

RESEARCH ARTICLE

Influence of sex, menstrual cycle, and oral contraceptives on cerebrovascular resistance and cardiorespiratory function during Valsalva or standing

Syed Abidi,¹ Misha Nili,¹ Stephania Serna,¹ Simon Kim,¹ Christopher Hazlett,¹ and Heather Edgell^{1,2}

¹School of Kinesiology and Health Sciences, York University, Toronto, Ontario, Canada; and ²Muscle Health Research Centre, York University, Toronto, Ontario, Canada

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Abidi S, Nili M, Serna S, Kim S, Hazlett C, Edgell H. Influence of sex, menstrual cycle, and oral contraceptives on cerebrovascular resistance and cardiorespiratory function during Valsalva or standing. *J Appl Physiol* 123: 375–386, 2017. First published May 18, 2017; doi:10.1152/jappphysiol.00035.2017.—Women experience orthostatic intolerance more than men, and they experience faintness more in the early follicular [i.e., low-hormone (LH)] than luteal [i.e., high-hormone (HH)] phase of the menstrual cycle. Men ($n = 13$, 25.8 ± 1.8 yr old) and women in the LH (days 2–5; placebo) and HH (days 18–24; high dose) phases of the menstrual cycle with (OC; $n = 14$, 22.0 ± 0.8 yr old) or without (NOC; $n = 12$, 21.8 ± 0.5 yr old) oral contraceptive (OC) use underwent the Valsalva maneuver and a supine-sit-stand protocol. Blood pressure, normalized stroke volume [stroke volume index (SVi)], cardiac output index, heart rate, end-tidal CO₂, and middle cerebral artery (MCA) blood flow velocity were measured. When subjected to the Valsalva maneuver, all women had a greater increase in diastolic and mean MCA blood flow velocity than men ($P \leq 0.065$), with no significant effect of menstrual cycle phase or OC use. When subjected to the supine-sit-stand protocol, men had lower MCA blood flow velocity ($P < 0.038$) than all women, and SVi was higher in men than in the NOC group in all postures ($P < 0.011$) and in the OC group in the LH phase of the menstrual cycle during standing ($P = 0.010$). Only men experienced higher resistance index ($P < 0.001$) and pulsatility index ($P < 0.001$) with standing. The OC group had lower end-tidal CO₂ ($P = 0.002$) than the NOC group ($P = 0.030$) and men ($P \leq 0.067$). SVi ($P = 0.004$) and cardiac output index ($P = 0.008$) were higher in the OC than NOC group. A tendency toward a lower mean MCA blood flow velocity ($P = 0.058$) and higher SVi ($P = 0.059$) and pulsatility index ($P = 0.058$) was noted in the HH than LH phase. Mean arterial pressure was higher in the OC than NOC group in the LH phase ($P = 0.049$) and lower in the HH than LH phase ($P = 0.014$). Our results indicate that cycling estrogens/progestins can influence ventilatory, cardiovascular, and/or cerebrovascular physiology.

NEW & NOTEWORTHY We have found sex differences in the cerebrovascular response to the Valsalva maneuver and standing. Men have greater cerebral vasoconstriction (or women have greater cerebral vasodilation) during late phase II of the Valsalva maneuver, and the cerebrovascular resistance index increases in men, but not in women, during standing. Furthermore, our findings indicate that both the menstrual cycle phase and oral contraceptive use can influence cardiovascular function both at rest and during active standing.

hemodynamics; end-tidal gases; center of balance; heart rate variability; cardiovagal baroreceptor sensitivity

WOMEN ARE KNOWN TO HAVE GREATER orthostatic hypotension (i.e., drop in blood pressure upon erect posture) (1, 11), as well as a greater propensity for postural orthostatic tachycardia syndrome (30, 31), than men. Although the exact mechanisms for the greater incidence of orthostatic hypotension in women are unclear, differently regulated cardiovascular responses to physiological stressors between men and women and throughout the menstrual cycle could play a role. Indeed, women have a greater increase in heart rate (HR) and a greater reduction of stroke volume (SV) during standing than age-matched men (17). Furthermore, a greater incidence of lightheadedness has been observed during menses (i.e., the early follicular phase of the menstrual cycle, when estrogen or progesterone levels are very low) than in the late follicular (high-estrogen) and luteal (high-estrogen and high-progesterone) phases in healthy women and in those with vasovagal syncope (35) or postural orthostatic tachycardia syndrome (38). Lower sympathetic nerve activity in the early follicular than luteal phase during orthostatic stress could be a contributing factor (19).

