Menstrual cycle and sex affect hemodynamic responses to combined orthostatic and heat stress


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Menstrual cycle and sex affect hemodynamic responses to combined orthostatic and heat stress. Am J Physiol Heart Circ Physiol 289: H631–H642, 2005. First published March 18, 2005; doi:10.1152/ajpheart.00029.2005.—Women have decreased orthostatic tolerance compared with men, and anecdotal evidence suggests women are more susceptible to orthostatic intolerance in warm environments. Because estrogen and progesterone affect numerous physiological variables that may alter orthostatic tolerance, the purpose of our study was to compare orthostatic tolerance across the menstrual cycle phases in women during combined orthostatic and heat stress and to compare these data with those of men. Eight normally menstruating women and eight males (22 ± 4.0 and 23 ± 3.5 yr, respectively) completed the protocol. Women were studied during their early follicular (EF), ovulatory (OV), and midluteal (ML) phases. Men were studied twice within 2–4 wk. Heart rate, cardiac output, blood pressure, core temperature (Tc), and cutaneous vascular conductance (CVC) were measured during three head-up tilt tests, consisting of two tilts in the thermoneutral condition and one tilt after a 0.5°C rise in Tc. There was no difference in orthostatic tolerance across the menstrual cycle phases, despite higher CVC in the ML phase after heating (EF, 42.3 ± 4.8; OV, 40.1 ± 3.7; ML, 57.5 ± 4.5; P < 0.05). Orthostatic tolerance in the heat was greater in men than women (P < 0.05). These data suggest that although many physiological variables associated with blood pressure regulation fluctuate during the menstrual cycle, orthostatic tolerance in the heat remains unchanged. Additionally, our data support a clear sex difference in orthostatic and heat tolerance in women and to compare these data with those of men. Eight normally menstruating women and eight males (22 ± 4.0 and 23 ± 3.5 yr, respectively) completed the protocol. All subjects were normally active (exercise 1 h), nonsmokers, and not currently taking medications. All subjects refrained from consuming alcoholic beverages within 24 h of participating in the study. Studies began at the same time of day for each individual subject to avoid complications of circadian variation (34, 35). All studies started between the hours of 6 and 8 AM. Women took a pregnancy test and showed negative results before the beginning of the protocol on each study day. Approval of this investigation was granted by the institutional review board of the University of Oregon.

METHODS

Eight women and eight men between the ages of 19–32 yr completed the protocol. All subjects were normally active (exercise ~1–3 days/wk for ≤1 h), nonsmokers, and not currently taking medications. All subjects kept a log of food and liquid consumption the day before the first study day and followed the same regimen before reporting to the laboratory on subsequent study days. Additionally, all subjects refrained from consuming alcoholic beverages within 24 h of participating in the study. Studies began at the same time of day for each individual subject to avoid complications of circadian variation (34, 35). All studies started between the hours of 6 and 8 AM. Women took a pregnancy test and showed negative results before the beginning of the protocol on each study day. Approval of this investigation was granted by the institutional review board of the University of Oregon.

Numerous investigators (3, 7, 12, 27, 32, 40) have found that women have lower orthostatic tolerance than men, and anecdotal evidence (1) suggests that otherwise healthy women more commonly faint when exposed to quiescent standing in warm environments. Orthostatic stress tends to be exacerbated in the heat (21) due to the large redistribution of central blood volume to the cutaneous vascular bed for thermoregulation. This peripheral blood volume shift may predispose women to orthostatic intolerance at greater rates than observed in men. However, this has not been investigated. Fluctuations of the female sex hormones estrogen and progesterone accompanying the menstrual cycle have been shown to have numerous effects on physiological variables associated with both orthostatic tolerance and temperature regulation in women. Specifically, hormonal changes during the menstrual cycle have been shown to impact such factors as sympathetic baroreflex sensitivity (25, 31), circulating catecholamines concentrations (2, 11, 16, 25), sensitivity of sympathetic adrenergic receptors (6), plasma volume (28, 33, 39), resting core body temperature (13, 14), and skin blood flow (14, 15). Independently, a number of the reported changes in cardiovascular regulation during the menstrual cycle could contribute to changes in orthostatic tolerance across the menstrual cycle, as well as to increased rates of orthostatic intolerance in women. However, no study has definitively determined whether orthostatic tolerance changes during the menstrual cycle. Part of the problem is that no study has brought women to the point of syncope, or to the onset of presyncopal symptoms, during various phases of the menstrual cycle within individual subjects. Furthermore, no study has tested orthostatic tolerance in young, healthy women in hot environmental conditions. We sought to bring women to the point of presyncopal symptoms during three specific phases of the menstrual cycle to investigate potential menstrual cycle related changes in orthostatic tolerance. We exploited the fact that orthostatic tolerance is less in the heat to bring a greater number of subjects to the point of presyncope.

