Menstrual cycle alters sympathetic neural responses to orthostatic stress in young, eumenorrheic women

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Carter JR, Lawrence JE, Klein JC. Menstrual cycle alters sympathetic neural responses to orthostatic stress in young, eumenorrheic women. Am J Physiol Endocrinol Metab 297: E85–E91, 2009. First published April 28, 2009; doi:10.1152/ajpendo.00019.2009.—Sympathetic baroreflex sensitivity (BRS) and muscle sympathetic nerve activity (MSNA) responses during early follicular (EF) and midluteal (ML) phases of the menstrual cycle are controversial. We hypothesize an augmented sympathetic BRS and MSNA response to orthostatic stress during the ML phase of the menstrual cycle. MSNA, mean arterial pressure (MAP), and heart rate (HR) were recorded during progressive lower body negative pressure (LBNP) (–5, –10, –15, –20, –30, and –40 mmHg; 3 min/stage) in 13 healthy, eumenorrheic women (age 21 ± 1 yr). Sympathetic BRS was assessed by examining relations between spontaneous fluctuations of diastolic arterial pressure and MSNA at rest and during progressive LBNP. Plasma estradiol (42 ± 6 vs. 112 ± 12 pg/ml; P < 0.01) and progesterone (2 ± 0 vs. 10 ± 2 ng/ml; P < 0.04) were elevated during the ML phase. Resting MSNA (8 ± 1 vs. 11 ± 1 bursts/min), MAP (79 ± 2 vs. 78 ± 2 mmHg), and HR (58 ± 2 vs. 60 ± 2 beats/min) were not different during EF and ML phases. MSNA and HR increased during progressive LBNP (P < 0.001), and the increases in MSNA burst frequency (bursts/min) and HR were similar during both phases. In contrast, increases in total MSNA (arbitrary units) during progressive LBNP were augmented during the ML phase (P < 0.04), but this response does not appear to be linked to differences in sympathetic BRS. Progressive LBNP did not change MAP during either phase. Our results demonstrate an augmentation of the MSNA response to progressive LBNP during the ML phase of the menstrual cycle. These findings suggest that hormonal fluctuations of eumenorrheic women may influence sympathoexcitation during an orthostatic challenge, but not through sympathetic baroreflex-mediated pathways.

METHODS

Subjects. Thirteen healthy women (age 21 ± 1 yr, height 169 ± 3 cm, weight 70 ± 4 kg) participated in the study. All subjects were nonsmokers, nondiabetics, and were not taking oral contraceptives and/or other hormonal supplementations. All subjects were instructed to abstain from exercise and caffeine for 12 h before laboratory testing. Only subjects with consistent and regular menstrual cycles were included in the study (26–30 days in length). The Michigan Technological University Human Subjects Committee approved the experimental protocols, and all participants signed a written informed consent.

Experimental design. Subjects were tested once during the EF phase (3 ± 0 days after start of menstruation) and again during the ML phase (23 ± 0 days after start of menstruation). The initial testing phase of the menstrual cycle was randomized. On the days of testing, the subjects reported for a blood draw to document levels of estradiol and progesterone. All subjects were tested during the same time of day for both trials. The experiment was conducted with subjects lying comfortably in the supine position. Baseline values were recorded for 5 min. Following baseline, 3-min stages of progressive LBNP were applied at –5, –10, –15, –20, –30, and –40 mmHg. MSNA, heart rate (HR), and beat-to-beat arterial blood pressure were acquired throughout the experiment.

LBNP. All subjects wore a neoprene skirt designed to form an airtight seal between the subject and the LBNP chamber. The application of LBNP (below the iliac crest) resulted in a redistribution of blood away...
from the upper body to the abdomen and lower extremities. Progressive LBNP was applied at −5, −10, −15, −20, −30, and −40 mmHg in 3-min stages after a 5-min baseline. These levels of LBNP do not typically induce presyncope, but subjects were monitored closely, and the experiment would have been stopped if any presyncopal signs were observed [i.e., symptoms of lightheadedness, nausea, diaphoresis, or a decrease of systolic arterial pressure (SAP) to <80 mmHg]. No subjects experienced presyncope during the present study.