This evidence of a potential role for cycling female sex hormones in presyncope suggests that the role of oral contraceptives (OC) should also be considered. A recent national survey concerning contraceptive use in Canada found that 43.7% of sexually active women between 15 and 50 yr of age who were trying not to conceive were taking OC (6). This high rate of pharmaceutical use highlights the importance of investigating the effect of OC on the physiological responses of women. Data on the effects of OC on cardiovascular and autonomic responses to upright posture are limited. In their investigation of blood pressure, HR, and sympathetic nerve activity in response to lower-body negative pressure in women taking OC (9), Carter et al. found no effect of OC use between the high-hormone (HH, maximal-dose pill) and low-hormone (LH, placebo) phases. However, unlike standing, lower-body negative pressure does not activate skeletal muscle in the lower body, which influences total peripheral resistance (TPR) via vasodilation. SV index (SVi) and cardiac output (\dot{Q}) index (\dot{Q}_i) were not reported in their study. The finding of Limberg et al. that increases in forearm vascular conductance due to isoproterenol (i.e., a β -adrenergic receptor agonist), acetylcholine, and nitroprusside were greater in OC users than non-OC users (28) implies that OC users could have a greater reduction of peripheral resistance during standing, thus reducing blood pressure and/or increasing \dot{Q} .

Many investigations using orthostatic stress do not concurrently measure indexes of cerebrovascular resistance (CVR) or end-tidal CO₂ (ETCO₂) (9, 10, 19, 20). Upright posture leads to a reduction of ETCO₂ (17, 40, 47), which, in turn, contributes to

Address for reprint requests and other correspondence: H. Edgell, 355 Bethune College, York University, 4700 Keele St., Toronto, ON, Canada M3J 1P3 (e-mail: edgell@yorku.ca).

the reduction of brain blood flow. While recent evidence suggests that there is no difference in this reduction of ET_{CO_2} between men and women (17), women were only studied from *day 8* to *day 11* of the menstrual cycle, and all were taking cyclic types of OC (i.e., all were taking the 1st wk of OC pills consisting of estrogen analog and the lowest dose of progesterone). Cerebrovascular reactivity to CO_2 has been shown to be greater in women than men (24), implying that, for each decrement in ET_{CO_2} , women would have a greater drop in brain blood flow/higher CVR and, potentially, an increase in the incidence of presyncope or fainting during upright posture.

The current study has investigated the role of sex difference, menstrual cycle phase, and OC use on cardiovascular, respiratory, and cerebrovascular responses to the Valsalva maneuver or orthostatic stress. These variables have been concurrently measured in men and women previously (17), but not during investigation of the menstrual cycle and OC use. Because of the lower sympathetic activity previously observed in the early follicular than luteal phase during upright posture (19), we hypothesized that women will exhibit a smaller HR response, smaller increase in blood pressure, and smaller increase in brain blood flow velocity during late phase II of the Valsalva maneuver in the early follicular phase/placebo pill week (i.e., LH phase) than during the luteal/maximal-dose week (i.e., HH phase). Furthermore, because of smaller left ventricular dimensions in women (4), greater cerebrovascular reactivity to changes in ET_{CO_2} in women (24), and reduced vasodilatory capacity from low estrogen levels in the LH phase of the menstrual cycle (25), we also hypothesized that, in the upright posture, 1) women will exhibit lower SV_i , lower Q_i , and higher CVR than men and 2) women (OC and non-OC users) will have lower SV_i , lower Q_i , and higher CVR in the LH than HH phase. Lastly, because of previous observations of greater vasodilatory capacity in OC users (28), we hypothesized that OC users will have higher SV_i , higher Q_i , and lower CVR than non-OC users.