We investigated the responses to progressive head-up tilting in both thermoneutral and passively heated conditions during the early follicular (EF), ovulatory (OV), and midluteal (ML) phases of the menstrual cycle. Additionally, we studied a group of men to identify potential sex differences. We hypothesized that orthostatic tolerance would fluctuate throughout the menstrual cycle in women, with the lowest rates occurring during the ML menstrual cycle phase. Additionally, we hypothesized that women would have decreased orthostatic tolerance in the heat compared with men.

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Oregon. The protocol was explained to each subject, and verbal as well as written consent was given by each subject before participation. All women were normally menstruating (6 never took oral contraceptives, 1 used no type of oral contraceptive for 2 yr, and 1 used no type of oral contraceptive for 4 mo) with regular menstrual cycles lasting 28 ± 3 days in duration. All women completed the protocol during their EF, OV, and ML menstrual cycle phases. With the first day of menstruation defined as day 1 of the cycle, the menstrual cycle phases studied were characterized as follows: EF (days 1–3), OV (between 24 and 48 h of a luteinizing hormone surge), and ML (8–10 days postovulation). The order of experimental study days was counterbalanced for the normally menstruating women. Three women were studied first during their EF phase, three women during their OV phase, and two women during their ML phase. The surge in luteinizing hormone before ovulation was detected via ovulation prediction kits (Clearblue Easy Ovulation Test Pack, Unipath Diagnostics, Waltham, MA). All menstrual cycle phases were verified by measuring circulating concentrations of estradiol and progesterone on each study day. All men completed the protocol twice within 2 to 4 wk to mimic the general time between study days during which women participated.

Protocol

The experimental protocol consisted of one preliminary screening day followed by three study days for women and two study days for men. On screening days subjects reported to the laboratory to review their health history with the investigators, to become familiar with the laboratory and laboratory equipment, and, if female, to map out their past menstrual cycles with the investigators to approximate potential study dates. Subjects were given two or three ingestible pills (CoreTemp2000, HQ, Palmetto, FL) for measuring core body temperature (Tc) and were instructed to ingest one thermister on the night before each study day.

On arriving at the laboratory on the study days, subjects’ height and weight measurements were taken in minimal clothing before the preliminary tilt test (SECA 700 column scale, SECA, Hamburg, Germany). The men wore only shorts, and women wore shorts and sports bras. The experimental protocol consisted of three tilt tests: a preliminary tilt, a thermoneutral tilt, and a heated tilt. This three-tilt protocol was repeated on each subsequent study day.

Preliminary tilt. The preliminary tilt was performed to collect cardiac output (Q) data on all groups in the thermoneutral condition. This tilt consisted of fewer stages of progressive tilt compared with the other tilts, (see Thermoneural and heated tilts) because we did not want any of the subjects to become presyncopal during this preliminary tilt. Subjects were positioned supine on an automatic tilt table (model CM6121, Colin Medical Instruments, San Antonio, TX) with their feet flush with the foot plate. Subjects’ arms were positioned outstretched to their sides at heart level and secured in the tilt table armrests. Subjects were instrumented for measurement of Tc, skin temperature (Tsk), skin blood flow (SkBF), HR, beat-to-beat arterial blood pressure, and arm blood pressure via automated brachial auscultation. Thermoneural water (~34°C) was pumped through the suit to maintain initial Tc. After instrumentation, 30-min rest, and 5 min of supine baseline measurements, subjects underwent the thermoneural tilt test, which consisted of progressive 5-min stages at 15°, 30°, 45°, and 60° head-up tilt. All subjects were then returned to the supine position and given a 30-min recovery period. The water circulating through the water-perfused suit was then changed from thermoneural (~34°C) to hot (~50°C), and subjects were draped with a plastic suit to reduce heat loss via evaporation. After a 0.5°C rise in Tc, the plastic was removed from all subjects, and the water temperature was reduced from 50° to 46°C to minimize additional increases in Tc during the heated tilt test. The heated tilt test was identical to the thermoneutral tilt with the exception of an additional 10 min at 60° head-up tilt if the subject was able to complete the first 5 min at 60°. Arm blood pressure was measured via automated brachial auscultation at minute 3 of each tilt stage, while HR, SkBF, Tc, Tsk, and beat-to-beat arterial blood pressure were measured continuously throughout the two tilt tests and during whole body heating. All subjects were returned to the supine position and immediately cooled by pumping thermoneral water (~34°C) through the water-perfused suit after subjects presented physiological signs of presyncope or on their request due to symptoms such as light-headedness, dizziness, tunnel vision, or nausea. Physiological signs of presyncope were defined as a drop in systolic blood pressure (~15 mmHg) or a sudden drop in HR (~15 beats/min). Once in the supine position, all subjects were given a 10-min recovery period before the temperature of a local heater placed on the forearm was raised to achieve maximum cutaneous dilatation at the site in which SkBF was measured. Throughout all studies, the laboratory temperature was maintained between 22° and 24°C.

