Effects of the ovarian cycle on sympathetic neural outflow during static exercise

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Ettinger, Steven M., David H. Silber, Kristen S. Gray, Michael B. Smith, Qing X. Yang, Allen R. Kunselman, and Lawrence I. Sinoway. Effects of the ovarian cycle on sympathetic neural outflow during static exercise. J. Appl. Physiol. 85(6): 2075-2081, 1998.-We compared reflex responses to static handgrip at 30% maximal voluntary contraction (MVC) in 10 women (mean age 24.1 ± 1.7 yr) during two phases of their ovarian cycle: the menstrual phase (days 1-4) and the follicular phase (days 10-12). Changes in muscle sympathetic nerve activity (MSNA; microneurography) in response to static exercise were greater during the menstrual compared with follicular phase (phase effect P = 0.01). Levels of estrogen were less during the menstrual phase (75 \pm 5.5 vs. 116 \pm 9.6 pg/ml, days 1-4 vs. days 10-12; P = 0.002). Generated tension did not explain differences in MSNA responses (MVC: 29.3 \pm 1.3 vs. 28.2 \pm 1.5 kg, *days* 1–4 vs. *days 10–12*; P = 0.13). In a group of experiments with the use of ³¹P-NMR spectroscopy, no phase effect was observed for H⁺ and $H_2PO_4^-$ concentrations (n = 5). During an ischemic rhythmic handgrip paradigm (20% MVC), a phase effect was not observed for MSNA or H^+ or $H_2PO_4^-$ concentrations, suggesting that blood flow was necessary for the expression of the cycle-related effect. The present studies suggest that, during static handgrip exercise, MSNA is increased during the menstrual compared with the follicular phase of the ovarian cycle.

sympathetic nerve activity; estrogen

THE PHYSIOLOGICAL RESPONSES seen with static exercise consist in part of increases in heart rate (HR), blood pressure, and myocardial contractility (2, 54, 56, 57). Part of the rise in these responses is due to activation of the sympathetic nervous system (37, 39, 40, 53). Changes in the concentrations of various by-products of cellular metabolism are thought to result in stimulation of metabolite-sensitive afferent fibers (metaboreceptors) located within the interstitium of the skeletal muscle (6, 15, 23, 24, 32). During bouts of static handgrip exercise in humans, metaboreceptor activation is thought to play a primary role in evoking the exercise pressor reflex and in increasing muscle sympathetic nerve activity (MSNA) (31, 55).

Previously, we demonstrated attenuated MSNA responses in women compared with men during a static handgrip exercise paradigm performed at 30% maximal voluntary contraction (MVC) (12). This gender effect was independent of differences in muscle mass, training status, and absolute workload. In a separate group of experiments with the use of a similar exercise paradigm, NMR spectroscopy demonstrated an attenuation in the development of intracellular acidosis and $H_2PO_4^-$ in the exercising muscle of the women compared with the men (12).

Tarnopolsky et al. (49) demonstrated gender-related differences in cellular metabolism in response to prolonged submaximal exercise [65% maximal O_2 consumption ($\dot{V}O_{2max}$)]. Compared with men, women studied during the midfollicular phase preferentially oxidized lipids and demonstrated a reduced reliance on carbohydrate and protein metabolism. As women were studied during only one phase of the ovarian cycle, potential cycle-related effects on cellular metabolism were not addressed. Subsequent work by Tarnopolsky et al. (50) demonstrated increased muscle glycogen (vastus lateralis) and greater plasma lactate concentrations in men compared with women in response to a fatiguing exercise paradigm (75% VO_{2max}) performed after a carbohydrate load.

Experimental evidence suggests that the ovariancycle phase in women may influence cellular metabolism. During heavy and exhaustive exercise (66 and 90% VO_{2max}), the production of lactate in premenopausal women varied with the cycle phase such that increased estrogen concentrations were associated with attenuated lactate production (luteal vs. follicular phase) (21).