MATERIALS AND METHODS

Participant Description

Young healthy participants with no history of cardiovascular or respiratory disease were recruited for the study [13 men, 12 women not taking OC (NOC group), and 14 women taking OC (OC group); Table 1]. The types of OC include a placebo week, where pills contain no estrogen or progesterone analogs (menses occurs) and then, in the subsequent 3 wk, pills contain both estrogen and progesterone analogs. In noncyclic types of OC, the dose of estrogen/progesterone analogs is the same in every pill; in cyclic types of OC, the dose of estrogen analog is the same in every pill, but the dose of progesterone

analog increases with each week. In the OC group, six women were taking Tri-Cyclen [0.18, 0.215, and 0.250 mg of noregestimate and 0.035 mg of ethinyl estradiol (EE) (cyclic)], one was taking Tri-Cyclen Lo [0.18, 0.215, and 0.250 mg of noregestimate and 0.025 mg of EE (cyclic)], five were taking Alesse [100 μ g of levonorgestrel and 0.020 mg of EE (noncyclic)], one was taking Marvelon [0.15 mg of desogestrel and 0.03 mg of EE (noncyclic)], and one was taking Novo-Cyprotenone/EE [2 mg of cyproterone acetate and 0.035 mg of EE (noncyclic)]. Women were tested twice during the menstrual cycle at the same time of day. The LH phase was defined as *days 2–5* in the NOC group and during the placebo week in the OC group. The HH phase was defined as *days 18–24* in the NOC group and the last week of pills containing both EE and progesterone analog in the OC group. These phases were determined by self-report with guidance from the researchers. Participants were not fasted but were asked to refrain from eating fatty food, to abstain from caffeine and alcohol, and to refrain from physical exercise for ≥ 12 h before assessment. All participants gave written informed consent to participate in the study, which was approved by the Office of Research Ethics at York University.

Hemodynamic Measurements

HR was determined using the R-R interval of a standard electrocardiogram. Beat-to-beat blood pressure and \dot{Q} were determined using a noninvasive blood pressure device (Finometer Pro, Finapres Medical Systems, Amsterdam, The Netherlands). Resting blood pressure was calibrated with a standard manual blood pressure measurement. SV was calculated as $\dot{Q} \div HR$. SV and \dot{Q} were normalized to body surface area. TPR index (TPR_i) was calculated as mean arterial pressure (MAP) $\div Q_i$. Cerebral perfusion pressure (CPP) was calculated as $MAP - 0.7355 \text{ mmHg/cmH}_2\text{O} \times \text{distance from the transcranial Doppler probe to the heart during upright posture}$.

Transcranial Doppler

A 2-MHz ultrasound probe (Multigon Industries, Yonkers, NY) was positioned on the left temple with an adjustable headband and used to measure blood flow velocity of the middle cerebral artery (MCA). The CVR index (CVR_i) was calculated as $CPP \div \text{mean MCA blood flow velocity}$. Resistance index (RI) was calculated as $RI = (MCA_{\text{systolic}} - MCA_{\text{diastolic}}) / MCA_{\text{systolic}}$. Pulsatility index (PI) was calculated as $PI = (MCA_{\text{systolic}} - MCA_{\text{diastolic}}) / MCA_{\text{mean}}$.

End-Tidal Gases

Participants were fitted with a nasal cannula to continuously sample end-tidal gases (O_2 and CO_2) for analysis via infrared spectroscopy (VacuMed, Ventura, CA). Respiratory rate was determined from the rate of CO_2 peaks.

