Sex differences in the modulation of vasomotor sympathetic outflow during static handgrip exercise in healthy young humans

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PREAMENOPAUSAL WOMEN TYPICALLY have lower resting blood pressure (BP) (9, 14, 17) and lower incidence of cardiovascular disease (24) than similarly aged men, but the underlying mechanisms are not completely understood. The hormone profile of premenopausal women suggests that estrogen and/or progesterone provide a degree of cardioprotection (27, 39). BP reactivity has been described as one predictor of the future development of hypertension (1, 8). Based on this, we would expect to see sex differences in BP responses to tests such as static handgrip exercise, which is one way to assess BP control when changes were examined as a percent increase from baseline (18). In contrast, Ettinger et al. (6) reported that cardiovascular and vasomotor sympathetic responses to static handgrip were attenuated in women. Additionally, the same group reported that increases in MSNA during static handgrip varied with the phase of the menstrual cycle (7). MSNA, but not cardiovascular responses, were attenuated during the late follicular phase (10 to 12 days after the onset of menstruation, low estrogen/low progesterone) compared with the early follicular phase (EF; 1 to 4 days after the onset of menstruation, high estrogen/low progesterone) of the menstrual cycle (7). These studies, however, are difficult to interpret and do not allow for a direct comparison as they have not controlled for menstrual cycle status (18), use of hormonal birth control (18), use of hormone replacement therapy (6), have not performed static handgrip to fatigue to reach a common metabolic endpoint (6, 7), or have not provided a male cohort for direct sex comparisons (7).

Thus, based on the current literature, it is not clear whether true sex differences exist and how both estrogen and progesterone influence static handgrip outcomes. Our study differs from those conducted previously because we have 1) controlled for hormone status by excluding individuals taking oral contraceptives and hormone replacement therapies; 2) tested young premenopausal women during the low and high hormone phases of the menstrual cycle; 3) included a cohort of men for sex comparisons; and 4) had subjects perform static handgrip until fatigue so that all subjects reached a common metabolic endpoint (6, 7), or have not provided a male cohort for direct sex comparisons (7).

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MATERIALS AND METHODS

Subjects

Twenty-one (11 women, 10 men) healthy volunteers were studied. Descriptive characteristics of the subjects are outlined in Table 1. Exclusionary criteria included: significant medical history, smoking, recreational drug use, hormonal contraceptive use within the previous 6 months, and current pregnancy. Additionally, subjects that were endurance trained were excluded from participation. All female participants were normally menstruating (i.e., ~28-day cycle). All subjects gave written informed consent to participate in the study, which was approved by the Institutional Review Boards at the University of Texas Southwestern Medical Center and Texas Health Presbyterian Hospital Dallas.

Measurements

HR and BP. HR was determined from lead II of the electrocardiogram (ECG). BP was assessed using two methods. Beat-by-beat arterial pressure [systolic BP (SBP); diastolic BP (DBP)] was estimated noninvasively by using finger photoplethysmography (model 1, Nexcel HD monitor; BMYE, Amsterdam, The Netherlands). This method was used to examine BP changes during static handgrip to fatigue and the cold pressor test (CPT). BP was also obtained via electrophygmomanometry (model 4240; SunTech Medical Instruments, Raleigh, NC) with a microphone placed over the brachial artery to detect Korotkoff sounds. This method was used to obtain baseline BP values when the subject was initially instrumented in our laboratory to ensure a resting steady state had been achieved before the onset of menstruation and by detection of the luteinizing hormone surge by an ovulation prediction kit (OvuQuick; Quiel, San Diego, CA). Hormone concentrations were verified on each study day. For the 2 days prior to testing, all subjects were on an isocaloric constant diet consisting of 200 meq sodium, 100 meq potassium, and 1,000 mg calcium, while water intake was ad libitum. Female subjects submitted a urine sample for pregnancy testing prior to any of the experimental procedures.