Whether cellular metabolism is influenced by the ovarian cycle and contributes to gender-related differences in the exercise pressor reflex remains to be evaluated. The purpose of the present study was to determine whether MSNA responses during static handgrip exercise varied with the cycle phase. Additionally, we sought to determine the effects of the cycle phase on blood pressure responses, as prior studies have demonstrated that estrogen increases, decreases, or does not influence this parameter (8, 47, 61). We examined healthy premenopausal women during two stages of their ovarian cycle: days 1-4 (the menstrual phase) and days 10-12 (the follicular phase). These times were selected in an effort to study the effects of high (days 10-12) and low (days 1-4) concentrations of estrogen and to minimize potential confounding effects of progesterone, which has been shown to influence cellular metabolism (20). Changes in progesterone concentra-

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tions during *days* 1-12 are minimal, whereas estrogen concentrations gradually increase as ovulation nears (59). Additionally, a separate group of experiments was performed with the use of an ischemic rhythmic handgrip exercise in an effort to examine effects of blood flow on sympathetic neural outflow as related to the ovarian cycle.

The results of our experiments demonstrate that MSNA varies with the ovarian-cycle phase, as sympathetic neural outflow was greater during the menstrual (low estrogen) compared with follicular (high estrogen) stage. This finding was not observed during the ischemic handgrip exercise paradigm, suggesting that blood flow was necessary for the manifestation of the cycle-related effect. A cycle-related effect on blood pressure responses was not observed. Finally, NMR studies demonstrated no difference in intracellular metabolism between the two phases, suggesting that factors aside from cellular by-product concentrations contribute to the effects of the cycle phase on MSNA.

METHODS

Subjects

All studies were approved by the Institutional Review Board of The Milton S. Hershey Medical Center, and all subjects signed informed consent. We studied 10 women (age 24.1 \pm 1.7 yr) during two phases of their ovarian cycle: *days* 1-4 (menstrual) and *days* 10-12 (follicular). We were able to obtain hemodynamic measurements and microneurographic recordings in eight women during both phases of the ovarian cycle. In two women, we were able to obtain microneurographic recordings during only one phase of the cycle, but we included their hemodynamic measurements during both phases [mean arterial blood pressure (MAP) and HR] for statistical analysis. All subjects were in good health, were not taking medications, and had regular menstrual cycles (cycle length \sim 28 days). Additionally, none of the subjects was on hormone-replacement therapy within the preceding 6 mo. The order of the cycle phase that was studied varied (menstrual vs. follicular).

Exercise Protocols

Subjects were placed in the supine position, and MVC was obtained by a brief (<5-s) contraction by using a handgrip dynamometer. Nonischemic static handgrip exercise was performed at 30% MVC for 2 min (nondominant arm) followed by a 2-min period of posthandgrip circulatory arrest (PHG-CA). By the removal of the effects of central command, this maneuver serves to isolate the metaboreceptor contribution to the MSNA response (31). During the exercise paradigm, we measured HR (electrocardiogram), MAP (Dinamap, Critikon, FL), and MSNA (microneurography). Respiratory movements (pneumograph) were monitored, and subjects were instructed to avoid Valsalva and breath-holding maneuvers.

The subjects also performed an ischemic rhythmic handgrip exercise previously used by our laboratory (12). After 5 min of rest, the nondominant forearm was made ischemic for 6 min by inflation to suprasystolic pressure (250 mmHg) of a circulatory occlusion cuff placed around the arm. Individuals then began a rhythmic handgrip exercise at 20% MVC until fatigue (30 contractions/min). This was followed by an additional 1-min period of circulatory arrest. This paradigm enabled us to examine MSNA responses in the absence of potential cycle-related flow effects.

At the completion of the exercise protocols, we performed a cold-pressor test to assess whether the two phases of the ovarian cycle were associated with different levels of sympathoexcitation. For these studies, MSNA, MAP, and HR were continuously recorded. After 3 min of baseline, ice was applied to the subject's forehead for 90 s. The 20-s period with the greatest nerve activity was normalized for 1 min, and the increase in MSNA was compared with baseline values.