HR Variability and Cardiovascular Baroreceptor Sensitivity

HR variability (HRV), an indicator of the autonomic control of HR (39), was determined using 5 min of data in each of the supine, sitting,

Table 1. Anthropometric data

	Women					Significance
	Men (n = 13)	NOC (n = 12)		OC (n = 14)		
		LH	HH	LH	HH	
Age, yr	25.8 \pm 1.8	21.8 \pm 0.5		22.0 \pm 0.7		*Men vs. OC; Men vs. NOC (P = 0.07)
Height, cm	176.7 \pm 2.1	157.8 \pm 1.6		164.0 \pm 1.7		*Sex; *OC vs. NOC
Weight, kg	83.3 \pm 2.9	61.0 \pm 3.1	59.7 \pm 3.3	58.9 \pm 2.0	60.4 \pm 1.9	*Sex
BMI, kg/m ²	26.6 \pm 0.6	24.5 \pm 1.0	24.1 \pm 1.3	21.8 \pm 0.4	22.3 \pm 0.4	*OC vs. NOC; *Men vs. OC

Values are means \pm SE. NOC, non-oral contraceptive users; OC, oral contraceptive users; LH and HH, low- and high-hormone phases of the menstrual cycle; BMI, body mass index. *P < 0.05.

and upright postures. HRV (time domain and spectral analysis) was analyzed using the HRV module of LabChart Pro 8.0 software (ADInstruments, Colorado Springs, CO).

Cardiovascular baroreceptor sensitivity was determined (lag 0 data) using the sequence method on 5 min of uninterrupted beat-by-beat blood pressure and ECG recordings in the supine, seated, and upright postures (3, 5).

Stress Questionnaires

To control for effects of psychological stress on the physiological responses during repeat visits, female participants were asked to complete the Cohen perceived stress scale questionnaire (13) at each visit. There were no effects of OC ($P = 0.26$) or menstrual cycle phase ($P = 0.65$) on the perceived stress scale; scores were 18.6 ± 1.7 for NOC-LH, 17.3 ± 1.4 for NOC-HH, 16.3 ± 1.8 for OC-LH, and 16.7 ± 1.9 for OC-HH (a maximum score is 40). These results indicate that there was no confounding effect of psychological stress between visits in the women.

Wii Balance Board

For the stand portion of the supine-sit-stand test, participants stood on a Wii balance board to obtain their lateral and medial movement. The maximal range of movement in the lateral (i.e., side-to-side) and medial (i.e., front-to-back) directions for the last 30 s of the stand test was determined using LabChart software.

Experimental Protocol

Valsalva. In the supine position, participants exhaled into a tube attached to a pressure gauge and maintained a pressure of 40 mmHg for 15 s (visual feedback of exhalation pressure was provided). The Valsalva HR ratio was calculated as maximal HR during Valsalva \div lowest HR within 30 s of the end of exhalation. Late phase II of the Valsalva maneuver is defined as the blood pressure increase in the latter half of exhalation (Fig. 1).

Supine-sit-stand. Measurements were obtained for 5 min of supine rest. Participants then moved to the seated position for 5 min. If needed, assistance was provided by a researcher. Lastly, participants stood on a Wii balance board for 10 min. The test was terminated early if systolic pressure fell below 70 mmHg or if the participant experienced dizziness, nausea, or light-headedness. The test was terminated early for two men, and data for the standing position were averaged for the 1 min before termination.

Data and Statistical Analysis

All signals were collected using PowerLab data acquisition and LabChart software (ADInstruments). During the supine-sit-stand protocol, 1-min averages were obtained at the end of each posture for presentation.

Valsalva maneuver, balance, and MCA blood flow velocity slopes. Two-way repeated-measures ANOVAs were used to compare responses between OC and NOC groups (OC use and menstrual cycle