The experiment was performed ≥ 2 h after a light meal and ≥ 48 h after the last caffeinated or alcoholic beverage was consumed. The laboratory was environmentally controlled with an ambient temperature of ~25°C. Each subject was studied in the supine position. Prior to microneurography, subjects performed three brief (~3 s) maximal contractions with his/her dominant hand to determine his/her maximal voluntary contraction (MVC) by using a handgrip dynamometer. Baseline data collection began at least 10 min after an acceptable nerve recording was obtained.

CPT. We used the CPT as a secondary probe of sympathetic neural control between the sexes, as others have used it to assess the central integration of vasomotor processes and their efferent pathways (26, 31, 37). CPT-induced increases in BP and HR involve the activation of the rostral ventrolateral medulla and the nucleus ambiguous (26), two brain structures responsible for pressor and tachycardic responses. Following 1 min of baseline, the dominant hand was immersed up to the wrist in an ice water bath (4°C) for 2 min, which was followed by 3 min of recovery. Subjects were instructed to avoid breath holding (confirmed by nasal cannula) and to stay as relaxed as possible.

Static handgrip to fatigue. After at least a 5-min recovery phase from the CPT, static handgrip was performed at 40% of MVC until fatigue. This level of force was chosen since it has been previously shown that handgrip sustained at 40% and 60% of MVC elicited comparable increases in BP and MSNA at fatigue (30). Since individuals can sustain 40% of MVC for a longer period of time, this allowed for a better assessment of the time course, as well as for comparisons between studies (i.e., studies that stopped handgrip at 2 min). Once the exerted force declined to < 80% of the desired force for ≥ 2 s, a 2-min PECA (with an upper arm cuff inflated to 250 mmHg) phase began. During static handgrip exercise and PECA, subjects were instructed to avoid breath holding (confirmed by nasal cannula).

Data Analysis

Data were sampled at 625 Hz with a commercial data acquisition system (Biopac Systems, Santa Barbara, CA) and analyzed using LabView Software (National Instruments, Austin, TX). Beat-by-beat HR was calculated from the R-R interval of the ECG. Beat-by-beat SBP and DBP were estimated from the arterial waveforms.

MSNA bursts were identified by a computer program using a 3:1 signal-to-noise ratio threshold within a 0.5-s search window and an expected burst reflex latency of 1.3 s from the preceding R-wave (5). All bursts were confirmed by trained personnel. Burst areas of the integrated neurogram and BP were measured simultaneously on a beat-to-beat basis. Burst frequency (BF) was defined as the number of bursts per min, and burst incidence (BI) was used to normalize BF per 100 heart beats. Total activity was defined as the burst area of the rectified and integrated neurogram. We used a modified method developed by Sugiyama et al. (34) and Halliwill (13) where we developed by Sugiyama et al. (34) and Halliwill (13) where we assigned the largest burst amplitude during baseline a value of 100. Therefore, all other bursts within a testing session were normalized against this value. For comparisons between groups (men vs. women) and across sessions (EF vs. ML) we report the change in total activity from baseline (Atotal activity).

Baseline MSNA, HR, and BP for both the static handgrip exercise and CPT were averaged for 1 min. The total handgrip time was divided evenly into five stages, and the data were presented as 20, 40, 60, 80, and 100% (at fatigue) (30). Stages during handgrip were divided accordingly because subjects performed handgrip until fatigue.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EF</td>
<td>ML</td>
</tr>
<tr>
<td>Age, yr</td>
<td>33 ± 10</td>
<td>33 ± 10</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165.9 ± 5.9*</td>
<td>165.5 ± 6.1*</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.7 ± 7.5*</td>
<td>63.8 ± 7.7*</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.7 ± 0.1*</td>
<td>1.7 ± 0.1*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.1 ± 2.1</td>
<td>23.3 ± 2.1</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 11 women, 10 men. EF, early follicular phase; ML, midluteal phase. *Different from men, P < 0.01.
and this served as a way to normalize for intersubject variability. PECA 1, PECA 2, and recovery (REC 1, REC 2, REC 3) data were averaged each minute. Stages for the CPT were averaged every 30 s.

