 6.6 ± 0.6, OM: 6.7 ± 0.5, YF: 6.6 ± 0.4, and OF: 6.3 ± 0.3, P = 0.896).

4. Discussion

4.1. Major findings

The major findings of this study were as follows: 1) in terms of age differences, the magnitude of the increase in MAP from baseline (ΔMAP) during voluntary normocapnic incremental hyperpnea was greater in OF than YF, whereas no difference was found between OM and YM; and 2) concerning sex differences, there was no difference in ΔMAP during hyperpnea between OM and OF, whereas ΔMAP in YF was smaller than that in YM. These results suggest that the respiratory muscle-induced metaboreflex is attenuated in YF, but it could be normalized with advancing age in females. They also suggest that, in males, aging could have little effect on the respiratory muscle-induced metaboreflex when hyperpnea is induced at the same relative intensity to MVV12. Furthermore, sex differences in the respiratory

muscle-induced metaboreflex do not appear to exist in older adults.

4.2. Cardiovascular response to increased respiratory muscle work

Increasing respiratory muscle work elicits time-dependent increases in sympathetic vasomotor outflow with a corresponding increase in MAP (St Croix *et al.*, 2000; Sheel *et al.*, 2001; Katayama *et al.*, 2012; Katayama *et al.*, 2018). In animal studies, fatiguing diaphragm contractions via phrenic nerve stimulation, and the associated accumulation of metabolites led to an increase in type IV (primary metabosensitive) afferent discharge (Hill, 2000). This sympathoexcitation occurs through the respiratory muscle-induced metaboreflex (Hill, 2000; Sheel *et al.*, 2001; Dempsey *et al.*, 2008; Sheel *et al.*, 2018). Consistent with our previous studies (Itoh *et al.*, 2016; Shimizu *et al.*, 2018; Katayama *et al.*, 2019; Shimizu *et al.*, 2020), progressive increases in HR and MAP occurred during the IRET in all groups (Table 3 and Figures 2 and 3), suggesting that an inspiratory and expiratory muscle work-induced metaboreflex could be responsible for these responses.

4.3. Age differences in cardiovascular responses to enhanced respiratory muscle work

With advancing age, pulmonary system changes occur such as a decrease in elastic recoil (Gibson *et al.*, 1976; Knudson *et al.*, 1977), stiffening of the chest wall (Mittman *et al.*, 1965; Johnson *et al.*, 1994; Watsford *et al.*, 2007), and a decrease in expiratory flow rate (Knudson *et al.*, 1977; Smith *et al.*, 2017b). Accordingly, the work and cost of breathing for a given ventilation outcome are greater in older individuals than in younger individuals (Johnson & Dempsey, 1991; Johnson *et al.*, 1994; Molgat-Seon *et*

al., 2018). In addition, older subjects reportedly have reduced respiratory muscle fatigue resistance relative to younger individuals (Chen & Kuo, 1989). Based on these reports, it has been assumed that older individuals would exhibit a greater cardiovascular response to increased respiratory muscle work (Smith *et al.*, 2017a). In contrast to this assumption, Smith et al. (Smith *et al.*, 2017a) demonstrated no difference in the changes in MAP and limb vascular resistance during inspiratory resistive breathing (60%PI_{max}) between YM and OM. In the present study, we also found that ΔMAP in OM during voluntary incremental hyperpnea (i.e., low-resistance, high-speed inspiratory and expiratory muscle contractions) did not differ from those in YM (Figure 3). In contrast to males, we found that ΔMAP during voluntary incremental hyperpnea was significantly greater in OF than in YF, as shown in Figure 3. A larger ΔMAP during hyperpnea in OF in this study is in consistent with data from a previous study, which demonstrated a greater MAP response in OF compared to YF during inspiratory resistive breathing (Smith *et al.*, 2017a).

Potential mechanisms for the greater MAP response during hyperpnea in OF should be considered. A lack of studies on the mechanisms of age differences in cardiovascular responses to increased respiratory muscle work only allows a comparison with data obtained from limb skeletal muscle. A higher MAP during postexercise ischemia after a handgrip exercise in OF compared with YF has been reported (Choi *et al.*, 2012). Postexercise ischemia is used to isolate the activation of the muscle metaboreflex effect of neural and cardiovascular regulation from central command and the muscle mechanoreflex. Thus, these data indicate that metabolic stimuli produced by muscle

