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

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Running head: Exercise pressor reflex is attenuated in women

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Abstract

Sex differences in sympathetic neural control during static exercise in humans are few and the findings are inconsistent. We hypothesized women would have an attenuated vasomotor sympathetic response to static exercise, which would be further reduced during the high sex hormone (mid-luteal, ML) vs. the low hormone phase (early follicular, EF). We measured heart rate (HR), blood pressure (BP), and muscle sympathetic nerve activity (MSNA) in 11 women and 10 men during a cold pressor test (CPT) and static handgrip to fatigue with 2 min of post-exercise circulatory arrest (PECA). HR increased during handgrip, reached its peak at fatigue, and was comparable between sexes. BP increased during handgrip and PECA where men had larger increases from baseline. Mean (±SD) MSNA burst frequency (BF) during handgrip and PECA was lower in women (EF, p<0.05), as was ΔMSNA-BF smaller (main effect, both p<0.01). Δtotal activity was higher in men at fatigue (EF: 632±418 vs. ML: 598±342 vs. men: 1025±416 a.u./min, p<0.001 for EF and ML vs. men) and during PECA (EF: 354±321 vs. ML: 341±199 vs. men: 599±327 a.u./min, p<0.05 for EF and ML vs. men). During CPT, HR and MSNA responses were similar between sexes and hormone phases, confirming that central integration and the sympathetic efferent pathway was comparable between the sexes and across hormone phases. Women demonstrated a blunted metaboreflex, unaffected by sex hormones, which may be due to differences in muscle mass or fiber type and, therefore, metabolic stimulation of group IV afferents.

Key Words

Cold pressor test, muscle sympathetic nerve activity, metaboreflex
**Introduction**

Premenopausal 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 through a pressor response.

Static exercise invokes increases in heart rate (HR), BP, and muscle sympathetic nerve activity (MSNA) through two neural pathways—central command and the exercise pressor reflex (4, 12, 15). The exercise pressor reflex is a feedback system arising from mechano-sensitive (group III) and metabo-sensitive (group IV) afferent nerve endings within the skeletal muscle (4, 15). This feedback loop increases BP through increases in MSNA, which is one determinant of vasoconstriction in non-exercising muscles.

Previous studies regarding the effects of sex on sympathetic neural control during static exercise in humans are few and the findings are inconsistent. For example, it was found that women and men responded with comparable increases in MSNA during 1 min of static handgrip exercise, 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, high estrogen/low progesterone) when compared to the early follicular phase (1 to 4 days after the onset of menstruation, low 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 (30). The purpose of this study was to test the hypothesis that premenopausal women would demonstrate an attenuated cardiovascular and vasomotor sympathetic response during static handgrip to fatigue and post-exercise circulatory arrest period when
compared to men. We also hypothesized that the influence of the high sex hormone phase (mid-luteal, ML) of the menstrual cycle in women would result in further blunting of these responses when compared to the low hormone phase (early follicular, EF).

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 non-invasively by using finger photoplethysmography (model 1, BMEYE Nexfin HD monitor, 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 electrosphygmomanometry (model 4240, SunTech Medical Instruments Inc., Raleigh, NC, USA) 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 data collection began. The BP values we report were derived from the beat-by-beat determinations.

**MSNA.** MSNA signals were obtained using the microneurographic technique (35). Briefly, a recording electrode was placed in the peroneal nerve at the popliteal fossa and a reference electrode was placed subcutaneously 2-3 cm from the recording electrode. The nerve signals were amplified (gain 70,000-160,000), band-pass filtered (700-2,000 Hz), full-wave rectified, and integrated with a resistance-capacitance circuit (time constant 0.1 sec). Criteria for adequate MSNA recording included: 1) pulse synchrony; 2) facilitation during the hypotensive phase of the Valsalva maneuver and suppression during the hypertensive overshoot after release; 3) increases in response to breath holding; and 4) insensitivity to emotional stimuli (35).

**Protocol**

The first visit (screening) consisted of a 12-lead resting ECG and supine BP measurements. The second visit was comprised of static handgrip and cold pressor tests. Female subjects completed the second visit twice since they were tested once during the EF phase (days 1-4 when both estrogen and progesterone are low) of their menstrual cycles.
and once during the ML phase (days 19-22 when both sex hormones are high), with the order counterbalanced. Cycle phase was determined by the onset of menstruation and by detection of the luteinizing hormone surge by an ovulation prediction kit (OvuQuick, Quidel Corp., San Diego, CA, USA). 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 1000 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 ≥2hrs after a light meal and ≥48 hrs 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 sec) 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.