Microneurography

This technique served as our primary index of sympathoexcitation. The details of this technique have been described previously by Vallbo et al. (54). Briefly, a tungsten electrode (5-µm tip) was inserted percutaneously into the peroneal nerve below the fibular head. A reference electrode was placed a few centimeters away in the subcutaneous tissue. The recording signal was amplified ($50,000-100,000\times$), filtered (700-2,000 Hz), and integrated to obtain a mean voltage neurogram. Bursts were scored and counted by hand and are expressed as bursts per minute. Total amplitude was obtained for each minute by measuring the height of all bursts within that time period and are expressed as arbitrary units (AU; in mm).

³¹P-NMR Spectroscopy

The use of ³¹P-NMR spectroscopy to examine muscle metabolism during exercise has also been described previously by our laboratory (12, 42). The NMR spectrometer is a 1.9-T, 26-cm bore Oxford Instruments (Abbington, UK) superconducting magnet interfaced to a Nicolet (Madison, WI) radio-frequency transmitter-receiver. The 2.5-cm-diameter coil is placed over the flexor digitorum superficialis muscle in the forearm. The spectra are collected at 32.5 MHz (resonance frequency for phosphorous nuclei) and represent the Fourier transformation of 32 transients averaged over 60 s.

Relative concentrations of P_i and phosphocreatine were calculated from the relative areas under the curve of their respective resonance. The value of P_i at rest was expressed as 1 AU. The relative concentration of $H_2PO_4^-$ ($[H_2PO_4^-]$) was determined from the cell pH (see below), the P_i concentration, and the acidic dissociation constant for the conversion of $HPO_4^{2^-}$ to $H_2PO_4^-$ by using the following equation: $[1/(1 + 10^{pH} - 6.75)]$ relative P_i (60). Muscle cell pH was derived from the chemical shift of P_i relative to the fixed-peak position of phosphocreatine (33).

Hormone Measurements

Venous blood samples were obtained via an intravenous catheter inserted in an antecubital vein placed in the dominant nonexercising arm. Blood samples were obtained after the subject completed the exercise paradigms and had rested for several minutes. Samples were analyzed for estradiol, estrone, and progesterone during the two phases of the ovarian cycle. Estradiol and progesterone were measured by a rapid-method, no-extraction ¹²⁵I radioimmunoassay (Coat-A-Count, Diagnostic Products, Los Angeles, CA). Estrone was measured by a radioimmunoassay ([³H]estrone) after extraction with ether from activated charcoal (1).

Statistical Analysis

Descriptive data are presented as means \pm SE. MSNA data are presented in terms of changes (Δ) from baseline as measured by both total amplitude (Δ MSNA; units = AU/min)

and burst count (Δ burst; units = bursts/min). Two methods were used to analyze these data: a two-way analysis of variance for comparing the various hemodynamic and NMR indexes and a repeated-measures analysis of variance to compare the two phases of the ovarian cycle.

The longitudinal design of the ovarian-cycle data consisted of two factors repeated for each subject: phase and exercise paradigm. The Satterthwaite approximation was utilized to determine the appropriate degrees of freedom for testing the main effects of phase and exercise paradigm, as well as predetermined simple-effects comparisons, in the repeatedmeasures models. Because it was determined to test all simple effects a priori and because the simple effects are all orthogonal contrasts, no adjustment for multiple testing was done. All main effects, as well as simple effects, were considered statistically significant if the *P* value was ≤ 0.05 . The data were analyzed by using the MIXED procedure from the SAS statistical software (SAS Institute, Cary, NC).

RESULTS

Handgrip Protocols

The age, MVC, and weight for all subjects performing the 30% MVC handgrip protocol are shown in Table 1. MVC was similar during the two phases of the ovarian cycle (29.3 \pm 1.3 vs. 28.2 \pm 1.5 kg, *days* 1–4 vs. *days* 10–12; P = 0.13). As mentioned previously, adequate microneurographic recordings were achieved in eight women during both phases of their ovarian cycle, and in two women adequate recordings could only be obtained during one cycle phase. Because we sought to study the effects of cycle phase on MAP responses in addition to sympathetic neural outflow, data from these two subjects were included in the statistical analysis of the hemodynamic parameters.