Statistical Analysis

Data are expressed as mean ± SD. A two-way repeated-measures ANOVA [group (men, EF, ML) × stage] was used to examine cardiovascular and MSNA responses to the static handgrip exercise and CPT between the sexes and menstrual phases. A separate two-way repeated-measures ANOVA [group (men, EF, ML) × stage] was used to assess differences between the sexes in terms of their response to these perturbations by examining the change (Δ) from baseline for each variable. Tukey’s post hoc analysis was used when significance was found. All statistical analyses were performed using SigmaStat 3.11 (Systat Software, San Jose, CA). A P value of <0.05 was considered statistically significant.

RESULTS

Hormone Analyses

Estrogen was lower during the EF phase compared with the ML phase (32.4 ± 8.9 vs. 91.8 ± 46.7 pmol/l; P < 0.01). Progesterone was also lower during the EF phase (0.9 ± 0.5 vs. 11.1 ± 5.7 nmol/l; P < 0.01). Low vs. high sex hormone status did not influence the absolute BP or the magnitude of the change in BP in women.

Static Handgrip to Fatigue

Figure 1 illustrates that the men had higher MVC compared with the women during both phases of the menstrual cycle (both P < 0.05) and that menstrual cycle phase did not influence MVC in the women (P = 0.43). The time to fatigue (Fig. 1) was comparable between the sexes and menstrual phases (P = 0.61).

HR

The cardiovascular variables in response to static handgrip exercise are illustrated in Table 2 and Fig. 2. HR increased progressively during handgrip and reached its peak at fatigue in all the subjects (P < 0.001). The absolute HR at fatigue was not different between the sexes; however, the increase in HR from baseline to fatigue (Table 2) was greater in men than in women (both phases P < 0.01). There was no difference in HR response from baseline to fatigue between the two phases of the menstrual cycle (P = 0.47). Finally, HR returned to baseline values during PECA in both women and men, with no difference between phases of the menstrual cycle.

BP

The BP responses to static handgrip and circulatory arrest are shown in Table 2 and Fig. 2. SBP and DBP in all groups was higher at fatigue (all P < 0.001) and during PECA (all P < 0.001) compared with baseline. The men had higher SBP and DBP compared with women at fatigue and PECA (all P < 0.01), as well as demonstrated a greater ΔSBP and ΔDBP from baseline to fatigue and during PECA (all P < 0.01). Low vs. high sex hormone status did not influence the absolute BP or the magnitude of the change in BP in women.

MSNA

MSNA (Table 2 and Fig. 3) progressively increased during static handgrip in both sexes, reached the peak at fatigue, and remained elevated during PECA compared with baseline (all P < 0.05). The absolute MSNA-BF during handgrip was lower in women during the low hormone phase (EF, P = 0.01), as was the ΔMSNA-BF smaller (main effect, both P < 0.01) compared with the men. AMSNA-BI was attenuated in women compared with the men during both the low and high hormone phases (main effect, both P < 0.05). The women had a smaller Δtotal activity (for both EF and ML) compared with the men (all P < 0.05). The low vs. high sex hormone phase did not influence any of the MSNA responses to static handgrip or PECA.

Vascular Transduction

During static handgrip (at fatigue and during PECA 2) the change in MSNA and DBP from baseline was lower in the women. However, there was no difference in the vascular transduction (ΔDBP/Δtotal activity) between the sexes in either condition (EF: 0.03 ± 0.02 units vs. ML: 0.04 ± 0.05 units vs. men: 0.03 ± 0.01 at fatigue, P = 0.66; EF: 0.04 ± 0.04 vs. ML: 0.05 ± 0.07 vs. men: 0.04 ± 0.02 units during PECA2, P = 0.69).

CPT

HR. Figure 4 shows the cardiovascular responses to the CPT. HR increased immediately and was significantly
The principal findings demonstrated higher BP than the women. Similarly, PECA induced higher BP during the high hormone phase compared with the low hormone phase in the women, there was no statistical difference. The women demonstrated similar increases (Δ) in SBP and DBP during the both low and high hormone phases of the menstrual cycle.