283 contraction contribute to the higher MAP in postmenopausal females (Choi *et al.*, 2012).
284 Additionally, greater sympathetic vasoconstriction during exercise has also been
285 reported in OF compared with YF (Fadel *et al.*, 2004). Another possible mechanism is a
286 difference in peripheral transduction of sympathetic vasomotor outflow to the peripheral
287 vasculature. Hart et al. (Hart *et al.*, 2011) compared the ability of β-adrenergic receptors
288 to offset the transduction of sympathetic vasomotor outflow into vasoconstrictor tone
289 among YM, YF, and postmenopausal females. They found that the β-adrenergic
290 receptors offset α-adrenergic vasoconstriction in YF but not in YM or postmenopausal
291 females. Therefore, it seems likely that the difference in adrenergic receptor sensitivity
292 in YF does not persist with aging. The mechanism underlying the respiratory
293 muscle-induced metaboreflex enhancement of MAP would include not only a decrease
294 in peripheral vascular conductance but also an increase in central hemodynamic
295 responses. In the present study, HR was elevated in both OF and YF, and there was no
296 significant difference in ΔHR between the two groups (Figure 2). Therefore, a large
297 increase in MAP during hyperpnea without a concomitant change in HR in OF implies
298 that larger MAP responses via the metaboreflex are modulated by peripheral
299 vasoconstriction with aging (Choi *et al.*, 2012; Schneider *et al.*, 2018). Estrogen has a
300 major effect on the regulation of MAP and vascular tone (Joyner *et al.*, 2016), so
301 estrogen deficiency may be one reason for the greater MAP response to increased
302 respiratory muscle work in OF. An increase in arterial stiffness with aging in females
303 may also contribute to the heightened muscle metaboreflex. With aging, arterial
304 stiffness increases more in females than in males (Coutinho *et al.*, 2013; DuPont *et al.*,

2019), and muscle metaboreflex activation causes increased arterial stiffness (Davies *et al.*, 2007). Relatedly, increased stiffness of the large arteries is associated with reduced baroreceptor sensitivity, which may alter sympathetic tone (Kingwell *et al.*, 1995). However, whether neural interaction between the respiratory muscle-induced metaboreflex and arterial baroreceptors is altered with age is as yet unknown. Another possible mechanism for the difference in MAP during hyperpnea between YF and OF is central respiratory motor output (central command). If central respiratory motor output during hyperpnea was greater in OF than in YF, this might result in a larger increase in sympathetic vasomotor outflow. However, this would be not the case in the present study, as dyspnea immediately after the IRET did not differ between OF and YF. St Croix (St Croix *et al.*, 1999) also provided evidence against a significant effect from respiratory motor output on sympathetic vasomotor outflow. Therefore, it is unlikely that central respiratory output is related to the larger MAP response during hyperpnea in OF.

4.4. Sex differences in cardiovascular response to increased respiratory muscle work

Because the work and cost of breathing are higher for YF for a given level of ventilation (Guenette *et al.*, 2007), it has been supposed that respiratory muscle fatigue and the respiratory muscle-induced metaboreflex would be exaggerated in YF compared with age-matched males. In contrast to this supposition, exercise-induced diaphragmatic fatigue in YF was less than that in age-matched males (Guenette *et al.*, 2010). Furthermore, the magnitude of the increases in MAP during inspiratory resistive

breathing was lower in YF than in YM (Smith *et al.*, 2016). Our group also found that the magnitude of the increase in MAP and sympathetic vasomotor outflow in response to increased respiratory muscle work at rest and during exercise were lower in YF than in age-matched males (Katayama *et al.*, 2018; Shimizu *et al.*, 2018). Similar to these previous studies, we found that ΔMAP during the IRET was lower in YF than in YM, as shown in Figure 3. These results suggest that YF exhibit a blunted respiratory muscle-induced metaboreflex compared with YM.

What are the possible mechanisms for the attenuated respiratory muscle metaboreflex in YF? Ettinger et al. (Ettinger *et al.*, 1996) found that the development of metabolites was attenuated and sympathetic vasomotor outflow during static handgrip exercise was decreased in premenopausal females compared with age-matched males. Therefore, it is conceivable that metabolic stimuli in inspiratory and expiratory muscles are diminished in YF compared to YM (Smith *et al.*, 2016). Another possible mechanism would be a difference between YF and YM in the peripheral transduction of sympathetic vasomotor outflow to the peripheral vasculature. Similar increases in sympathetic vasomotor outflow in males and females reportedly resulted in a smaller increase in limb vascular resistance in females (Hogarth *et al.*, 2007). This relative insensitivity to sympathetic vasoconstriction likely results from the offset of α-adrenergically mediated vasoconstriction by augmented β-adrenergic vasodilator effects (Joyner *et al.*, 2016). These differences could contribute to the blunted respiratory muscle-induced metaboreflex in YF compared with YM. In YF, IRET was conducted during the early follicular phase of their menstrual cycle. We previously

reported no difference in the increase in MAP during the IRET between the early follicular and midluteal phases in YF (Shimizu *et al.*, 2020). Thus, it seems likely that the menstrual cycle does not appear to affect the respiratory muscle-induced metaboreflex in YF.

4.5. Limitations

We did not assess sympathetic vasomotor outflow (muscle sympathetic nerve activity) during hyperpnea. Measurements of sympathetic activation would provide additional valuable information regarding the underlying mechanisms responsible for the differences observed between OF and YF (Smith *et al.*, 2017a). In previous studies, esophageal or mouth pressure was recorded during voluntary hyperpnea and exercise, thereby allowing the work exerted during breathing to be calculated (Johnson & Dempsey, 1991; Johnson *et al.*, 1994; Dominelli *et al.*, 2015; Molgat-Seon *et al.*, 2018). In those studies, the work of breathing at a given ventilation was higher in older individuals than in younger individuals and was higher in females than in males. Further research is necessary to elucidate the relationship between work of breathing and cardiovascular responses in older and younger individuals.