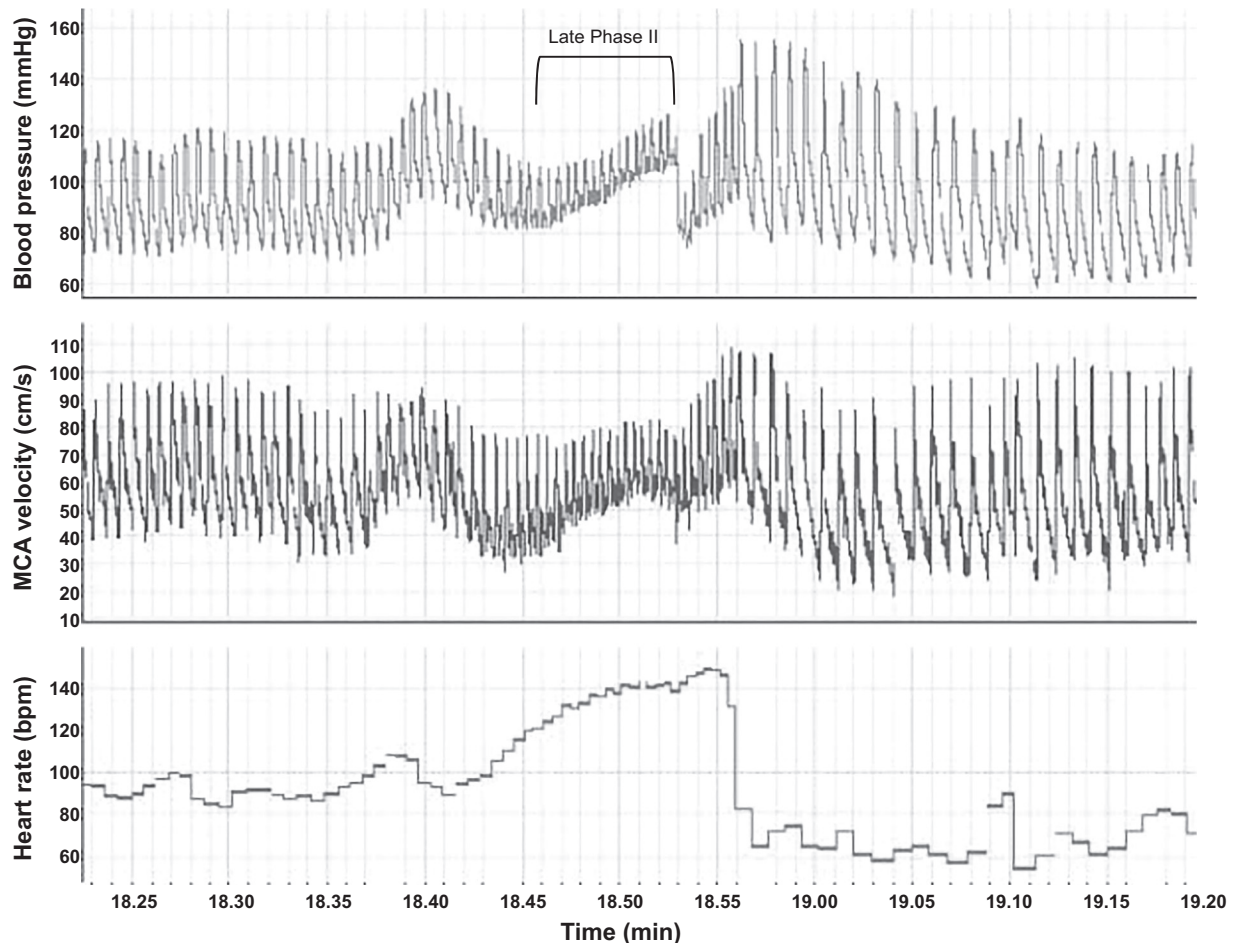


Fig. 1. Raw blood pressure, middle cerebral artery (MCA) blood flow velocity, and heart rate data collected during the Valsalva maneuver in a female participant in the high-hormone phase of the menstrual cycle.

phase as repeated measures). One-way ANOVAs were used to compare responses between men and each of the OC-LH, OC-HH, NOC-LH, and NOC-HH groups (sex as nonrepeated measure).