30% MVC static protocol. Baseline total amplitude and burst counts were not statistically different during the two cycle phases (*days 1–4* vs. *days 10–12*, MSNA: 86 ± 15 vs. 123 ± 30 AU/min, cycle effect P = 0.13; bursts: 13 ± 2 vs. 18 ± 4 bursts/min, cycle effect P =0.13). Baseline MAP (n = 10) was similar between the menstrual and follicular phases (85 \pm 2 vs. 84 \pm 2 mmHg, *days* 1-4 vs. *days* 10-12, cycle effect P = 0.48). Baseline HR measurements (n = 10) were similar between the menstrual and follicular phases (68 ± 3 vs. 67 ± 2 beats/min, *days* 1–4 vs. *days* 10–12, cycle effect P = 0.36). Δ MSNA, Δ MAP, and Δ HR responses in the women during the two phases of the ovarian cycle are shown in Fig. 1. Δ MSNA responses (*n* = 8) were greater in the women during the menstrual phase (days 1-4) compared with the follicular phase (days 10-12). This was observed whether we used Δ amplitude or Δ bursts for analysis (cycle effect P = 0.019 and 0.017, respectively). Throughout the exercise paradigm, changes in blood pressure (Δ MAP) were similar between cycle phases with the maximal increase being observed during the second minute of handgrip for both phases $(14.6 \pm 2.2 \text{ vs.} 12.2 \pm 1.9 \text{ mmHg}, days 1-4 \text{ vs.} days$ 10-12; P = 0.24). HR responses increased throughout the handgrip exercise and approached baseline values during the period of PHG-CA.

Ischemic rhythmic 20% MVC protocol. We observed no significant difference in baseline MSNA, MAP, or HR

Table 1. Anthropomorphic data

		Weight	MV	C, kg
Subject No.	Age, yr	lb.	Days 1-4	Days 10-12
1	25	129	30	27
2	20	150	32	33
3	23	160	29	31
4	27	115	30	25
5	27	130	35	34
6	21	110	22	20
7	26	215	35	35
8	20	132	28	27
9	21	120	27	26
10	31	160	25	24
$Means \pm SE$	24.1 ± 1.7	142.1 ± 9.8	$\textbf{29.3} \pm \textbf{1.3}$	$\textbf{28.2} \pm \textbf{1.5}$

MVC, maximal voluntary contraction during menstrual phase (*days* 1–4) and follicular phase (*days* 10–12).

(*n* = 5) between *days* 1–4 and *days* 10–12 (MSNA: 95 ± 20 vs. 179 ± 47 AU/min, cycle effect P = 0.08; bursts: 12 ± 2 vs. 22 ± 6 bursts/min, cycle effect P = 0.10; MAP: 84 ± 2 vs. 82 ± 2 mmHg, cycle effect P = 0.49; HR: 65 ± 2 vs. 66 ± 1 beats/min, cycle effect P = 0.48). No cycle-related effect was observed for Δ MSNA (Δ amplitude or Δ bursts), Δ MAP, or Δ HR responses (Fig. 2).

There was no difference between cycle phases in the time to fatigue (ischemic endurance) (133 \pm 15 vs. 140 \pm 17 s, *days* 1–4 vs. *days* 10–12; P = 0.3).

NMR Spectroscopy Experiments

For the 30% MVC static protocol and the 20% MVC rhythmic protocol, baseline values for pH, $H_2PO_4^-$, HR, and MAP were similar between *days* 1–4 and *days* 10–12 (n = 5). No cycle-related effect was observed for either metabolic or hemodynamic parameters during the 30% static and 20% rhythmic exercise paradigms (Fig. 3).