4.6. Perspectives and significance

Postmenopausal females have been reported to exhibit an enhanced MAP response to whole-body exercise (Julius *et al.*, 1967; Martin *et al.*, 1991; Ogawa *et al.*, 1992; Stratton *et al.*, 1994; Fisher *et al.*, 2007). The mechanisms are not fully understood, and impairments of cardiac function, an active limb muscle metaboreflex, an arterial baroreflex, and arterial stiffness could contribute to an exaggerated elevation in MAP

during dynamic exercise (Joyner, 2006; Sharman *et al.*, 2018). In the present study, we found a larger MAP response during hyperpnea in OF than in YF (Figure 3). This larger respiratory muscle metaboreflex is likely related to the enhanced MAP response to whole-body exercise in OF. Clinically, respiratory muscle activity plays an important role in limiting oxygen delivery in patients with chronic obstructive pulmonary disease (COPD) (Amann *et al.*, 2010) and chronic heart failure (Johnson *et al.*, 2000). Recent studies show a progressively higher prevalence of COPD in females (Thun *et al.*, 2013). However, the previous studies were conducted in male COPD patients or in a mixed population of both sexes, without consideration of potential differences between males and females (Ausin *et al.*, 2017). Therefore, it is necessary to explore the effect of increased inspiratory muscle activation on MAP and sympathetic vasomotor outflow during exercise in both sexes.

4.7. Conclusion

In the present study, we found that the magnitude of the increase in MAP from baseline during voluntary normocapnic incremental hyperpnea did not differ between YM and OM, whereas it was larger in OF than in YF. No significant difference in ΔMAP during the IRET was observed between OM and OF. These results suggest that the respiratory muscle-induced metaboreflex is blunted in YF, but it could be normalized with advancing age. In males, aging has little effect on the respiratory muscle-induced metaboreflex. Furthermore, these results show no sex difference in the respiratory muscle-induced metaboreflex in older adults.

Competing interests

None declared.

Author contributions

Conception of work: K.S., K.S., K.I., M.S., H.A., and K.K. Data acquisition, analysis, and interpretation of the data, K.S., K.S., K.I., M.S., S.M., and K.K. Drafting of the article, K.S., K.S., K.I., M.S., S.M., H.A., and K.K. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Figure legends

Figure 1. Representative recordings of flow and arterial blood pressure (BP) during the incremental respiratory endurance test (IRET) in a younger female (A) and an older female (B).

Figure 2. Absolute (A) and percent (B) changes in heart rate (ΔHR) from baseline during the incremental respiratory endurance test (IRET). Values are presented as the mean ± SD.

Figure 3. Absolute (A) and percent (B) changes in mean blood pressure (ΔMAP) from baseline during the incremental respiratory endurance test (IRET). Values are presented as the mean ± SD. *$P < 0.05$ YM vs. YF. †$P < 0.05$ YF vs. OF. Differences between YM and OF and between OM vs. YF are not shown in this figure.

Table 1. Physical characteristics, pulmonary function, and respiratory muscle strength.

	YM (n = 14)	OM (n = 13)	YF (n = 13)	OF (n = 14)
Age (years)	20.6±2.1	70.6±2.6 *	20.4±1.7	69.9±1.9 †
Height (cm)	171.2±5.2	168.8±6.7	157.2±5.9 *	153.8±4.7 #
Body mass (kg)	62.1±7.2	67.8±10.0	53.1±6.2 *	49.3±7.3 #
VC (l)	4.6±0.7	3.7±0.8 *	3.1±0.4 *	2.6±0.4 #
FVC (l)	4.5±0.7	3.3±0.6 *	3.1±0.4 *	2.4±0.4 †#
FEV1.0 (l)	4.0±0.6	2.7±0.5 *	2.8±0.3 *	2.0±0.3 †#
$FEV_{1.0}$/FVC (%)	90.7±5.7	81.8±5.4 *	89.2±4.2	82.8±4.9 †
MVV_{12} (l/min)	187.0±28.7	112.1±27.4 *	109.7±13.6 *	82.8±15.7 †#
PImax (cmH_2O)	138.0±32.0	87.0±6.6 *	87.5±21.8 *	70.9±15.1
PEmax (cmH_2O)	155.2±32.4	147.1±48.2	99.2±24.6 *	104.0±28.9 #

Values are mean±SD. YM, younger males; OM, older males; YF, younger females, OF, older females; VC, vital capacity; FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in 1 s; MVV_{12}, maximal voluntary ventilation; PImax, maximal inspiratory pressure; PEmax, maximal expiratory pressure. *P < 0.05 vs. YM. †P < 0.05 vs. YF. #P < 0.05 vs. OM. Differences between YM and OF and OM and YF do not show in this table.

Table 2. Respiratory variables during the incremental respiratory endurance test.