Supine-sit-stand protocol. Three-way repeated-measures ANOVAs were used to compare responses between OC and NOC groups (OC use, menstrual cycle phase, and posture as repeated measures). Two-way repeated-measures ANOVAs were used to compare responses between men and each of the OC-LH, OC-HH, NOC-LH, and NOC-HH groups (sex as nonrepeated measure and posture as repeated measure).

Where main or interaction effects were significant, Holm-Sidak post hoc tests were used. All statistical tests were performed with Sigmaplot 13.0 (Systat Software, San Jose, CA). Significance was set at $P \leq 0.05$, and trends are noted when $P < 0.07$. Values are means \pm SE.

RESULTS

Cardiovascular Responses to Valsalva

There were no significant effects of OC use, menstrual cycle phase, or sex difference on the increase in diastolic pressure, systolic pressure, and MAP during late phase II of the Valsalva maneuver (Table 2), nor were there any significant effects of OC use, menstrual cycle phase, or sex difference on the Valsalva HR ratio (Table 2).

Cerebrovascular Responses to Valsalva

During late phase II of the Valsalva maneuver, men had a significantly smaller increase in diastolic MCA blood flow velocity than OC users (both phases) and the NOC-HH group (Fig. 2B) and a significantly smaller increase in mean MCA blood flow velocity than the NOC-HH group (Fig. 2C). There were no significant sex differences in CVRi (Fig. 2D), RI (Fig. 2E), or PI (Fig. 2F). There were no significant effects of OC use or menstrual cycle phase on cerebrovascular measurements during late phase II of the Valsalva maneuver (Fig. 2).

Cardiovascular Responses to Posture Change

SVi decreased with upright posture in all groups (Fig. 3A). The OC group had significantly higher SVi than the NOC group, and men had significantly higher SVi than the NOC group (both phases) in all postures and the OC-LH group in the standing posture only (Fig. 3A). HR increased with upright posture equally in all groups and was higher in the HH than LH phase of the menstrual cycle (Fig. 3B). Qi was significantly higher in the OC than NOC group and significantly higher in the HH than LH phase (Fig. 3C). Men had significantly higher Qi than the NOC-LH group in all postures and the OC-LH group during standing (Fig. 3C). TPRi increased with upright posture in all groups (Fig. 3D). TPRi was lower in the HH than LH phase, and during standing, TPRi was lower in men than in

the OC-LH group (Fig. 3D). MAP increased with upright posture in all groups (Fig. 3E). In the LH phase, OC users had higher MAP than the NOC group and higher MAP than in the HH phase (Fig. 3E). The OC-HH group also had lower MAP than men (Fig. 3E). CPP decreased significantly with upright posture only in men (Fig. 3F). Similar to the MAP responses, OC users had higher CPP in the LH than HH phase (Fig. 3F).

Cerebrovascular Responses to Posture Change

Systolic MCA blood flow velocity decreased with upright posture in all groups and was lower in men than all groups of women (Fig. 4A). Diastolic MCA blood flow velocity decreased significantly with upright posture in men, but not in women (Fig. 4B). Men had lower diastolic MCA blood flow velocity than the OC-LH group in all postures and the NOC-LH group during standing (Fig. 4B). Mean MCA blood flow velocity decreased with upright posture in all groups (Fig. 4C) was lower in men than in all groups of women (Fig. 4C). The CVRi increased with posture in men only and was higher in men than all groups of women (Fig. 4D). PI increased with upright posture in men only and was higher in men than in the OC-LH group during standing (Fig. 4E). RI increased with upright posture in men only and was higher in men than in the OC-LH group (Fig. 4F). There were no significant differences between groups for the relationships between mean MCA blood flow velocity and either ETco₂ or CPP during the supine-sit-stand test (Table 3).

Respiratory Responses to Posture Change

ETco₂ decreased with upright posture in all groups (Fig. 5A) and was significantly lower in the OC than NOC group (Fig. 5A). End-tidal O₂ (ETo₂) increased with upright posture in all groups and was significantly higher in the OC than NOC group (Fig. 5B). Respiratory rate decreased with upright posture in all groups of women, but not in men (Fig. 5C).