Measurement Techniques

HR and blood pressure. HR was determined by ECG (Cardiocap, Datex Ohmeda, Louisville, CO). Beat-to-beat arterial blood pressure was measured continuously with a finger blood pressure cuff (Portapres model-2, TNO-TPD, Biomedical Instrumentation, Amsterdam, The Netherlands) placed on the middle or ring finger of the left hand. Blood pressures from the finger blood pressure cuff were corrected against arm blood pressure measured noninvasively from the left arm via automated brachial auscultation (Cardiocap, Datex-Ohmeda). Pulse pressure (PP) was calculated as systolic blood pressure minus diastolic blood pressure (PP = systolic blood pressure – diastolic blood pressure). Mean arterial pressure (MAP) was calculated as diastolic pressure plus one-third of the PP [MAP = diastolic blood pressure + 1/3 (systolic blood pressure – diastolic blood pressure)].

Qc and stroke volume. Qc was estimated by using the noninvasive open-circuit acetylene washin method (10, 17, 36). Subjects breathed a mixture of 0.6% acetylene, 9.0% helium, 20.9% oxygen, and balance nitrogen for 8–10 breaths via a two-way non-rebreathing valve. Breath-by-breath acetylene and helium uptake were measured by a pneumotach (model 3700, Hans Rudolph, Kansas City, MO) and a mass spectrometer (MGA 1100, Marquette, MA Tech Services, St. Louis, MO) interfaced with a custom data acquisition system. Stroke volume (SV) was calculated by dividing Qc by HR (SV = Qc/HR).

Total systemic peripheral resistance (TPR) was calculated as MAP divided by Qc (TPR = MAP/Qc).
SkBF. Red blood cell (RBC) flux was measured via Laser-Doppler flowmetry (DRT-4, Moor Instruments, Devon, UK) on the ventral forearm to gain an index of SkBF. RBC flux was divided by MAP to produce an index of cutaneous vascular conductance (CVC = RBC flux/MAP). CVC values are reported as a percentage of maximum CVC, which was achieved by locally heating each site to 43°C until a plateau was reached for a minimum of 10 min. This temperature has been previously shown to elicit maximal dilatation (19, 24, 38).

$T_s$, and mean $T_{sk}$. $T_s$ was measured via a wireless core body temperature monitoring system (CorTemp2000, HQ). All subjects swallowed a disposable temperature sensor 8–10 h before the protocol. An unweighted average of five copper-constantan thermocouples, placed on the skin of the chest, abdomen, back, thigh, and calf, were used to measure mean $T_{sk}$. We did not use a weighted average to calculate $T_{sk}$ because the thermocouples were placed under a water-perfused suit. Mean body temperature ($T_b$) was calculated as $(0.8 - T_s) + (0.2 - T_{sk})$.

Hormone and catecholamine profiles. Venous blood samples were collected from the antecubital vein from all subjects on each study day. Samples from men were collected for measurement of circulating levels of estradiol and progesterone to verify menstrual cycle phase and circulating catecholamine concentrations. Venous blood samples collected from men were used to measure the concentrations of testosterone, dihydrotestosterone, and catecholamines. All samples were collected after 30 min quiet rest in the supine position. Estradiol, progesterone, testosterone, and dihydrotestosterone samples were collected in 8.5-ml plastic collection tubes (BD Vacutainer SST Gel & Clot Activator, Becton Dickinson, Franklin Lakes, NJ), and catecholamine samples were collected in 10-ml glass collection tubes (BD Vacutainer heparin sodium, Becton Dickinson). Samples were separated via centrifuge at 1,300 g for 12 min at 4°C. Samples were stored frozen at −70°C until transported to Oregon Medical Laboratories (Eugene, OR) for serum analysis of estradiol, progesterone, testosterone, and dihydrotestosterone (all measured via radioimmunoassay) and plasma analysis of catecholamines (measured via high-performance liquid chromatography).

Orthostatic tolerance. Orthostatic tolerance was estimated during the heated tilts by using a cumulative stress index (CSI). The CSI allowed us to quantitatively compare the orthostatic tolerance between visits in subjects as well as across subject groups. The CSI was calculated by summing the products of the sine of each tilt angle and the duration of time completed at that tilt stage. A higher CSI indicates a greater orthostatic tolerance, with the maximum score equaling 20.4% gravitational stress times minutes for subjects who completed the full heated tilt test.