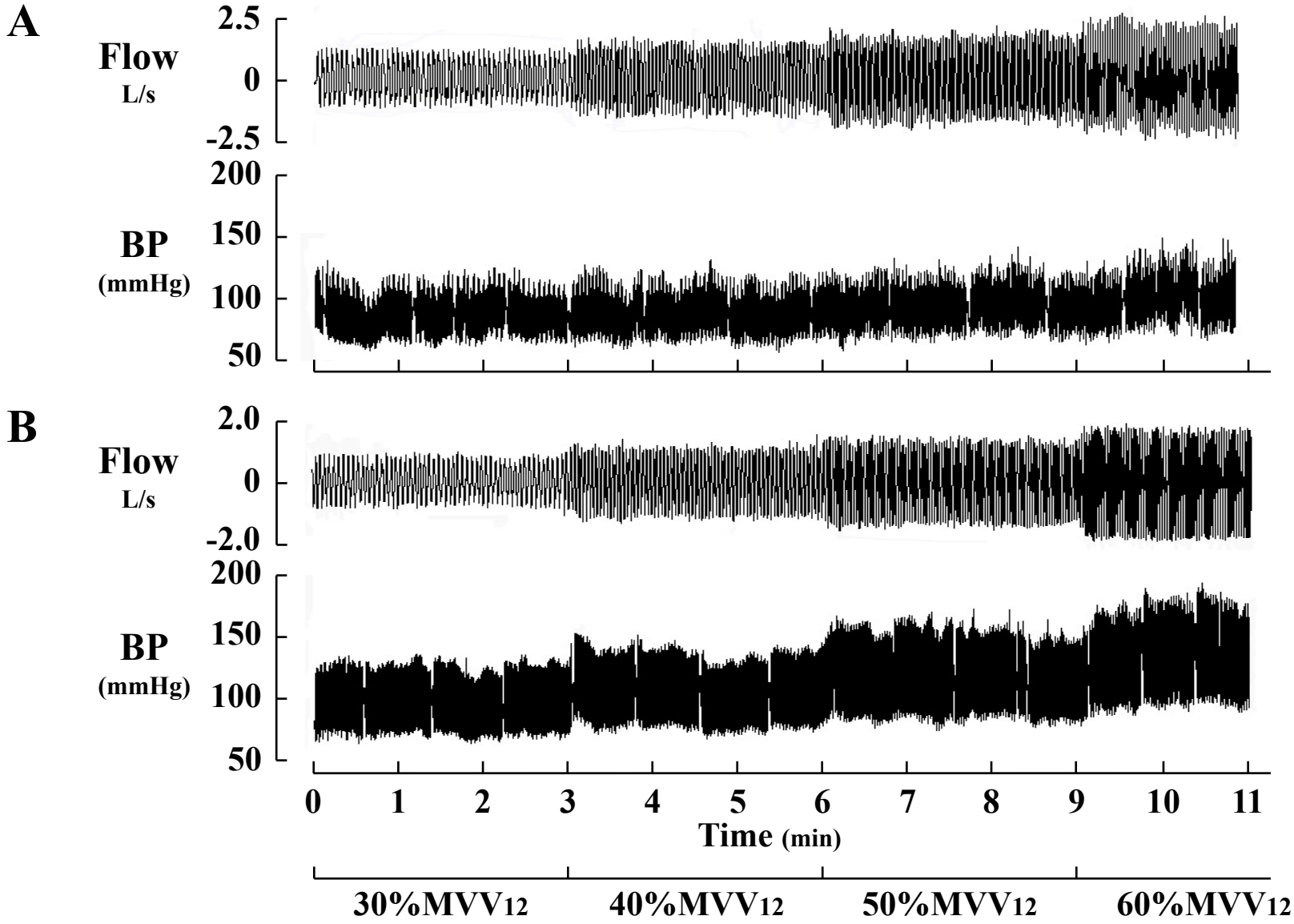
	Groups	Baseline	Incremental respiratory endurance test								
			1 min	2 min	3 min	4 min	5 min	6 min	7 min	Statistics	End
$\dot{V}_E$ (l/min)	YM	8.7 ±1.7	63.7 ±10.3	63.8 ±10.5	63.8 ±10.4	84.1 ±14.7	85.0 ±15.2	84.7 ±15.1	105.5 ±17.7	Three-way ANOVA RM F=5.90 P<0.001 Two-way ANOVA RM YM vs. OM, F=29.2 P<0.001 YF vs. OF, F=19.2 P<0.001 YM vs. YF, F=37.6 P<0.001 OM vs. OF, F=16.5 P<0.001	136.0 ±22.7
	OM	8.6 ±1.3	43.6 ±11.6*	43.7 ±11.5*	43.7 ±11.5*	56.2 ±13.1*	56.1 ±13.2*	56.1 ±13.1*	70.1 ±15.8*		84.7 ±15.7*
	YF	8.3 ±1.5	39.1 ±4.9*	39.2 ±4.9*	39.3 ± 5.1*	51.6 ±6.8*	51.6 ±6.8*	51.9 ±6.8*	64.7 ±8.4 *		81.6 ±12.8*
	OF	7.3 ±1.7#	27.6 ±6.3†#	27.7 ±6.3†#	27.6 ±6.4†#	36.5 ±8.6†#	36.6 ±8.3†#	36.6 ±8.5†#	45.4 ±10.9†#		54.0 ±13.9†#
V_T (l)	YM	0.7 ±0.1	3.1 ±0.6	3.1 ±0.5	3.1 ±0.6	3.2 ±0.6	3.2 ±0.6	3.2 ±0.6	3.2 ±0.5	Three-way ANOVA RM F=1.3 P=0.268 Two-way ANOVA RM YM vs. OM, F=1.0 P=0.457 YF vs. OF, F=0.68 P=0.663 YM vs. YF, F=1.8 P=0.098 OM vs. OF, F=0.5 P=0.840	3.1 ±0.6
	OM	0.7 ±0.1	2.5 ±0.5	2.5 ±0.5	2.5 ±0.5	2.5 ±0.4	2.5 ±0.5	2.5 ±0.4	2.5 ±0.4		2.5 ±0.5*
	YF	0.6 ±0.2	2.2 ±0.3	2.2 ±0.3	2.2 ±0.3	2.2 ±0.3	2.2 ±0.3	2.2 ±0.3	2.2 ±0.3		2.2 ±0.3*