MSNA. MSNA is illustrated in Fig. 5. MSNA-BF was significantly higher during the CPT compared with baseline (P < 0.001). MSNA-BI showed a similar trend (P < 0.001). MSNA-BF was similar between the sexes; however, the men had a significantly greater MSNA-BI compared with women in the ML phase because women had higher HRs (Table 2, P < 0.05). ΔTotal activity was not different between the sexes. Low vs. high sex hormone status did not affect the responses to either static handgrip or PECA in women. REC, recovery. *Group difference compared with BL, P < 0.05. **Difference from BL (all groups), P < 0.05. †Sex difference within stage, P < 0.05. ‡Group difference (main effect), P < 0.05. 321* 675

The present study is the first to comprehensively examine responses to static handgrip exercise and a cold pressor stimulus in a group of men and women, while controlling for low vs. high hormone phases in the women. The principal findings higher compared with baseline during the CPT (P < 0.001). The change from baseline (ΔHR, Table 2) was similar between men and women, as well as between phases of the menstrual cycle.

BP. SBP and DBP (Table 2 and Fig. 4) also increased during the CPT in both groups (all P < 0.05). The magnitude of the change compared with baseline (ΔSBP and ΔDBP, Table 2) was significantly higher in the men during the peak response (at 1.5 min) compared with the women during both phases of the menstrual cycle (all P < 0.05). The absolute DBP was higher in men compared with women in the EF during the CPT (all P < 0.05). Although it appears that absolute SBP and DBP was higher during the high hormone phase compared with the low hormone phase of the menstrual cycle in the women, there was no statistical difference. The women demonstrated similar increases (Δ) in SBP and DBP during the both low and high hormone phases of the menstrual cycle.

DISCUSSION

Table 2. Cardiovascular and vasomotor sympathetic outflow changes during static handgrip exercise and cold pressor test (CPT)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Static Handgrip</th>
<th></th>
<th>CPT Peak</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>100%, at fatigue PECA 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔHR, beats/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, EF</td>
<td>15 ± 7*</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Women, ML</td>
<td>12 ± 9*</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Men</td>
<td>24 ± 6</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>ΔSBP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, EF</td>
<td>22 ± 13*</td>
<td>17</td>
<td>12*</td>
</tr>
<tr>
<td>Women, ML</td>
<td>16 ± 12*</td>
<td>15</td>
<td>13*</td>
</tr>
<tr>
<td>Men</td>
<td>43 ± 13</td>
<td>31</td>
<td>10</td>
</tr>
<tr>
<td>ΔDBP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, EF</td>
<td>16 ± 6*</td>
<td>9</td>
<td>5*</td>
</tr>
<tr>
<td>Women, ML</td>
<td>12 ± 7*</td>
<td>9</td>
<td>7*</td>
</tr>
<tr>
<td>Men</td>
<td>29 ± 6</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>ΔMSNA-BF, bursts/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, EF</td>
<td>18 ± 7*</td>
<td>11</td>
<td>8*</td>
</tr>
<tr>
<td>Women, ML</td>
<td>20 ± 12*</td>
<td>10</td>
<td>7*</td>
</tr>
<tr>
<td>Men</td>
<td>30 ± 7</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>ΔMSNA-BI, bursts/100 heart beats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, EF</td>
<td>20 ± 10</td>
<td>17</td>
<td>14*</td>
</tr>
<tr>
<td>Women, ML</td>
<td>24 ± 16</td>
<td>16</td>
<td>13*</td>
</tr>
<tr>
<td>Men</td>
<td>29 ± 7</td>
<td>31</td>
<td>14</td>
</tr>
<tr>
<td>ΔTotal activity, a.u./min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, EF</td>
<td>632 ± 418*</td>
<td>354</td>
<td>321*</td>
</tr>
<tr>
<td>Women, ML</td>
<td>598 ± 342*</td>
<td>341</td>
<td>199*</td>
</tr>
<tr>
<td>Men</td>
<td>1025 ± 416</td>
<td>599</td>
<td>327</td>
</tr>
</tbody>
</table>

All values are the differences from baseline, means ± SD. PECA, postexercise circulatory arrest; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MSNA, muscle sympathetic nerve activity; BF, burst frequency; BI, burst incidence; a.u., arbitrary units. *Difference from men within stage, P < 0.05. Peak for CPT was defined as: HR, 0.5 min; SBP and DBP, 1.5 min; MSNA, 1.5 min.