Data Analysis

The ECG, beat-to-beat arterial blood pressure, RBC flux, $T_s$, and $T_{sk}$ signals were all digitized and stored on a computer at 250 Hz for later analysis with signal processing software (Windaq, DataQ Instruments, Akron, OH). These data were averaged over the last 2 min of each completed tilt stage throughout the progressive preliminary, thermoneutral, and heated tilt tests. In addition, data were also averaged in 30-s intervals over the 2 min before presyncope in all women who failed to complete the full heated tilt tests. The intervals are as follows: 120–90 s before presyncope (−90), 90–60 s before presyncope (−60), 60–30 s before presyncope (−30), and 30 s before point of presyncope (0). This heat-to-presyncope comparison was performed on seven of the eight women because one woman completed the full heated tilt on two out of three study days and was therefore omitted from this comparison. Additionally, men were not included in this comparison because the majority of men completed the full heated tilt. All subjects completed the thermoneutral tilt test with the exception of one man who showed signs of presyncope and was returned to the supine position 1 min before completion of the full 60° head-up tilt. Data from the 60° thermoneutral head-up tilt in this subject were averaged over the stable period of minute 4 before the onset of presyncopal signs and symptoms. During the heated tilt test, the majority of women were unable to complete the heated tilt stages past 30° head-up tilt. Thus because of the large percentage of drop-out data, we were only able to analyze the heated tilt test at baseline, and 15° and 30° head-up tilt stages. Only one woman was unable to complete the full 30° head-up tilt. As she completed 4 min at 30°, her data at 30° head-up tilt were averaged over the stable section of minute 4.

Statistics

One-way repeated measures ANOVAs were used to compare subject characteristics and orthostatic tolerance between visit 1 and visit 2 in men. Men’s data from the preliminary tilt test, thermoneutral tilt test, thermoneutral to heat period, and heated tilt test were compared using two-way repeated measures ANOVAs (visit × tilt stage) to determine differences in response to tilt between visits.

Subject characteristics. Subject characteristics were analyzed across menstrual cycle phases in women using one-way repeated measures ANOVAs and between sexes using one-way ANOVAs.

Tilting. Two-way repeated measures ANOVAs (menstrual cycle phase × tilt stage) were performed on all variables within each tilt test (i.e., preliminary, thermoneutral, and heated) to distinguish differences across menstrual cycle phases in women in response to the preliminary tilt test, thermoneutral tilt test, and heated tilt test. Two-way ANOVAs (sex × tilt stage) were used to test for sex differences in response to tilting at the same time periods as the women.

Heating and presyncope. The supine thermoneutral to supine heat time period was compared across the menstrual cycle using two-way repeated measures ANOVAs (menstrual cycle phase × time) and between sexes using two-way ANOVAs (sex × time). Two-way repeated measures ANOVAs (menstrual cycle phase × time) were also used to compare the heat to presyncope time period across the menstrual cycle in women.

Orthostatic tolerance. Orthostatic tolerance was compared across menstrual cycle phases in women and between sexes during the heated tilt test using one-way repeated measures ANOVAs. When significance was achieved using ANOVAs, the Holm-Sidak post hoc test was performed to locate the differences. The level of significance was set at $P < 0.05$. Subject characteristics are presented as means ± SD. All additional data are presented as means ± SE.

RESULTS

There were no significant differences in subject characteristics or responses to the preliminary tilt, thermoneutral tilt, thermoneutral to heat period, and heated tilt tests between visit 1 and visit 2 in men for any of the measured variables. Therefore, we averaged these data to compare against the EF menstrual cycle phase in women to test for sex differences.

Subject Characteristics

Table 1 summarizes the physical characteristics of all subject groups. There was no significant difference in age or height between the men and women, although the men tended to be taller and weigh more than the women. As predicted, estrogen and progesterone concentrations fluctuated across the menstrual cycle phases, consistent with normal values. Estradiol was significantly higher in the normally menstruating women during the OV and ML phases of the menstrual cycle compared with the EF phase ($P < 0.001$). Progesterone concentration was low during the EF and OV menstrual cycle phases and peaked during the ML phase, where it was significantly greater than both the EF and OV phases ($P < 0.001$).
Testosterone and dihydrotestosterone were consistent on visit 1 and visit 2 in men. Resting plasma norepinephrine was significantly higher during the ML phase compared with the EF phase ($P < 0.05$). However, resting plasma epinephrine did not differ across the menstrual cycle phases. There were no differences in resting plasma norepinephrine and epinephrine concentrations between visit 1 and visit 2 in men ($P > 0.05$) or between sexes ($P > 0.05$).

**Preliminary Tilt**

The cardiovascular and hemodynamic responses to the preliminary tilt test are illustrated in Fig. 1. From baseline, all groups showed a progressive decrease in $SV (P < 0.001)$ and increase in $HR (P < 0.001)$ with increasing tilt stage. However, the increase in $HR$ did not prevent a progressive decrease in $Q_c (P < 0.001)$. $TPR$ increased progressively with increasing tilt stage ($P < 0.001$) in all groups, whereas PP decreased ($P = 0.003$), and MAP remained constant.

There were no main menstrual cycle phase differences or significant interactions of any of the measured variables and menstrual cycle phases in women, because women responded similarly to the preliminary tilt regardless of menstrual cycle phase. There was a main effect of sex on $SV (P < 0.001)$, $HR (P = 0.013)$, $Q_c (P < 0.001)$, $TPR (P < 0.001)$, and $PP (P < 0.001)$, such that $SV$, $Q_c$, and $PP$ were higher in men than women in the supine position and remained higher throughout the preliminary tilt test. Furthermore, $HR$ and $TPR$ were lower in men compared with women in the supine position and during all preliminary tilt stages. Despite numerous main sex effects, no interaction effects were observed. Thus the changes in each variable across the preliminary tilt were similar between sexes.