Autonomic Responses to Posture Change

The time domain of HRV (SDRR), the high-frequency component of the frequency domain of HRV (HF), and cardiovagal baroreceptor sensitivity significantly decreased with upright posture in all groups (Table 4). The low-frequency component of HRV variability (LF) and the LF-to-HF ratio increased with upright posture in all groups (Table 4). SDRR was significantly higher in the OC than NOC group (Table 4).

Center of Balance

OC use significantly increased the maximal range of movement of lateral (side-to-side) and medial (front-to-back) center

Table 2. Blood pressure responses to late phase II of the Valsalva maneuver

	Women					Significance
	Men	NOC		OC		
		LH	HH	LH	HH	
Change in DBP, mmHg	19.6 \pm 4.0	18.4 \pm 3.2	20.2 \pm 4.2	20.2 \pm 4.0	25.0 \pm 4.2	Phase ($P = 0.061$)
Change in SBP, mmHg	21.9 \pm 4.9	16.0 \pm 4.6	21.1 \pm 6.5	20.7 \pm 5.4	22.8 \pm 4.3	Phase ($P = 0.065$)
Change in MAP, mmHg	20.3 \pm 4.2	17.6 \pm 3.5	20.5 \pm 4.9	20.4 \pm 4.4	24.3 \pm 4.0	Phase ($P = 0.051$)
Valsalva HR ratio	2.1 \pm 0.1	2.1 \pm 0.1	2.1 \pm 0.1	2.1 \pm 0.1	1.9 \pm 0.1	

Values are means \pm SE. DBP, diastolic blood pressure; SBP, systolic blood pressure; MAP, mean arterial pressure; HR, heart rate.