Fig. 2. Cardiovascular responses during static handgrip exercise and postexercise circulatory arrest (PECA). Static handgrip elicited increases in heart rate (HR), systolic blood pressure (SBP), and diastolic BP (DBP) where men demonstrated higher BP than the women. Similarly, PECA induced higher BP compared with baseline (BL) in both sexes with the women showing an attenuated response compared with the men. Low vs. high hormone status did not affect the responses to either static handgrip or PECA in women. REC, recovery. *Group difference compared with BL, P < 0.05. **Difference from BL (all groups), P < 0.05. †Sex difference within stage, P < 0.05. ‡Group difference (main effect), P < 0.05.
from the present study are that 1) women demonstrate attenuated BP and MSNA responses during static handgrip to fatigue and during PECA compared with men; 2) women and men demonstrate comparable increases in MSNA (ΔMSNA-BF and Δtotal activity) in response to a cold pressor stimulus; and 3) low vs. high sex hormone exposure does not influence the cardiovascular or vasomotor sympathetic responses to static handgrip exercise or to the CPT in women. Our results support the hypothesis that women would demonstrate a blunted sympathetic response during static handgrip to fatigue compared with the men; however, our results do not support the hypothesis that the high sex hormone phase results in further blunting of sympathetic responses to either static handgrip exercise or the CPT in the women.

Fig. 3. Muscle sympathetic nerve activity (MSNA) responses during static handgrip exercise and PECA. Static handgrip elicited increases in MSNA-BF and MSNA-BI where men demonstrated higher values than the women. ΔTotal activity was also greater in the men compared with the women. Similarly, PECA induced higher MSNA compared with BL in both sexes with the women showing an attenuated response compared with the men. Low vs. high hormone phase of the menstrual cycle did not affect the responses to either handgrip or PECA in women. a.u., Arbitrary units. BF, burst frequency; BI, burst incidence. *Group difference compared with BL, $P < 0.05$. **Difference from BL (all groups), $P < 0.05$. †Sex difference within stage, $P < 0.05$. ‡Group difference (main effect), $P < 0.05$.

Fig. 4. Cardiovascular responses during the cold pressor test (CPT). The cold pressor stimulus elicited increases in HR, SBP, and DBP. HR responses were comparable between the sexes and between the low vs. high hormone phases of menstrual cycle. However, men demonstrated greater changes from BL in SBP and greater DBP compared with the women. *Group difference compared with BL, $P < 0.05$. **Difference from BL (all groups), $P < 0.05$. †Sex difference within stage (absolute difference), $P < 0.05$.
We found that women demonstrated a similar contraction-induced peak HR during static handgrip to fatigue. Since this occurred with a concomitantly lower BP and vasomotor sympathetic response during the CPT, a nonexercise pressor stimulus, central processing and the efferent arm of the sympathetic nervous system are assumed to be comparably intact between the two groups. That is, the women are capable of achieving similar levels of MSNA as the men but the static handgrip to fatigue and PECA do not elicit the same response. Thus, the blunted response demonstrated by the women is likely to be explained by differences in the metaboreflex arising from the exercising muscle. The following section relates our principal findings in the context of the contributions of the mechanoreflex and the metaboreflex during static handgrip exercise and the PECA phase.