**Thermoneutral Tilt**

Figure 2 displays the cardiovascular, hemodynamic, and temperature data for all groups in response to the thermoneutral tilt test. In women, there was a main effect of tilt stage on $T_c (P < 0.001)$, $T_b (P < 0.001)$, $HR (P < 0.001)$, $CVC (P = 0.003)$, and $PP (P < 0.05)$. $T_c$, $T_b$, and $HR$ progressively increased, whereas $CVC$ and $PP$ decreased with increasing tilt stage. Men had similar tilt stage responses, because there was a main tilt stage effect of increased $T_c$, $T_b$, $HR$ (all variables: $P < 0.001$), and $PP (P < 0.05)$ with increasing tilt stage. $MAP$ remained constant throughout the thermoneutral tilt in all groups.

Consistent with the preliminary tilt, there was no main effect of menstrual cycle phase on any of the measured variables, and no interactions were observed. Initial supine $T_c$ and $T_b$ were not significantly different across the menstrual cycle but showed expected trends as $T_c$ and $T_b$ were lower during the OV phase and higher during the ML phase compared with the EF phase.

We identified a main effect of sex on $T_c (P < 0.001)$, $T_b (P < 0.002)$, $HR (P < 0.001)$, $MAP (P < 0.001)$, and $PP (P < 0.001)$ during the thermoneutral tilt. $T_c$, $T_b$, and $HR$ were significantly lower in men in the supine position and remained lower throughout the tilt compared with women. In contrast, $MAP$ and $PP$ were higher in men compared with women in the supine position throughout the thermoneutral tilt. There were no main effects of sex on $CVC$ because both groups showed similar baseline values and gradual decreases in $CVC$ throughout the thermoneutral tilt. No significant interactions were observed between sexes in response to the thermoneutral tilt.

**Passive Heating and Heated Tilt**

Figure 3 illustrates the cardiovascular, hemodynamic, and temperature data of all groups from thermoneutral to heat in the supine position through 30° head-up tilt in the heat. As predicted, there were main effects of heating on $T_c$, $T_b$, $HR$, $CVC$ (all variables: $P < 0.001$), and $PP (P < 0.004)$ in all groups, as all variables increased significantly during heating. Moreover, there were main effects of tilting in the heat on $T_c$, $T_b$, $HR$, $CVC$ (all variables: $P < 0.001$), and $PP (P < 0.05)$. $T_c$, $T_b$, and $HR$ continued to increase from the supine heated position with increasing tilt stage, whereas $CVC$ and $PP$ progressively decreased in all groups. We observed no main heating or heated tiling effects on $MAP$.

There were no main effects of menstrual cycle phase on $T_c$, $T_b$, $HR$, or $MAP$ during heating. However, there was a significant main effect of menstrual cycle phase on $CVC$ from thermoneutral to heat in the supine position ($P = 0.026$), because women in the ML phase had higher $CVC$ compared...
with women in the EF and OV menstrual cycle phases after heating. Additionally, there was a significant interaction between the menstrual cycle phase and CVC ($P = 0.023$), such that women in the ML menstrual cycle phase had a greater rise in CVC in response to a 0.5°C rise in $T_c$ than women in the EF and OV menstrual cycle phases. Upon the onset of tilting, there was a significant interaction between menstrual cycle phase and CVC ($P = 0.023$). The ML phase showed a greater drop in CVC from the heated supine position to 30° head-up tilt. There was also a main menstrual cycle phase effect on HR ($P = 0.031$) during the heated tilt test as HR was lower in the OV phase compared with EF and ML menstrual cycle phases. There were no additional main effects of menstrual cycle phase during the heated tilt test on $T_c$, $T_b$, CVC, MAP, or PP.

Fig. 1. Hemodynamic responses to progressive stages of head-up tilt during the early follicular (EF; ●), ovulatory (OV; ○), and midluteal (ML; ▽) menstrual cycle phases in women and the mean hemodynamic response from visit 1 and visit 2 in men (△) during the preliminary tilt. Values are means ± SE; bpm, beats/min; *significant main effect of tilt; †significant main effect of sex.
Furthermore, there were no interactions between menstrual cycle phase and \( T_c \), \( T_b \), HR, MAP, or PP during the heated tilt test.

There was a main effect of sex on \( T_c \) (\( P = 0.020 \)), HR (\( P = 0.004 \)), MAP (\( P = 0.023 \)), and PP (\( P < 0.001 \)) during heating. \( T_c \) and HR were lower in men compared with women, whereas MAP was higher. No interactions were observed between sexes for any of the measured variables during heating, showing that both sexes responded similarly to a 0.5°C rise in \( T_c \). During the heated tilt test, there was a main sex effect on HR.