Fig. 1. Effects of ovarian-cycle phase (\bigcirc , *days* 1–4; \bullet , *days* 10–12) on efferent sympathetic nerve activity and hemodynamic parameters during 30% maximal voluntary contraction static handgrip exercise paradigm. Δ MSNA, change in muscle sympathetic nerve activity from baseline (in arbitrary units, AU); Δ MAP, change in mean arterial blood pressure (in mmHg); Δ HR, change in heart rate (in beats/min); G1 and G2, static handgrip *minutes* 1 and 2, respectively; CA1 and CA2, posthandgrip circulatory arrest *minutes* 1 and 2, respectively; R, recovery (mean value during 5 min); ns, not significant; *n*, no. of women. Values are means \pm SE. *P* values show main effects of ovarian cycle on various exercise parameters. * *P* < 0.05.



Fig. 2. Effects of ovarian-cycle phase (\bigcirc , *days* 1–4; \bullet , *days* 10–12) on hemodynamic parameters during 20% maximal voluntary contraction ischemic rhythmic handgrip exercise paradigm. MSNA values are in mm/min. CAx, circulatory arrest before rhythmic handgrip exercise (mean value during 6 min); EG, end handgrip (obtained during last full minute of rhythmic handgrip); PHG-CA, posthandgrip circulatory arrest (obtained during 1 min of continued circulatory arrest after completion of exercise). Values are means ± SE; n = 5 women.

Cold-Pressor Test

There was no difference in the maximal Δ MSNA response between *days* 1–4 and *days* 10–12 during cold-pressor testing (n = 5) (171 ± 71 vs. 183 ± 33 AU; P = 0.9; Fig. 4).

Estrogen Measurements

Levels of estrogen (estradiol and estrone) were significantly lower during the menstrual compared with the follicular phase (*days 1–4* vs. *days 10–12*, estrogen: 75 ± 5.5 vs. 116 ± 9.6 pg/ml, P < 0.05; estradiol: 34 ± 2.5 vs. 57 ± 5.7 pg/ml, P < 0.05; estrone: 41 ± 4.6 vs. 60 ± 4.8 pg/ml, P < 0.05). Levels of progesterone were



Fig. 3. Effects of ovarian-cycle phase $(\bigcirc, days 1-4; \bullet, days 10-12)$ on ³¹P-NMR parameters (pH and $H_2PO_4^-$) during 30% maximal voluntary contraction static handgrip (*A*) and 20% maximal voluntary contraction ischemic rhythmic handgrip exercise paradigms (*B*). B, baseline value (mean value during 5 min before exercise); CA, posthandgrip circulatory arrest (obtained during 1 min of continued circulatory arrest after completion of exercise). Values are means \pm SE; n = 5 women.



Fig. 4. Effects of cold-pressor test on MSNA during ovarian cycle (*days 1–4*, menstrual phase vs. *days 10–12*, follicular phase). Values for Δ MSNA are in AU. Values are means \pm SE.

not different during the two phases (0.5 ± 0.1 vs. 0.8 ± 0.4 ng/ml, *days 1–4* vs. *days 10–12*; P = 0.56).

DISCUSSION

The present study demonstrates that, in healthy premenopausal women, MSNA responses to nonfatiguing static handgrip exercise are greater during the menstrual phase (days 1-4) compared with the follicular phase (*days 10–12*). Blood flow appears necessary for the full manifestation of this cycle effect, as MSNA responses did not differ during the ischemic exercise paradigm (20% MVC rhythmic handgrip). MSNA responses during cold-pressor testing were similar between the two phases, suggesting that differences in sympathetic responsiveness were not due to nonspecific generalized influences of the ovarian cycle on sympathetic activation. Finally, concentrations of H⁺ ([H⁺]) and $[H_2PO_4^-]$ as measured by ³¹P-NMR spectroscopy were not different during the exercise paradigm performed during *days* 1-4 compared with *days* 10-12. We believe these results are consistent with two potential explanations: 1) some metaboreceptor stimulant not measured by NMR spectroscopy was preferentially altered by the ovarian cycle, or 2) the ovarian cycle did not affect metabolism but rather the "washout" of muscle metabolites from the cell to the muscle interstitium.