	OF	0.6 ±0.2	1.6 ±0.3	1.6 ±0.4	1.6 ±0.4	1.6 ±0.4	1.6 ±0.3	1.6 ±0.4	1.6 ±0.4		1.6 ±0.4†#
fb (bpm)	YM	13.1 ±3.9	20.5 ±2.6	20.5 ±2.6	20.5 ±2.5	26.9 ±3.1	27.1 ±3.2	27.1 ±3.2	33.8 ±4.2	Three-way ANOVA RM F=3.6 P=0.002 Two-way ANOVA RM YM vs. OM, F=12.0 P<0.001 YF vs. OF, F=1.1 P=0.345 YM vs. YF, F=4.5 P<0.001 OM vs. OF, F=0.6 P=0.703	44.1 ±7.8
	OM	12.8 ±3.1	17.3 ±3.2*	17.3 ±3.2*	17.3 ±3.2*	22.3 ±3.9*	22.3 ±3.9*	22.3 ±3.9*	27.8 ±4.9*		34.0 ±6.4*
	YF	13.8 ±2.3	18.1 ±2.5*	18.1 ±2.5*	18.1 ±2.5*	23.9 ±3.4*	23.8 ±3.4*	23.9 ±3.4*	29.8 ±4.1*		37.4 ±4.9*
	OF	12.2 ±2.6	17.1 ±2.0	17.1 ±1.9	17.1 ±2.0	22.5 ±2.9	22.6 ±2.8	22.6 ±2.8	28.0 ±3.7		33.4 ±6.2
P_{ETCO_2} (torr)	YM	40.2 ±0.7	40.3 ±1.6	40.1 ±1.3	39.9 ±1.2	39.9 ±1.0	40.4 ±1.1	40.4 ±0.6	39.9 ±0.6	Three-way ANOVA RM F=0.1 P=0.998 Two-way ANOVA RM YM vs. OM, F=0.4 P=0.882 YF vs. OF, F=0.7 P=0.670 YM vs. YF, F=0.3 P=0.922 OM vs. OF, F=0.4 P=0.891	40.3 ±1.6
	OM	40.1 ±0.5	40.0 ±0.8	40.3 ±0.5	40.1 ±0.8	39.9 ±0.5	40.3 ±0.6	40.3 ±1.0	39.9 ±0.7		40.2 ±0.5
	YF	39.9 ±1.0	40.0 ±1.1	39.7 ± 0.9	40.0 ±1.2	39.8 ±1.1	40.2 ±1.2	39.8 ±1.1	39.7 ±1.2		39.7 ±1.0
	OF	40.1 ±1.4	40.0 ±1.6	40.2 ±1.0	40.2 ±1.4	40.2 ±1.0	40.3 ±1.1	40.2 ±0.9	40.1 ±1.1		40.1 ±1.0