Measurements. Multifiber recordings of MSNA were made by inserting a tungsten microelectrode in the peroneal nerve of a resting leg. A reference electrode was inserted subcutaneously 2–3 cm from the recording electrode. Both electrodes were connected to a differential preamplifier, and then to an amplifier (total gain of 80,000) where the nerve signal was band-pass filtered (700–2,000 Hz), and integrated (time constant, 0.1) to obtain a mean voltage display of the nerve activity. Satisfactory recordings of MSNA were defined by spontaneous, pulse synchronous bursts that increased during end-expiratory apnea, and did not change during auditory stimulation.

Arterial blood pressure was measured three consecutive times during both the EF and ML phases of the menstrual cycle. Progressive LBNP was performed in the ML phase. Resting baseline values during the EF and ML phases are illustrated in Fig. 1, which demonstrates a more dramatic increase of burst area when LBNP was performed in the ML phase.

Table 1. Baseline values for the early follicular and midluteal phases of the menstrual cycle

<table>
<thead>
<tr>
<th>Variable</th>
<th>EF</th>
<th>ML</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen, pg/ml</td>
<td>42±5</td>
<td>109±13*</td>
<td>0.003</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>2±0</td>
<td>9±3</td>
<td>0.038</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>8±1</td>
<td>11±1</td>
<td>0.254</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>105±3</td>
<td>104±3</td>
<td>0.569</td>
</tr>
<tr>
<td>DAP, mmHg</td>
<td>66±1</td>
<td>65±2</td>
<td>0.549</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>79±2</td>
<td>78±2</td>
<td>0.403</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>58±2</td>
<td>60±2</td>
<td>0.128</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 subjects unless noted. MSNA, muscle sympathetic nerve activity (n = 7); SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial blood pressure; HR, heart rate; EF, early follicular; ML, midluteal. *P < 0.05 vs. corresponding EF value.
Increases of sympathetic baroreflex sensitivity during LBNP were not different between phases, whether assessed as burst incidence or total MSNA (Fig. 4). Linear regression slopes for “burst strength” did not yield correlation coefficients >0.5; thus, they are not reported. Representative data from one subject are presented in Fig. 5, illustrating that sympathetic baroreflex sensitivities increase (as a function of the MSNA-DAP slope) during progressive LBNP.

**DISCUSSION**

This study investigated sympathetic neural and cardiovascular responses to orthostatic stress during the EF and ML phases of the menstrual cycle. Based on previous data indicating increased sympathetic baroreflex sensitivity during the ML phase of the menstrual cycle (18), we hypothesized an augmented sympathoexcitation to LBNP during this phase in young, eumenorrheic women. Our findings support this hypothesis by demonstrating an augmentation of MSNA (when expressed as total activity) during the ML phase of the menstrual cycle. However, this augmented sympathoexcitation during the ML phase does not appear to be due to increased sympathetic baroreflex sensitivity as originally hypothesized. Increases of sympathetic baroreflex sensitivity during orthostatic stress were similar between the EF and ML phases. Our findings, taken in conjunction with a recent report by Fu et al. (8), provide strong evidence that orthostatic stress elicits altered patterns of sympathoexcitation during the menstrual cycle, but these altered patterns are not modulated by sympathetic baroreflex sensitivity. The augmentation of total MSNA during the ML phase occurred despite no change in MSNA burst frequency, suggesting that the fluctuations of reproductive hormones during the menstrual cycle preferentially modulate the neural recruitment patterns as opposed to simply the number of bursts produced during an orthostatic challenge. Furthermore, increased sympathetic baroreflex sensitivity during LBNP demonstrated in the present study challenges a recent report indicating no change in sympathetic baroreflex sensitivity during progressive LBNP (10).
This finding is in conflict with Minson et al. (18), who demonstrated that hypothalamic baroreflex sensitivities were not different between menstrual phases during resting conditions, 30° HUT, or 60° HUT. This finding is in conflict with Minson et al. (18), who demonstrated that hypothalamic baroreflex sensitivities were not different between menstrual phases during resting conditions, 30° HUT, or 60° HUT.

Fu et al. (8) recently examined MSNA responses to HUT during the EF and ML phases, and reported that the sympathetic baroreflex sensitivities were not different between menstrual phases during resting conditions, 30° HUT, or 60° HUT. This finding is in conflict with Minson et al. (18), who demonstrated a significant increase of sympathetic baroreflex sensitivity during the ML phase. Our results support the findings of Fu et al. (8), since we report that progressive LBNP elicits similar increases in sympathetic baroreflex sensitivity during orthostatic stress. The increases in sympathetic baroreflex sensitivity were not different between the EF and ML phases. Reported as means ± SE (n = 7).