The remainder of the discussion will focus on potential implications and explanations of our results in addition to the limitations of our findings.

Study Implications and Explanations

Previously, we observed decreased MSNA responses in a group of pre- and postmenopausal women compared with a group of age-matched men during a bout of static handgrip exercise (12). This finding was independent of differences in muscle mass, level of MVC, or training status. In a separate group of experiments that used a similar exercise paradigm, intracellular concentrations of specific metabolic markers ([H⁺] and [H₂PO₄⁻]) were greater in men compared with women. The present group of experiments extends our initial observation of a gender effect to include an ovarian cycle effect on sympathetic neural outflow during static handgrip exercise.

One potential explanation for our finding relates to a direct effect of estrogen on skeletal muscle metabolism (11, 21, 36). Biopsied vastus lateralis samples have confirmed gender-related differences in muscle fiber types and enhanced glycolytic enzyme activity in men compared with women. Conversely, women demonstrated greater enzyme potential for oxidation of fatty acids (16, 29, 41). We speculate that these established differences in skeletal muscle enzymatic activity between men and women may be further "enhanced" by the presence and effects of estrogen. In animals, biopsy samples obtained from skeletal and cardiac tissue after treadmill exercise demonstrated attenuated glycogen utilization after estrogen supplementation. Additionally, this glycogen-"sparing" effect was associated with an increase in the oxidation of fatty acids (11, 20, 26, 27).

In the present study, whereas we observed an ovarian cycle-related effect on MSNA responses during static handgrip, we did not find a similar effect on the indexes of cellular metabolism, specifically [H⁺] and $[H_2PO_4^-]$. This may in part be explained by changes in specific by-products of cellular metabolism not measured by NMR spectroscopy that have been linked to metaboreceptor activation and the exercise pressor reflex, i.e., potassium (13, 38), bradykinins (44), adenosine (4, 7), or prostaglandins (45). Alternatively, changes in hormone concentrations, aside from estrogen, that occur throughout the ovarian cycle may affect cellular metabolism or alter plasma volume and influence MSNA responses, i.e., cortisol (51), aldosterone (51), growth hormone (14, 17), ACTH (28), and insulin (22, 43). Whether cycle-related attenuations in metaboreceptor activity are linked to alterations in cellular metabolism and/or changes in hormone concentrations independent of estrogen will require further investigation.

Another potential explanation is that NMR spectroscopy serves as an index of cellular metabolism and does not accurately reflect changes in the interstitium. This is critical because the free nerve endings of metaboreceptors terminate in the skeletal muscle interstitium near vascular structures (3). On first glance one might anticipate that changes in interstitial concentrations of various substances would be reflective of changes in the respective intracellular concentrations. However, the work of Stewart (46) suggests that changes in interstitial concentrations are largely affected by the need to maintain charge neutrality. Under these circumstances, "surprising" changes in interstitial concentrations of important metabolites may be encountered. For example, recent preliminary work by MacLean et al. (30) suggests that the triceps twitch contraction evokes alkalosis during contraction with acidosis seen only during recovery. Experiments that utilize invasive techniques (i.e., microdialysis) to monitor changes in the interstitium are necessary to evaluate this concept.

If skeletal muscle metabolism is not affected by the ovarian cycle, a second possible explanation for our finding relates to potential differences in skeletal muscle blood flow. If blood flow to exercising muscle is greater during days 10-12 compared with days 1-4, then the washout of stimulating metabolites may be enhanced and metaboreceptor activation reduced. Increased washout during the follicular phase would be consistent with our observations of attenuations in MSNA responses despite similar intracellular $[H^+]$ and $[H_2PO_4^-]$. Recent studies have demonstrated a link between endotheliumdependent, flow-mediated dilatation and the ovarian cycle. After a maximal vasodilating stimulus (circulatory arrest for 5 min), changes in brachial artery diameter were observed to be greater during the follicular and luteal phases of the ovarian cycle compared with the menstrual phase (18, 25). Supplementation with estrogen has also been shown to alter the vasoconstrictor response to norepinephrine, enhance basal plasma levels of nitric oxide, and attenuate the vasoconstrictor response to angiotensin II (5, 47, 48). Further investigation will be necessary to study whether this modulation in vascular tone and peripheral blood flow significantly impacts interstitial metabolite concentrations and whether this affects metaboreceptor activation.