Values are expressed as the mean±SD. YM, younger males; OM, older males; YF, younger females; OF, older females; $\dot{V}_E$, expired minute ventilation; V_T, tidal volume; fb, breathing frequency; P_{ETCO_2}, end-tidal partial pressure of CO_2. *P < 0.05 vs. YM. †P < 0.05 vs. YF. #P < 0.05 vs. OM. Differences between YM and OF and between OM and YF do not show in this table.

Table 3. Cardiovascular variables during the incremental respiratory endurance test.

	Groups	Baseline	Incremental respiratory endurance test								
			1 min	2 min	3 min	4 min	5 min	6 min	7 min	Statistics	End
HR (bpm)	YM	73.8 ±9.1	86.7 ±11.5	84.3 ±11.3	84.4 ±11.4	89.1 ±11.4	88.8 ±11.1	89.2 ±11.3	96.8 ±11.8	Three-way ANOVA RM F=0.8 P=0.536 Two-way ANOVA RM YM vs. OM, F=1.5 P=0.177 YF vs. OF, F=0.5 P=0.828 YM vs. YF, F=2.8 P=0.014 OM vs. OF, F=0.5 P=0.792	110.3 ± 13.9
	OM	70.7 ±11.4	76.4 ±12.7	76.2 ±12.1	76.0 ±11.3	80.2 ±12.4	81.0 ±12.7	81.7 ±12.2	85.1 ±13.3		94.3 ± 14.6
	YF	67.6 ±9.4	77.9 ±9.5	76.8 ±9.5	76.8 ±9.8	81.0 ±10.2	81.6 ±10.0	81.6 ±10.6	85.0 ±10.8		93.5 ± 11.9
	OF	71.8 ±9.6	76.5 ±12.2	77.1 ±12.0	76.6 ±11.5	79.7 ±12.9	80.0 ±12.4	80.4 ±12.8	83.7 ±13.8		89.6 ± 16.1
SAP (mmHg)	YM	118.3 ±9.6	131.7 ±18.3	135.2 ±15.7	138.6 ±18.6	143.3 ±19.4	144.5 ±20.8	148.9 ±19.5	154.8 ±20.8	Three-way ANOVA RM F=5.1 P<0.001 Two-way ANOVA RM YM vs. OM, F=1.1 P=0.341 YF vs. OF, F=8.9 P<0.001 YM vs. YF, F=12.1 P<0.001 OM vs. OF, F=0.2 P=0.979	184.9 ± 25.2
	OM	130.2 ±6.3 *	148.3 ±22.2*	151.0 ±22.6*	154.7 ±21.5*	161.4 ±25.8*	163.9 ±25.2*	167.3 ±26.7*	173.1 ±30.1*		189.7 ± 35.0
	YF	113.8 ±7.2	115.2 ±12.6*	117.0 ±9.2*	118.5 ±8.7*	121.7 ±10.8*	123.1 ±11.6*	124.4 ±11.4*	126.3 ±12.9*		139.6 ± 16.8 *
	OF	122.2 ±9.1	138.2 ±20.2†	139.3 ±19.5†	146.5 ±19.7†	154.1 ±17.4†	157.1 ±17.3†	159.1 ±18.0†	165.5 ±15.7†		183.5 ± 23.4 †
DAP (mmHg)	YM	75.7 ±6.5	82.4 ±10.5	86.5 ±10.3	88.1 ±11.1	90.4 ±11.1	93.0 ±13.4	93.2 ±14.4	93.9 ±14.7	Three-way ANOVA RM F=2.4 P=0.021 Two-way ANOVA RM YM vs. OM, F=1.8 P=0.095 YF vs. OF, F=0.7 P=0.690 YM vs. YF, F=6.6 P<0.001 OM vs. OF, F=0.4 P=0.901	110.4 ± 15.6
	OM	79.3 ±6.6	84.9 ±9.8	87.7 ±10.8	88.6 ±9.8	91.9 ±10.9	91.0 ±11.4	91.6 ±12.7	93.8 ±12.7		103.3 ± 15.6
	YF	66.2 ±7.5*	67.4 ±8.6*	69.8 ±8.5*	71.2 ±7.9*	74.3 ±8.7*	74.6 ±8.9*	73.6 ±8.6*	75.4 ±9.2*		81.8 ± 10.8 *
	OF	76.6 ±7.3†	88.4 ±12.2†	92.1 ±14.1†	93.4 ±12.1†	97.8 ±13.0†	97.6 ±15.9†	98.4 ±16.3†	101.2 ±15.0†		106.5 ± 14.6 †
MAP (mmHg)	YM	89.9 ±7.1	98.8 ±12.7	102.7 ±11.5	104.9 ±13.0	108.1 ±13.4	110.2 ±15.4	111.7 ±15.4	114.2 ±16.1	Three-way ANOVA RM F=3.4 P=0.001 Two-way ANOVA RM YM vs. OM, F=1.6 P=0.144 YF vs. OF, F=3.1 P=0.004 YM vs. YF, F=10.6 P<0.001 OM vs. OF, F=0.2 P=0.975	135.3 ± 17.9
	OM	96.3 ±6.0	106.1 ±13.3	108.8 ±14.1	110.6 ±13.0	115.1 ±14.8	115.3 ±14.7	116.9 ±16.2	120.2 ±17.1		132.1 ± 21.2
	YF	82.1 ±5.6*	83.3 ±8.2*	85.6 ±6.6*	87.0 ±6.3*	90.1 ±7.4*	90.8 ±7.7*	90.5 ±7.2*	92.4 ±8.3*		101.1 ± 11.6 *
	OF	91.8 ±6.7†	105.0 ±13.7†	107.8 ±15.1†	111.1 ±13.1†	116.5 ±13.0†	117.4 ±15.2†	118.6 ±15.4†	122.7 ±14.1†		132.2 ± 16.1 †