**Cold pressor test.** We used the cold pressor test (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 5 min of a 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 sec, a 2-min post-exercise circulatory arrest (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 System, Santa Barbara, CA, USA) and analyzed using LabView Software (National Instruments,
Austin, TX, USA). 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 sec search window and an expected burst reflex latency of 1.3 sec 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 burst frequency 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 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 ($\Delta$total 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 and this served as a way to normalize for inter-subject 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 sec.

**Statistical analysis**

Data are expressed as mean±SD. A two-way repeated measures analysis of variance [group (men, EF, ML) × stage; ANOVA] 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, Inc., San Jose, CA, USA). A $P$ value of <0.05 was considered statistically significant.

**Results**

**Hormone analyses**

Estrogen was lower during the EF phase when compared to 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; $p<0.01$).

**Static handgrip to fatigue**

Figure 1 illustrates that the men had higher MVC compared to 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 (Figure 1) was comparable between the sexes and menstrual phases (p=0.61).

**Heart rate**

The cardiovascular variables in response to static handgrip exercise are illustrated in Table 2 and Figure 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). Lastly, HR returned to baseline values during PECA in both women and men, with no difference between phases of the menstrual cycle.

**Blood pressure**

The BP responses to static handgrip and circulatory arrest are shown in Table 2 and Figure 2. SBP and DBP in all groups was higher at fatigue (all p<0.001) and during PECA (all p<0.001) when compared to baseline. The men had higher SBP and DBP compared to 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.
**Muscle sympathetic nerve activity**

MSNA (Table 2 and Figure 3) progressively increased during static handgrip in both sexes, reached the peak at fatigue, and remained elevated during PECA when compared to 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 \( \Delta \)MSNA-BF smaller (main effect, both p<0.01) when compared to the men. \( \Delta \)MSNA-BI was attenuated in women compared to the men during both the low and high hormone phases (main effect, both p<0.05). The women had a smaller \( \Delta \)total activity (for both EF and ML) compared to 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 (\( \Delta \)DBP/\( \Delta \)total activity) between the sexes in either condition (EF: 0.03±0.02 U vs. ML: 0.04±0.05 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 U during PECA2, p=0.69).

**Cold pressor test**

**Heart rate**

Figure 4 shows the cardiovascular responses to the CPT. HR increased immediately and was significantly higher compared to 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.

_Blood pressure_

SBP and DBP (Table 2 and Figure 4) also increased during the CPT in both groups (all p<0.05). The magnitude of the change compared to baseline (ΔSBP and ΔDBP, Table 2) was significantly higher in the men during the peak response (at 1.5 min) compared to the women during both phases of the menstrual cycle (all p<0.05). The absolute DBP was higher in men compared to 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 to 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.

_Muscle sympathetic nerve activity_

MSNA is illustrated in Figure 5. MSNA-BF was significantly higher during the CPT compared to baseline (p<0.001). MSNA-BI showed a similar trend (p<0.001). ΔMSNA-BF was similar between the sexes; however, the men had a significantly greater ΔMSNA-BF compared to women in the ML phase because women had higher heart rates (Table 2, p<0.05). Δtotal activity was not different between the sexes. Low vs. high sex hormone status did not affect any of the MSNA variables in the women.
**Discussion**

The current study is the first to comprehensively exam 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 from the current study are that: 1) women demonstrate attenuated BP and MSNA responses during static handgrip to fatigue and during PECA when compared to 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 hormones 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 to 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.

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 that persisted during PECA, when the contribution of central command and the stimulation of group III afferents was removed, it indicates that the disparate responses between the sexes are not likely due to the contribution of central command or the mechanoreflex. Moreover, it has been previously shown that central command contributes little to the rise in MSNA during static handgrip exercise (38). Since
women and men had similar vasomotor sympathetic responses during the CPT, a non-exercise 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.

*The 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 when compared to 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 inter-subject 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 maximal voluntary contraction (adductor pollicis) 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 intra-subject increases in MSNA but does not necessarily explain inter-subject 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 $H^+$ and $H_2PO_4^-$ in the muscle (6); $H^+$ and $H_2PO_4^-$ 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 $\rightarrow$ high type II) elicited dissimilar increases in MSNA. For example, forearm (high type II) static exercise elicited the largest increase in MSNA compared to the soleus (very low type II)
suggesting that type II fibers are associated with greater production and/or a decrease in clearance of metabolic byproducts compared to type I fibers. Indeed, animal studies have shown that lactate uptake is higher in type I fibers when compared to type II fibers. 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. Simoneau & Bouchard (32) studied biopsies of 270 healthy sedentary individuals (126 women, 144 men), reporting that while large inter-individual 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.

The influence of sex hormones on the 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 (pre-ovulatory) 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 to 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 (above 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 current 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 current 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 non-exercise control trial of their study, MSNA was suppressed, though not statistically significant, compared to 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, premenopausal 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).