Fig. 2. Hemodynamic responses to progressive stages of head-up tilt during the EF (●), OV (○), and ML (▼) menstrual cycle phases in women and the mean hemodynamic response from visit 1 and visit 2 in men (△) during the thermoneutral tilt test. Values are means ± SE; %max, percentage of maximum. *Significant main effect of tilt; †significant main effect of sex.
Fig. 3. Hemodynamic responses to a 0.5°C rise in core temperature in the supine position followed by progressive stages of head-up tilt during the EF (●), OV (○), and ML (●) menstrual cycle phases in women and the mean hemodynamic response from visit 1 and visit 2 in men (●) during the heated tilt test. Data past 30° head-up tilt were excluded from analysis due to the large number of women who failed to complete the 45° head-up tilt stage before showing presyncopal symptoms. Values are means ± SE; TN, thermoneutral condition; Heat, heated condition. *Significant main effect of whole body heating and a significant main effect of tilt; tilt妇女 only, tilt affected only women; #significant interaction between the indicated variable and menstrual cycle phase; ‡significant main effect of menstrual cycle phase; †significant main effect of sex on each variable indicated. Main effects of menstrual cycle phase and sex are indicated for the whole body heating period (left) and for heated tilt (right).
(P < 0.001), MAP (P = 0.002), and PP (P = 0.013). HR was lower, whereas MAP and PP were higher in men compared with women in the heated supine position throughout all stages of heated tilt. Similar responses to heating and to the heated tilt test were observed between sexes as there were no significant interactions between sex and the measured variables.

**Heat to Presyncope**

Figure 4 shows the cardiovascular, hemodynamic, and temperature data across the menstrual cycle phases in women from the baseline supine position to presyncope in the heat. Tc, Tb, and HR increased, whereas CVC, MAP, and PP decreased significantly from the heated supine position to presyncope in all groups (P < 0.05). MAP remained reasonably steady in subjects before a rapid fall just before the subjects were returned to the supine position due to signs or symptoms of presyncope.

There was a main effect of menstrual cycle phase on HR from baseline heat to presyncope (P < 0.05). The OV phase had significantly lower HR than EF and ML menstrual cycle phases. There were no main menstrual cycle phase effects on Tc, Tb, CVC, MAP, or PP. However, there were significant interactions between the menstrual cycle phase and PP (P < 0.05) and between the menstrual cycle phase and CVC (P < 0.05) from the supine heated position to presyncope. Women in the ML phase had a greater decrease in PP from the supine heat position to presyncope compared with women in the EF and OV menstrual cycle phases. Additionally, women in their ML phase had a greater decrease in CVC during this time period, because CVC was significantly higher in the ML phase in the supine heated condition (P = 0.024) and decreased to values similar to the other menstrual cycle phases at the point of presyncope.

**Orthostatic Tolerance**

All subjects completed the thermoneutral tilt test, with the exception of one man who showed signs of presyncope and was returned to the supine position 1 min before completion. Thus because of the insufficiency of the thermoneutral tilt protocol to elicit presyncopal symptoms, orthostatic tolerance cannot be compared in the thermoneutral condition. Figure 5 shows the mean CSI, as a measure of orthostatic tolerance, from the heated tilt test for all groups. There was no difference in orthostatic tolerance across menstrual cycle phases in women in the heat. A power test with a desired power of 0.80 and significance of 0.05 determined that a minimum of 117 subjects would be needed to identify a difference in orthostatic tolerance across the phases. Therefore, we are confident that our data accurately reflect no difference in orthostatic tolerance across the menstrual cycle in the heated condition.

Orthostatic tolerance was not different between visits in men during the heated tilt test. However, there was an obvious sex difference in orthostatic tolerance in the heat. With the use of the CSI, the orthostatic tolerance of women was 52% lower than the orthostatic tolerance of men (P < 0.005). No women completed the full heated tilt test in all phases of the menstrual cycle. Only one woman completed the heated tilt test, which she completed on two of her three visits to the laboratory. In contrast, six of the eight men completed the full heated tilt test on both visits.

**DISCUSSION**

To our knowledge this is the first study to investigate hemodynamic responses in women during three separate phases of the menstrual cycle to progressive head-up tilting in a thermoneutral environment and during whole body heating. Our goal was to take women to the point of presyncope during three distinct phases of the menstrual cycle to evaluate the effects of the phases of the menstrual cycle on orthostatic tolerance. Additionally, we studied a group of men using the same protocol to identify potential sex differences in the responses to combined passive heating and orthostatic stress.

There were two main findings from the present study. First, despite differences in the SkBF response to passive heating, orthostatic tolerance in the heat did not differ across the menstrual cycle. Second, our study extends the observation of previous studies (3, 7, 12, 27, 32, 40) that found a distinct sex difference in orthostatic tolerance in the thermoneutral condition to show that there is a sex difference in orthostatic tolerance in the heat. Furthermore, our data suggest men have greater orthostatic tolerance than women (across all three phases of the menstrual cycle) during combined upright tilting and passive heating.