We speculate that the mechanistic effects of estrogen in altering sympathetic neural outflow are multifactorial and that changes in cellular metabolism, as well as modifications in skeletal muscle blood flow, are critical components. Whereas the present group of experiments studied the influences of estrogen in women, the effects of this hormone may be gender independent. In a recent study, genetic men undergoing sex hormone manipulation (long-term, high-dose estrogen therapy) were observed to have greater increases in brachial artery diameter during reactive hyperemia compared with a group of age-matched controls. The reactive hyperemic blood flow responses of the men receiving estrogen therapy were similar to those of a group of age-matched women (34).

A third explanation for our findings is that changes in estrogen directly affect the central neural response to exercise. Prior studies have demonstrated greater attenuations in plasma norepinephrine levels after the administration of a centrally acting α -agonist in premenopausal women compared with age-matched men (10). Attenuations in plasma norepinephrine and epinephrine concentrations after the administration of oral estrogen have also been observed in men in response to a mental stress test (9). Whether a similar phenomenon is seen with exercise will need to be investigated.

The findings in the present study are consistent with several earlier works that evaluated changes in muscle strength, hemodynamic parameters, and response to cold-pressor testing in women during different phases of their ovarian cycle. Petrofsky et al. (35) demonstrated that, during bouts of isometric exercise, strength, HR, and blood pressure responses were not affected by the ovarian-cycle phase. Hastrup and Light (19) demonstrated no cycle-related effect on cardiovascular reactivity in response to cold-pressor testing. These findings are consistent with results reported in the present study.

Study Limitations

The study was designed to evaluate the effects of the ovarian cycle on sympathetic nerve activity, not the effects of the hormone estrogen. Although the women were studied during two phases that are associated with significant changes in estrogen concentrations, we cannot conclude that the cycle effect was estrogen dependent. Given the diverse physiological changes that occur throughout the ovarian cycle, other mechanisms not studied could have contributed to our findings. Significant changes in plasma concentrations of cortisol and aldosterone have been demonstrated to occur throughout the ovarian cycle (51). To what effect these substances influence sympathetic nerve activity, either directly or indirectly by changes in volume status or vascular tone, will require further investigation.

Skeletal muscle forearm blood flow was not measured during the exercise paradigm. Although prior works have demonstrated a link between ovarian cycle and reactive hyperemic blood flow, to our knowledge the effects of cycle phase on forearm blood flow during static handgrip exercise have not been reported. In an important study by Sudhir et al. (47), forearm blood flow responses and total body norepinephrine spillover were attenuated in a group of perimenopausal women after estrogen supplementation. One potential explanation offered by the investigators relates to the regulation of skeletal muscle nitric oxide synthases (58) by estrogen and the hormone's effects on endothelial nitric oxide production and release (48). Whether the particular phases of the ovarian cycle in the present study are associated with differences in skeletal muscle blood flow will require further investigation.

Finally, we observed a dissociation between MSNA responses and MAP during the nonischemic static handgrip exercise. This finding has been observed in prior studies (12). This dissociation may be related to the concept that MSNA responses are predominantly reflective of events within the exercising muscle, whereas changes in blood pressure are due to myriad influences including changes in HR, stroke volume, and blood-vessel function.

In conclusion, the present study demonstrates that MSNA responses during static handgrip exercise are greater during the menstrual phase (*days* 1-4) compared with the follicular phase (*days* 10-12) in premenopausal women. This cycle-related effect is independent of changes in progesterone, intracellular [H⁺] and [H₂PO₄], and absolute workload.

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