Table 2. Sympathetic baroreflex sensitivity during the EF and ML phases of the menstrual cycle

<table>
<thead>
<tr>
<th>Menstrual Phase</th>
<th>Baseline</th>
<th>−5 mmHg</th>
<th>−10 mmHg</th>
<th>−15 mmHg</th>
<th>−20 mmHg</th>
<th>−30 mmHg</th>
<th>−40 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF</td>
<td>−2.3 ± 0.5</td>
<td>−2.8 ± 0.6</td>
<td>−3.0 ± 0.5</td>
<td>−3.2 ± 0.8</td>
<td>−2.8 ± 0.6</td>
<td>−3.7 ± 0.2</td>
<td>−3.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(0.70 ± 0.07)</td>
<td>(0.74 ± 0.03)</td>
<td>(0.70 ± 0.05)</td>
<td>(0.67 ± 0.07)</td>
<td>(0.63 ± 0.08)</td>
<td>(0.69 ± 0.09)</td>
<td>(0.66 ± 0.09)</td>
</tr>
<tr>
<td>ML</td>
<td>−2.6 ± 0.4</td>
<td>−2.5 ± 0.5</td>
<td>−2.8 ± 0.3</td>
<td>−2.8 ± 0.4</td>
<td>−2.8 ± 0.5</td>
<td>−4.1 ± 0.4</td>
<td>−2.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>(0.69 ± 0.05)</td>
<td>(0.67 ± 0.10)</td>
<td>(0.62 ± 0.09)</td>
<td>(0.61 ± 0.11)</td>
<td>(0.48 ± 0.11)</td>
<td>(0.62 ± 0.13)</td>
<td>(0.50 ± 0.14)</td>
</tr>
<tr>
<td></td>
<td>Burst incidence slope, bursts•100 heart beat⁻¹•mmHg⁻¹</td>
<td>Total MSNA slope, units•beat⁻¹•mmHg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF</td>
<td>−1.6 ± 0.4</td>
<td>−2.0 ± 0.4</td>
<td>−3.1 ± 1.2</td>
<td>−2.6 ± 0.6</td>
<td>−2.8 ± 1.0</td>
<td>−4.6 ± 1.9</td>
<td>−4.8 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>(0.75 ± 0.06)</td>
<td>(0.74 ± 0.05)</td>
<td>(0.78 ± 0.07)</td>
<td>(0.78 ± 0.04)</td>
<td>(0.75 ± 0.08)</td>
<td>(0.87 ± 0.03)</td>
<td>(0.90 ± 0.03)</td>
</tr>
<tr>
<td>ML</td>
<td>−1.7 ± 0.3</td>
<td>−2.6 ± 0.7</td>
<td>−2.5 ± 0.6</td>
<td>−3.0 ± 0.6</td>
<td>−4.7 ± 1.4</td>
<td>−5.5 ± 2.1</td>
<td>−5.3 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>(0.73 ± 0.04)</td>
<td>(0.75 ± 0.05)</td>
<td>(0.63 ± 0.08)</td>
<td>(0.73 ± 0.06)</td>
<td>(0.81 ± 0.08)</td>
<td>(0.79 ± 0.08)</td>
<td>(0.79 ± 0.03)</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 subjects. Correlation coefficients for linear regression slopes are provided in parentheses. Progressive lower body negative pressure increased sympathetic baroreflex sensitivity during both the EF and ML phases of the menstrual cycle (burst incidence condition, P < 0.05; total MSNA condition, P < 0.01), but responses were not different between phases.

Fig. 3. Changes in MSNA during progressive LBNP. Subjects were examined during the EF and ML phases of the menstrual cycle. Progressive LBNP elicited similar increases of MSNA burst frequency during the EF and ML phases. In contrast, progressive LBNP elicited a greater increase in total MSNA activity [arbitrary units (AU)] during the ML phases of the menstrual cycle. Reported as means ± SE (n = 7).