**Menstrual Cycle and Thermoneutral Orthostatic Stress**

Menstrual cycle phases had no effect on the preliminary and thermoneutral tilt tests. The hemodynamic responses to progressive stages of head-up tilt were strikingly similar across all menstrual cycle phases, showing similar decreases in SV and compensatory increases in HR, which were not sufficient to prevent similar decreases in Qc. All phases showed a progressive decrease in CVC and increase in TPR to maintain MAP. Furthermore, there were no differences observed in the decrease in PP across the menstrual cycle phases, which was characterized by similar decreases in systolic blood pressure and increases in diastolic blood pressure across all phases with increasing tilt stage.

Although HR is controlled by both sympathetic and parasympathetic nervous systems, HR remained less than 100 beats/min throughout the thermoneutral tilt, and thus the increase in HR observed in women during the thermoneutral tilt was primarily controlled by parasympathetic withdrawal (29, 30). There were no differences in the rise in HR in response to thermoneutral tilt across the menstrual cycle phases. This finding supports previous studies (4, 20, 25) reporting no change in cardiovagal baroreflex sensitivity across the phases of the menstrual cycle but disagrees with others (37), suggesting baroreflex sensitivity in response to a decrease in arterial blood pressure is greater during the EF phase than during the ML phase.

The similar decrease in CVC and the increase in TPR across the menstrual cycle phases during thermoneutral head-up tilt were surprising in light of numerous studies that have shown that the female reproductive hormones affect the sympathetic nervous system. Specifically, the female reproductive hormones estrogen and progesterone have been shown to influence muscle sympathetic nerve activity in response to pharmacological changes in blood pressure (25) and in response to static
Additionally, estrogen and progesterone have been shown to modify the sensitivity of sympathetic adrenergic receptors (6) and to influence plasma norepinephrine concentrations (2, 11, 16, 25). In the present study, resting norepinephrine concentrations were significantly higher in the ML compared with the EF and OV menstrual cycle phases. This finding agrees with previous studies that identified greater resting norepinephrine levels during the ML compared with the EF and OV menstrual cycle phases.

Fig. 4. Hemodynamic responses from the heated supine position to presyncope for the EF (●), OV (○), and ML (▼) menstrual cycle phases in women. Values are means ± SE. *Significant main effect of tilt; ‡significant main effect of menstrual cycle phase; #significant interaction between the indicated variable and menstrual cycle phase.
are means /H11006\phases, despite the same rise in Tc. This finding is consistent in the ML phase compared with EF and OV menstrual cycle and in response to the heated tilt. The rise in CVC was greater across the phases of the menstrual cycle during passive heating. Menstrual Cycle and Combined Orthostatic and Heat Stress regulate blood pressure in response to head-up tilt. Does not affect the ability of the sympathetic nervous system to across the menstrual cycle, it appears that the menstrual cycle there may be differences in the sympathetic nervous system effect on CVC and TPR in response to head-up tilt. Although present study, it is interesting that there appears to be no net nervous system seen at rest by previous investigators and in the light of these menstrual cycle differences in the sympathetic nitric oxide when estrogen and progesterone are present. In potential for greater concentrations of the local vasodilator ML phase to maintain vascular tone and blood pressure due to muscle sympathetic nerve activity may be greater during the ML phase to maintain vascular tone and blood pressure due to the potential for greater concentrations of the local vasodilator nitric oxide when estrogen and progesterone are present. In light of these menstrual cycle differences in the sympathetic nervous system seen at rest by previous investigators and in the present study, it is interesting that there appears to be no net effect on CVC and TPR in response to head-up tilt. Although there may be differences in the sympathetic nervous system across the menstrual cycle, it appears that the menstrual cycle does not affect the ability of the sympathetic nervous system to regulate blood pressure in response to head-up tilt.

Menstrual Cycle and Combined Orthostatic and Heat Stress

Unlike the thermoneutral tilts, differences were observed across the phases of the menstrual cycle during passive heating and in response to the heated tilt. The rise in CVC was greater in the ML phase compared with EF and OV menstrual cycle phases, despite the same rise in Tc. This finding is consistent with previous studies in which an augmented rise in SkBF was observed for a given rise in Tc during exercise (15) and passive heating (14) in the ML phase compared with the EF phase using the local heat clearance technique as an index of SkBF. It was speculated in previous studies that the sensitivity of vasodilation is increased during this phase to promote heat loss in the face of an elevated core body temperature, coinciding with elevated progesterone levels in the ML phase. Because a substantially greater increase in SkBF in the heat during the ML phase suggests a greater percentage of blood volume would be in the compliant cutaneous vascular bed, we predicted women would be more susceptible to orthostatic intolerance during this phase of the menstrual cycle. However, we observed a greater drop in CVC in the ML phase that eliminated any menstrual cycle phase difference in CVC by 15° head-up tilt.