**Disclosures:** N/A

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Table 1. Subject characteristics

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<th>Men (n = 10)</th>
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<td>Body mass index (kg/m²)</td>
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Values are mean±SD. EF, early follicular phase; ML, mid-luteal phase. *Different from the men, p<0.01.
**Table 2.** Cardiovascular and vasomotor sympathetic outflow changes during static handgrip exercise and CPT

<table>
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<td>Women, ML</td>
<td>20±12*</td>
<td>10±7*</td>
</tr>
<tr>
<td>Men</td>
<td>30±7</td>
<td>18±9</td>
</tr>
<tr>
<td>ΔMSNA-BI, bursts/100 heart beats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, EF</td>
<td>20±10</td>
<td>17±14*</td>
</tr>
<tr>
<td>Women, ML</td>
<td>24±16</td>
<td>16±13*</td>
</tr>
<tr>
<td>Men</td>
<td>29±7</td>
<td>31±14</td>
</tr>
<tr>
<td>Δtotal activity, a.u./min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, EF</td>
<td>632±418*</td>
<td>354±321*</td>
</tr>
<tr>
<td>Women, ML</td>
<td>598±342*</td>
<td>341±199*</td>
</tr>
<tr>
<td>Men</td>
<td>1025±416</td>
<td>599±327</td>
</tr>
</tbody>
</table>

All values are the differences from baseline, mean±SD. *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.
Figure legends

**Figure 1.** MVC and time to fatigue. MVC (panel A) was greater in men compared to the women during both the low and high hormone phases of the menstrual cycle. The time to fatigue (panel B) during the static handgrip exercise was neither different between the groups, nor affected by menstrual cycle phase. *Difference from the men, p<0.05.

**Figure 2.** Cardiovascular responses during static handgrip exercise and PECA. Static handgrip elicited increases in HR, SBP, and DBP where men demonstrated higher BP than the women. Similarly, PECA induced higher BP compared to baseline in both sexes with the women showing an attenuated response compared to the men. Low vs. high hormone status did not affect the responses to either static handgrip or PECA in women. *Group difference compared to baseline, p<0.05. **Difference from baseline (all groups), p<0.05. †Sex difference within stage, p<0.05. ‡Group difference (main effect), p<0.05. BL, baseline; PECA, post-exercise circulatory arrest; REC, recovery.

**Figure 3.** 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 to the women. Similarly, PECA induced higher MSNA compared to baseline in both sexes with the women showing an attenuated response compared to the men. Low vs. high hormone phase of the menstrual cycle did not affect the responses to either handgrip or PECA in women. *Group difference compared to baseline, p<0.05. **Difference from baseline (all
groups), p<0.05. †Sex difference within stage, p<0.05. ‡Group difference (main effect),
p<0.05. BL, baseline; PECA, post-exercise circulatory arrest; REC, recovery.

**Figure 4.** Cardiovascular responses during the 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 baseline in SBP and greater DBP compared to the
women. *Group difference compared to baseline, p<0.05. **Difference from baseline (all
groups), p<0.05. †Sex difference within stage (absolute difference), p<0.05. BL, baseline;
CPT, cold pressor test; REC, recovery.

**Figure 5.** MSNA responses during the CPT. The cold pressor stimulus elicited increases
in MSNA-BF and MSNA-BI. No cycle phase × stage interaction was present, indicating
MSNA responses were comparable between the sexes and between the low vs. high
hormone phases of the menstrual cycle. ΔMSNA-BI was significantly different between
the men and women during the ML phase, which likely the result of a higher HR in the
women. ΔTotal activity was similar between the sexes and menstrual phases. **Difference
from baseline (all groups), p<0.05. BL, baseline; CPT, cold pressor test; REC, recovery.
References


Figure 1. MVC and time to fatigue. MVC (panel A) was greater in men compared to the women during both the low and high hormone phases of the menstrual cycle. The time to
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MSNA burst frequency (bursts/min)

- Women (EF)
- Women (ML)
- Men

*group, p=0.112
*stage, p<0.001
*group*stage, p=0.046

MSNA burst incidence (bursts/100 heart beats)

*group, p=0.083
*stage, p<0.001
*group*stage, p=0.071

Total activity (a.u./min)

*group, p=0.004
*stage, p<0.001
*group*stage, p=0.045

BL 20% 40% 60% 80% 100% PECA 1 PECA 2 REC 1 REC 2 REC 3
Figure 4. Cardiovascular responses during the 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 baseline in SBP and greater DBP compared to the women. *Group difference compared to baseline, p<0.05. **Difference from baseline (all groups), p<0.05. †Sex difference within stage (absolute difference), p<0.05. BL, baseline; CPT, cold pressor test; REC, recovery.