Fig. 4. Linear relations between MSNA and DAP during EF and ML phases of the menstrual cycle. Progressive LBNP elicited significant reductions in the MSNA-DAP slopes during both the EF and ML phases of the menstrual cycle, indicating increased sympathetic baroreflex sensitivities during orthostatic stress. The increases in sympathetic baroreflex sensitivity were not different between the EF and ML phases. Reported as means ± SE (n = 7).
influence sympathetic baroreflex sensitivity in young, healthy eumenorrheic women. Differences between Minson et al. (18) and the present study/Fu et al. (8) are likely due to the technique used to assess sympathetic baroreflex sensitivity. Minson et al. (18) assessed sympathetic baroreflex sensitivity by measuring changes in arterial blood pressure and MSNA during intravenous bolus injections of sodium nitroprusside and phenylephrine (i.e., modified Oxford technique). With the use of this technique, sympathetic baroreflex sensitivity was determined by comparing MSNA and DAP slopes. In contrast, the present study and Fu et al. (8) assessed sympathetic baroreflex sensitivity by examining the linear regression slopes between spontaneous fluctuations of MSNA and DAP. This method does not induce as dramatic increases and decreases in DAP as the modified Oxford technique, yet it typically examines arterial blood pressure fluctuations within a range of 20 mmHg, which appears to be a good reflection of arterial baroreflex control of MSNA under actual physiological conditions.

It is important to note that both the present study and the recent study by Fu et al. (8) demonstrate that orthostatic stress induces an augmentation of total MSNA during the ML phase of the menstrual cycle. Interestingly, both studies also report no change in burst frequency between phases during orthostatic stress. Thus, despite two different approaches (i.e., LBNP vs. HUT), it appears that the menstrual cycle alters sympathetic neural responses to orthostatic stress when the area of a burst is considered. The physiological significance of this alteration of neural recruitment during an orthostatic challenge in women during high and low hormonal phases remains unclear. Fu et al. (8) suggested differences in norepinephrine spillover during EF and ML phases may contribute to differences in total MSNA and burst frequency responses, but norepinephrine spillover was not measured. We agree, and our data allow us to expand upon this concept further. Fu et al. (8) examined the linear regression slope between total MSNA and DAP, but did not report burst incidence and burst strength slopes. In contrast, the present study analyzed total MSNA, burst incidence, and burst strength slopes. Figure 4 demonstrates that, when examined

burst incidence, changes in the linear regression slopes during the ML phase were generally more positive compared with the EF phase. In contrast, changes in the linear regression slopes during the ML phase were generally more negative compared with the EF phase. Burst strength slopes could not be reported because the correlation coefficients were well below our threshold of $r = 0.5$, but the directional shift of the total MSNA-DAP slope data with respect to the burst incidence-DAP slope data clearly indicates the average area of a burst (i.e., burst strength) was greater during the ML phase. Thus our data suggest that the amount of norepinephrine release per efferent sympathetic burst, not burst frequency, is altered by the menstrual cycle during an orthostatic challenge. Examinations of norepinephrine spillover during orthostatic stress in the high and low hormonal phases of the menstrual cycle are warranted. Additionally, measurements of single nerve fiber activity could provide valuable insight (16).

The present study contributes further by providing data during low and high levels of baroreceptor unloading. Specifically, the lower levels of the progressive LBNP protocol (i.e., $-5$ and $-10$ mmHg) are generally believed to primarily engage the cardiopulmonary baroreceptors, while the higher levels of LBNP ($-15$, $-20$, $-30$, and $-40$ mmHg) are generally recognized as levels that engage both the cardiopulmonary and arterial baroreceptors (24). Interestingly, our data reveal that MSNA responses to LBNP begin to differ between the EF and ML phases at the higher levels of baroreceptor unloading. The failure to observe phase differences in MSNA during $-5$ and $-10$ mmHg suggests that arterial baroreceptors may play a greater role than the cardiopulmonary baroreceptors in the augmentation of orthostatically mediated MSNA during the ML phase of the menstrual cycle.

Our progressive LBNP protocol increased sympathetic baroreflex sensitivities during both the EF and ML phases, and these increases were not different across phases. Ichinose et al. (10) recently reported that progressive LBNP did not increase sympathetic baroreflex sensitivity. Differences between our results and the findings of Ichinose et al. (10) are not immediately apparent, but may be due to the blood pressure binning techniques used. Ichinose et al. (10) utilized 1-mmHg DAP bins, and the present study utilized 3-mmHg bins, as outlined by Keller et al. (12). However, in addition to our reported 3-mmHg bin data, we also examined our data using 1-mmHg and 2-mmHg bins (data not included) to allow for more direct comparison with Ichinose et al. (10). Correlation coefficients for the 2-mmHg bin did not differ from the 3-mmHg bin, but correlations were generally a bit lower (but still above the threshold of $r = 0.5$). Slopes produced using 2-mmHg bins vs. 3-mmHg bins did not differ; thus, we chose to present our 3-mmHg data. In contrast, the 1-mmHg binning produced extremely poor correlation coefficients for our data, preventing us from utilizing data from the 1-mmHg bin as an index of sympathetic baroreflex sensitivity. We are uncertain why our correlation coefficients differed so much from Ichinose et al. (10), who demonstrated robust correlations with the 1-mmHg binning technique. Nevertheless, our data indicate an increase of sympathetic baroreflex sensitivity during progressive LBNP that was not observed by Ichinose et al. (10). Furthermore, previous studies have demonstrated an increase of sympathetic baroreflex sensitivity during mild levels of LBNP (11, 15). The increased sympathetic baroreflex sensitivities observed during