Values are expressed as the mean±SD. YM, younger males; OM, older males; YF, younger females; OF, older females; HR, heart rate; SAP, systolic arterial blood pressure; DAP, diastolic arterial blood pressure; MAP, mean arterial blood pressure. *P < 0.05 vs. YM. †P < 0.05 vs. YF. Differences between YM and OF and between OM and YF do not show in this table.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Time (min)
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60%MVV12

Figure 1

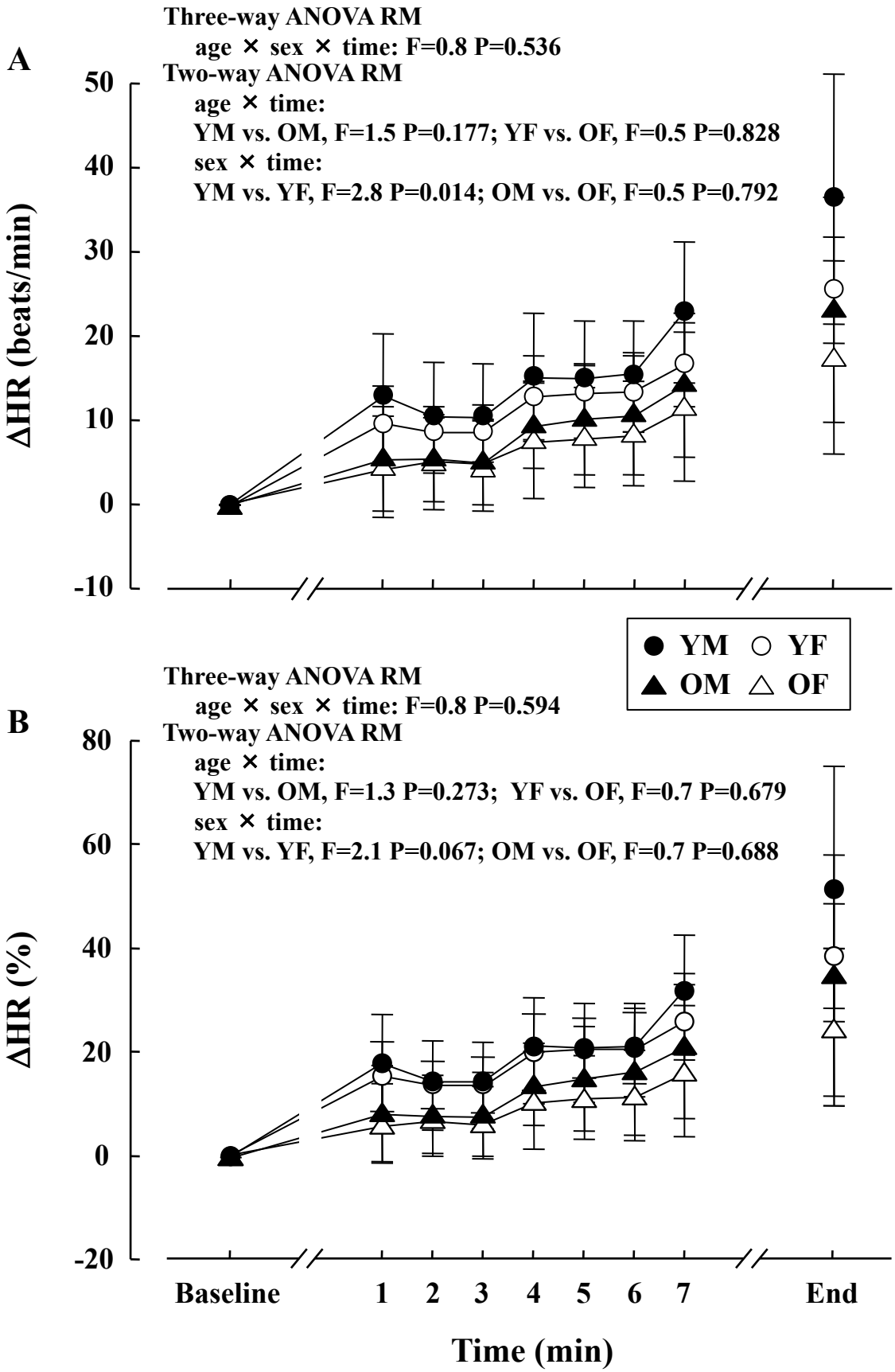
A
Three-way ANOVA RM
age × sex × time: F=0.8 P=0.536
Two-way ANOVA RM
age × time:
YM vs. OM, F=1.5 P=0.177; YF vs. OF, F=0.5 P=0.828
sex × time:
YM vs. YF, F=2.8 P=0.014; OM vs. OF, F=0.5 P=0.792
ΔHR (beats/min)
50
40
30
20
10
0
-10
YM
YF
OM
OF
B
Three-way ANOVA RM
age × sex × time: F=0.8 P=0.594
Two-way ANOVA RM
age × time:
YM vs. OM, F=1.3 P=0.273; YF vs. OF, F=0.7 P=0.679
sex × time:
YM vs. YF, F=2.1 P=0.067; OM vs. OF, F=0.7 P=0.688
ΔHR (%)
80
60
40
20
0
-20
Baseline
1
2
3
4
5
6
7
End
Time (min)