SkBF is controlled by two types of sympathetic nerves: sympathetic adrenergic nerves and sympathetic cholinergic nerves that control vasoconstriction and active vasodilation, respectively. Kellogg et al. (18) found that while the decrease in CVC observed during lower body negative pressure in the thermoneutral state was caused by an increase in sympathetic vasoconstriction, the decrease in CVC elicited by unloading the baroreceptors under heat stress is primarily due to the withdrawal of active vasodilation. Because of a greater percentage of blood volume within the compliant cutaneous vascular bed in the ML phase, it seems likely that women in the ML phase may have had a greater drop in end-diastolic pressure and SV at the onset of head-up tilt, eliciting greater unloading of the cardiopulmonary baroreceptors leading to greater withdrawal of cutaneous active vasodilation. Along these lines, it has been reported (25) that sympathetic baroreflex sensitivity is greater during the ML than EF phase of the menstrual cycle, although control of skin sympathetic nerve activity was not investigated in that study. Although not statistically significant, we observed a trend toward a greater drop in PP in response to the first stage of heated tilt during the ML phase, coinciding with the greater drop in CVC observed during this portion of the head-up tilt. The data compliment the CVC data from this group, portraying a greater increase in diastolic blood pressure due to greater withdrawal of cutaneous active vasodilation.

A difference in HR across the phases of the menstrual cycle was observed during the heated tilt test, as HR was significantly lower in the OV phase compared with the EF and ML phases. Interestingly, CVC was lowest in the OV phase throughout the heated tilt test, suggesting that the lower HR seen in this phase may be due to less cardiovascular strain during passive heating. That is, women in the OV phase may have had less of a drop in end-diastolic filling with tilt in the heat and therefore may have experienced less of a decrease in venous return and SV. Consequently, women in the OV phase of the menstrual cycle may not have required as great of a HR to maintain Qc and blood pressure.

Sex Difference and Orthostatic Tolerance

Our finding that women had significantly lower orthostatic tolerance in the heat than men is consistent with previous investigations (3, 7, 12, 27, 32, 40) in thermoneutral environments that have observed a sex difference in tolerance to lower-body negative pressure. In the present study, women had lower Qc, SV, PP, and MAP, but higher TPR and similar CVC during the preliminary and thermoneutral tilt tests. Likewise, during the heated tilt test HR was higher in women, but MAP and PP were lower, and CVC remained similar between sexes. No interactions were observed in the thermoneutral or heated tilt tests between men and women, indicating no significant difference in the rate of change of these cardiovascular variables between sexes.

A recent study by Fu et al. (8) proposed that a cardiac anatomical sex difference may underlie the sex difference in orthostatic tolerance. Specifically, the authors reported that women may have smaller, less distensible left ventricles, such
that the maximal slope of the Frank-Starling curve is greater in women than men, occurring at the lower range of pulmonary capillary wedge pressure (an index of left ventricular filling pressure). To date, this study seems to provide the most plausible argument to explain the sex difference in orthostatic tolerance and agrees with our results suggesting no sex differences in vascular responses to an orthostatic challenge. In our study, the change in left ventricular filling pressure during tilting in the thermoneutral condition may not have been great enough to observe a sex difference in SV during upright tilting. However, when passively heated, the peripheral displacement of blood volume to the cutaneous circulation most likely resulted in lower left ventricular filling pressures, such that when combined with upright tilt, a greater drop in SV occurred in women than in men. Unfortunately, we did not measure SV during the heated tilt test as orthostatic tolerance was our primary end point, and we were concerned that the technique for measuring Qc, and therefore SV, could impact orthostatic tolerance.

Recently, we (22) reported that lower leg venous compliance is less in women than in men and that it does not change during the menstrual cycle. In general, it may be thought that greater calf compliance would be associated with higher rates of orthostatic intolerance, but this has not proven to be the case (26). For example, lower leg venous compliance in women may be predicted to help preserve venous return in the face of venous pooling in the dependent limbs during the upright posture, and yet the rise in sympathetic outflow in response to pharmacological changes in blood pressure (23) and head-up tilt (32) have been reported to be less in women than in men. In contrast, other investigators (9) have observed similar increases in total muscle sympathetic nerve activity in response to head-up tilt between sexes. Despite these conflicting findings, changes in vascular resistance (8, 12, 32, 40) appear to be similar between sexes for a given level of orthostatic stress. In our study, the finding that most cardiovascular variables were similar between sexes during upright tilting, including CVC during combined heat stress and tilt, appears to be consistent with the concept that women are operating on a steeper portion of the Frank-Starling relationship at low filling pressures. Moreover, these findings demonstrate the complexity and integrated nature of orthostatic responses.

Two limitations must be considered when critically evaluating this study. First, our tilt protocol was not adequate to elicit presyncopal symptoms in our subjects in the thermoneutral environment and extend this observation to show a distinct sex difference in orthostatic tolerance in the heat.

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REFERENCES

Menstrual Cycle, Orthostatic Tolerance, and Heat Stress