![Fig. 5. Representative linear regression slopes from one subject during baseline, $-15$ mmHg, and $-30$ mmHg LBNP. Slopes became more negative with increasing levels of LBNP, indicating sympathetic baroreflex sensitivity increased with increasing levels of orthostatic stress. Data presented are from the EF phase, since results were similar between the EF and ML phases.](http://ajpendo.physiology.org/)
LBNP in the present study and others (11, 15) are suggestive that alterations of sympathetic baroreflex sensitivity during orthostatic stress are important in the maintenance of orthostatic tolerance. Our results indicate that such adjustments are not different between the EF and ML phases of the menstrual cycle.

The graded LBNP protocol used in the present study did not elicit presyncope in any subjects. This is not surprising considering subjects were exposed to a maximum of −40 mmHg and the relative short duration of the protocol. Thus it is unclear if sympathetic baroreflex sensitivities are similar between menstrual phases as subjects approach presyncope. Fu et al. (8) reported that 5 of 11 women experienced presyncope during the EF phase, whereas only 3 experienced presyncope during the ML phase. Three recent studies indicate no differences in orthostatic tolerance during high and low hormone phases of the menstrual cycle, but these studies did not assess sympathetic baroreflex sensitivity (2, 4, 17). Instead, cardiovascular baroreflex sensitivity was assessed and determined to be similar during the various phases of the menstrual cycle (2, 4, 17). The influence of menstrual phase on sympathetic baroreflex sensitivities during a maximal orthostatic stress test remains unknown.

Finally, the influence of the menstrual cycle on resting MSNA has been controversial. Ettinger et al. (6) was the first to report MSNA during altered phases of the menstrual cycle and reported no differences between the EF and follicular phases of the menstrual cycle. In contrast, Minson et al. (18) reported increased levels of resting MSNA during the ML phase compared with the EF phase. More recently, several studies have reported no changes in resting MSNA during the EF and ML phases (1, 8, 14). The present study once again reports no change in resting MSNA; thus, evidence is accumulating to suggest that hormonal fluctuations during the menstrual cycle do not modify MSNA at rest.

There are three limitations worth discussing. First, our MSNA sample size is modest (n = 7). Nevertheless, this number allowed us to detect significant differences in total MSNA responses to LBNP during the two menstrual phases. Additional MSNA signals would have been unlikely to change MSNA burst frequency responses, which were remarkably similar during EF and ML phases. Second, our resting MSNA levels are low (i.e., 8–10 bursts/min), but this is not uncommon with young, eumenorrheic women. In fact, the resting MSNA levels reported in the present study are similar to previous studies examining resting MSNA in young, eumenorrheic women (1, 14). Third, the interpretation of the MSNA-DAP slope analysis is limited to the spontaneous fluctuations of blood pressure (i.e., −20–40 mmHg range). Pharmacological manipulations of blood pressure, or the neck-chamber technique, would produce a wider range of blood pressure changes, but this would be outside the normal physiological range. Furthermore, examination of spontaneous fluctuations allows for analysis of burst incidence slopes. Thus there are advantages and limitations to using the spontaneous MSNA-DAP slope method. We limit our interpretations to sympathetic baroreflex sensitivity at or around the normal operating point of the baroreflex function curve.

In conclusion, our results demonstrate an augmentation of MSNA responses to LBNP during the ML phase of the menstrual cycle. This augmentation of MSNA during the ML phase is not linked to increases of sympathetic baroreflex sensitivity. The present study, coupled with the findings of Fu et al. (8), suggest that the increased incidence of orthostatic stress in women does not appear to be due to fluctuations of sympathetic baroreflex sensitivity during the menstrual cycle. Furthermore, the augmented total MSNA response to orthostatic stress during the ML phase of the menstrual cycle is in agreement with Fu et al. (8), but the physiological significance of this response remains unclear.

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GRANTS

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REFERENCES