Contributions of the Mechano- and Metaboreflex

The separate contributions of the mechanoreceptor and the metaboreceptor during static exercise are difficult to assess. Mechanical deformation stimulates group III afferents and metabolites stimulate group IV afferents (20), both of which occur during static handgrip. The size of the active muscle mass has been thought to be a potential contributor to the magnitude of the increase in MSNA (29). For example, Seals (29) demonstrated that isometric handgrip evoked a larger increase in MSNA compared with isometric abduction of the first dorsal interosseus of the hand in the same subject. It is difficult, however, to extrapolate the findings of Seals (29) to those of intersubject differences. That is, do the men have higher MSNA during static exercise simply because a larger muscle mass is engaged? Ettinger et al. (6) addressed this issue by matching the sexes for anthropometric variables, as well as MVC (adductor pollicus) and time to fatigue, showing that women still had an attenuated MSNA response. Taken together, these studies suggest that activation of a larger muscle mass is associated with greater intrasubject increases in MSNA but does not necessarily explain intersubject differences. When size discrepancies are accounted for, there still remains a sex difference in the neural response to static exercise, which suggests the metaboreflex may be a larger contributor to the increase in MSNA and BP than the mechanoreflex.

Our findings are consistent with others who showed 1) baseline MSNA was comparable between the sexes (3, 6, 10, 11); 2) the low and high hormone phases of the menstrual cycle did not influence baseline MSNA (2, 10, 22, 23); and 3) women had a smaller change in MSNA from baseline during circulatory arrest after exercise (6). We interpret these findings as women demonstrating a reduced metaboreceptor response, which is independent of exposure to low or high sex hormone concentrations. Indeed, others have previously found, using \[^{31}P\]-nuclear magnetic resonance spectroscopy, that women had lower concentrations of H\(^+\)/H\(_2\)PO\(_4\)/H\(_2\)PO\(_4\) in the muscle (6); H\(^+\) and H\(_2\)PO\(_4\) have been positively associated with increases in MSNA (33, 36).

Cellular metabolite concentration should be related to the net effect of production and clearance, which is influenced by muscle fiber type. Saito (28) showed that static exercise performed in muscle groups of differing fiber type composition (very low type II → high type II) elicited dissimilar increases in MSNA. For example, forearm (high type II) static exercise
elicited the largest increase in MSNA compared with the soleus (very low type II) (28), suggesting that type II fibers are associated with greater production and/or a decrease in clearance of metabolic byproducts compared with type I fibers. Indeed, animal studies have shown that lactate uptake is higher in type I fibers compared with type II fibers (19). These findings imply that fiber type distribution may vary between the sexes. In fact, human studies of biopsied vastus lateralis muscles have confirmed this hypothesis (32). Simoneau and Bouchard (32) studied biopsies of 270 healthy sedentary individuals (126 women, 144 men), reporting that while large interindividual differences exist, on the average women had a significantly higher percentage of type I fiber distribution. Taken together, these studies indicate that women have a higher proportion of type I fibers in the vastus lateralis, which may also be true of other muscle beds. The smaller proportion of type II fibers may lead to lower production of metabolites and the greater proportion of type I fibers may increase their capacity to buffer the metabolites, which may result in a smaller MSNA response in women during static exercise.

**Influence of Sex Hormones on Exercise Pressor Reflex**

Ettinger et al. (7) demonstrated that the estrogen fluctuations throughout the menstrual cycle did not influence baseline (resting) MSNA but did influence the response to static handgrip exercise. They showed that the increase in MSNA was attenuated during the high estrogen phase with unopposed progesterone (7). We were unable to replicate their finding; however, our study was consistent with another group that examined MSNA during ischemic, rhythmic handgrip exercise during the EF and ML phases of the menstrual cycle (25). Thus, one possible explanation for the disparate findings between our study and Ettinger et al. (7) could be related to the contribution of progesterone. Ettinger et al. (7) compared days 1–4 (EF) to days 10–12 (preovulatory) when estrogen, but not progesterone, was different. We compared days 1–4 (EF) to days 19–22 (ML) or during the low hormone phase and the high hormone phase for both estrogen and progesterone. The role of progesterone in cardiovascular control is not well defined and no consensus exists. However, the interaction of estrogen and progesterone is important as premenopausal women are exposed to both during the course of the menstrual cycle, where neither acts in true isolation. Thus, based on the differences between these studies (7, 25) and ours, it appears that the presence of progesterone may alter clearance of metabolic byproducts. We also cannot leave out the possibility that differences in experimental design (handgrip to fatigue vs. terminating handgrip at 2 min) and/or data analysis played a role in these observed differences.