Figure 2 R2

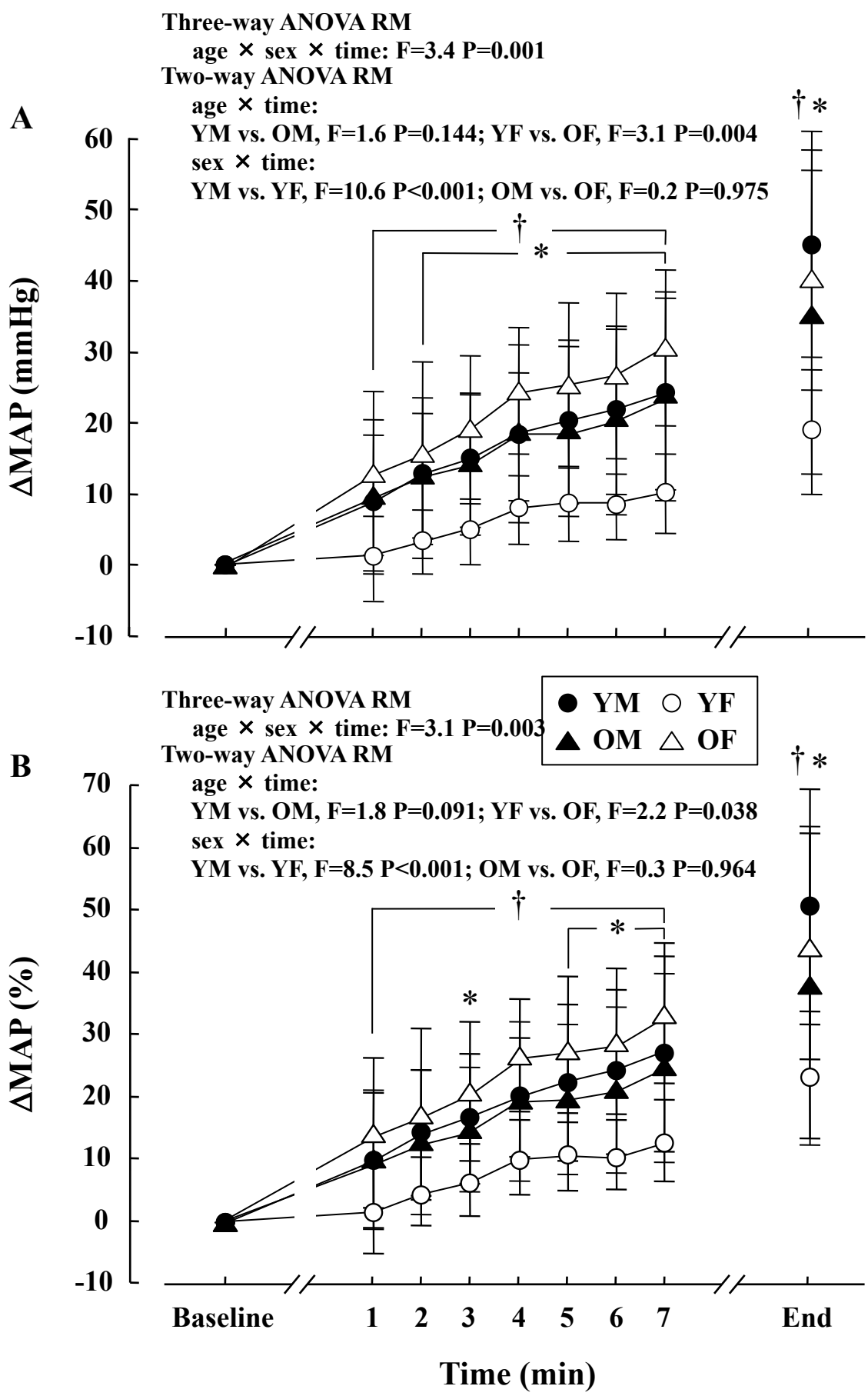
A
Three-way ANOVA RM
age × sex × time: F=3.4 P=0.001
Two-way ANOVA RM
age × time:
YM vs. OM, F=1.6 P=0.144; YF vs. OF, F=3.1 P=0.004
sex × time:
YM vs. YF, F=10.6 P<0.001; OM vs. OF, F=0.2 P=0.975
ΔMAP (mmHg)
60
50
40
30
20
10
0
-10
†
*
†*
B
Three-way ANOVA RM
age × sex × time: F=3.1 P=0.003
Two-way ANOVA RM
age × time:
YM vs. OM, F=1.8 P=0.091; YF vs. OF, F=2.2 P=0.038
sex × time:
YM vs. YF, F=8.5 P<0.001; OM vs. OF, F=0.3 P=0.964
YM
YF
OM
OF
ΔMAP (%)
70
60
50
40
30
20
10
0
-10
†
*
*
†*
Baseline
1
2
3
4
5
6
7
End
Time (min)

Figure 3 R3