Despite the large fluctuations in both estrogen and progesterone throughout the menstrual cycle, the response of the women remained similar between the low hormone and high hormone phases of the cycle in our study. Thus, findings from our study indicate that the acute alterations in sex hormones in women do not alter the cardiovascular and vasomotor sympathetic activity responses during static handgrip exercise. This does not exclude the long-term effects of cyclical exposure to estrogen and/or progesterone, which may contribute to the overall sex differences in the exercise pressor reflex.

**Sex Differences in BP Responsiveness During the CPT**

It is worth noting that the women demonstrated reduced DBP responses to the CPT compared with the men, despite similarities in MSNA. We, however, did not find sex differences in vascular transduction or differences between the low and high hormone phases of the menstrual cycle, which is consistent with others that have shown no difference between the EF and ML phases (25). Certainly there are other factors to consider that influence vascular tone and BP responsiveness. For example, it has been reported that women have greater $\beta_2$-adrenergic sensitivity (21). Additionally, DBP does not differentiate the degree of vasoconstriction in beds like the splanchnic and renal circulations but only provides an overall assessment of total peripheral resistance. Therefore, it is possible that MSNA alone (i.e., the skeletal muscle bed) does not reflect the activity of other circulations.

**Limitations**

There are at least four limitations in our study. First, we did not assess the separate roles of estrogen and progesterone by measuring responses in the late-follicular phase (high estrogen/low progesterone). Second, we cannot ignore that men produced a higher absolute force than women during static handgrip exercise. However, Seals (30) posited that at fatigue sympathetic outflow is independent of the level of force (>$20\%$ of MVC); therefore, having the subjects perform handgrip to fatigue served as a way of normalizing potential differences between the sexes. Third, we did not measure metabolic byproducts during handgrip and PECA in our subjects. This, however, does not change the overall finding that a sex difference exists in cardiovascular and neural responses during PECA, demonstrating that women have a blunted metaboreflex response. Fourth, we did not assess the interaction of the metaboreflex and the arterial baroreflex in the present study. Some studies (5, 16) have suggested that during PECA there is an increase in baroreflex sensitivity. We cannot reject the possibility that baroreflex sensitivity was altered differently between the women and men during PECA in the present study. However, Cui et al. (5) reported that the metaboreflex is more powerful in increasing MSNA than any baroreflex-mediated suppression of MSNA. During pharmacologic manipulation (bolus injection of sodium nitroprusside followed by phenylephrine) of BP, and, therefore, MSNA during PECA, Cui et al. (5) found that MSNA was still significantly higher than baseline during the phenylephrine portion of the maneuver. Yet, in the nonexercise control trial of their study, MSNA was suppressed, though not statistically significant, compared with baseline (5). Thus, it is not likely that the contribution of the baroreflex changes our findings. We acknowledge, however, that whether baroreflex sensitivity was differently altered during PECA between the two groups remains a separate and testable hypothesis, which was not the primary focus of this paper.

**Perspectives and Significance**

BP reactivity is one predictor for the future development of hypertension in young healthy individuals. Exposure to both estrogen and progesterone seem to confer a degree of cardioprotection in young premenopausal women. Indeed, premeno-
pausal women demonstrate lower resting BP and lower incidence of hypertension. Based on these findings, we surmise that this cardioprotection will be lost during menopause and that BP reactivity will be comparable or even greater in elderly women when the incidence of hypertension and cardiovascular disease is higher than in men (39).

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DISCLOSURES

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

REFERENCES