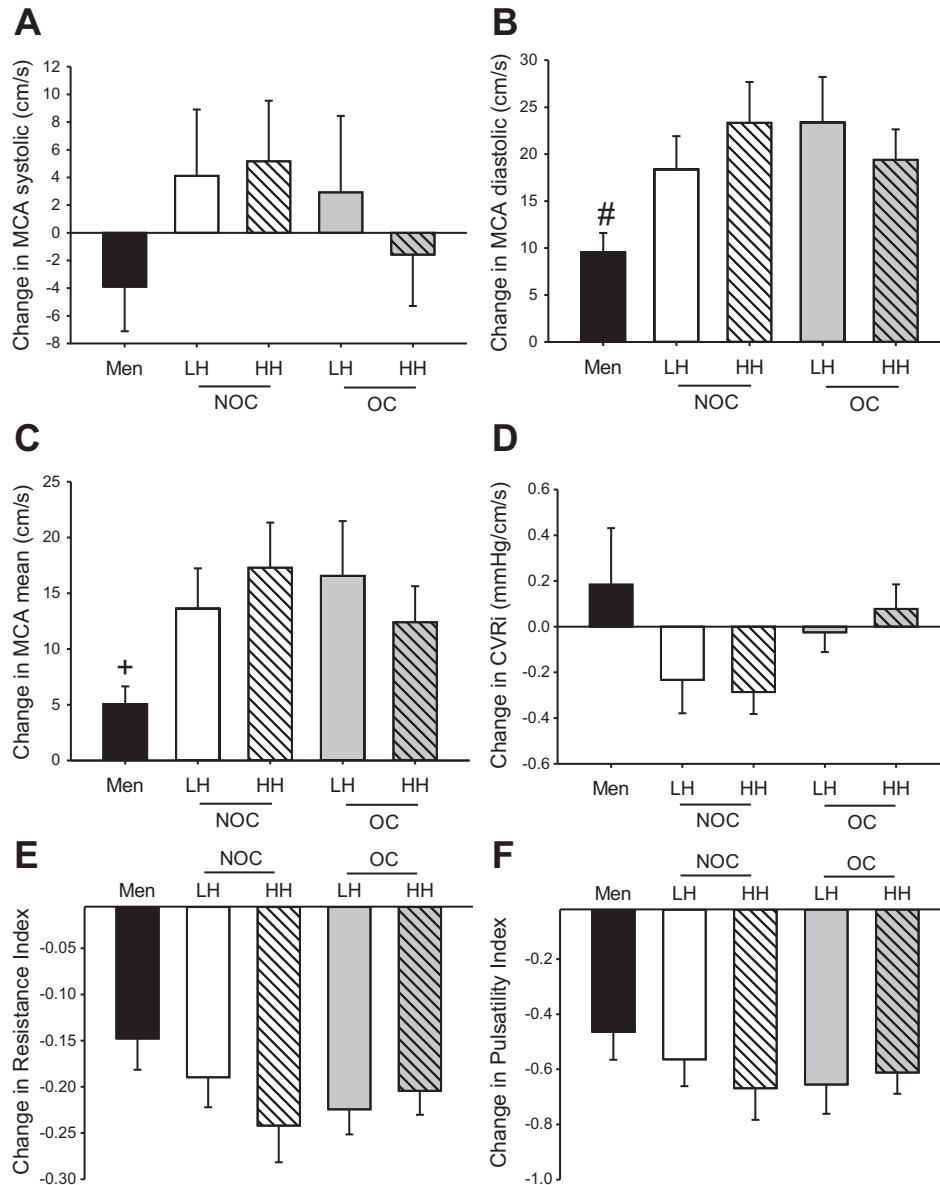


Fig. 2. Change in systolic, diastolic, and mean MCA blood flow velocity (A–C), cerebrovascular resistance index (CVRi; D), and resistance and pulsatility indexes (E and F) in response to late phase II Valsalva maneuver. LH, low hormone; HH, high hormone; NOC, non-oral contraceptive (OC) user; OC, OC user. #Significant difference ($P < 0.05$) between men and OC-LH, OC-HH, and NOC-HH, with a near-significant difference ($P < 0.06$) between men and NOC-LH. +Significant difference ($P < 0.05$) between men and NOC-HH, with a near significant difference ($P < 0.065$) between men and OC-LH, OC-HH, and NOC-LH.

of balance during the last 30 s of the stand test compared with the NOC group ($P < 0.05$). Men had significantly greater maximal range of movement than only the NOC-HH group in both the lateral and medial directions ($P < 0.05$): in the lateral direction, 24.5 ± 5.8 kg for men, 14.1 ± 3.0 kg for the NOC-LH group, 13.5 ± 2.5 kg for the NOC-HH group, 24.8 ± 4.3 kg for the OC-LH group, and 36.0 ± 4.5 kg for the OC-HH group; in the medial direction, 12.3 ± 3.4 kg for men, 6.1 ± 1.4 kg for the NOC-LH group, 4.8 ± 0.9 kg for the NOC-HH group, 8.5 ± 1.2 kg for the OC-LH group, and 10.1 ± 1.2 kg for the OC-HH group.

DISCUSSION

Summary

OC use did not significantly affect blood pressure, HR, or cerebrovascular responses to late phase II of the Valsalva maneuver in women. Similarly, menstrual cycle phase did not

significantly affect HR or cerebrovascular responses to the Valsalva maneuver; however, there was a strong tendency ($P = 0.051$) toward a greater increase in MAP during late phase II for women in the HH than women in the LH phase of the menstrual cycle. OC users (LH and HH phases) and non-OC users in the HH phase exhibited a greater increase in diastolic MCA blood flow velocity than men, suggesting a greater increase in brain blood flow in women during late phase II of the Valsalva maneuver. However, a concurrent reduction of CVR indexes in women was not observed.

Throughout the posture change protocol, women in the HH phase of the menstrual cycle had significantly higher HR and \dot{Q}_i with lower TPRi than women in the LH phase. OC users had significantly higher SVi and \dot{Q}_i with lower ET_{CO_2} and higher ET_{O_2} than non-OC users throughout the posture change protocol. In OC users, MAP and, therefore, CPP decreased in the HH phase. These results suggest that the presence of cycling estrogen and progesterone (HH phase) can result in

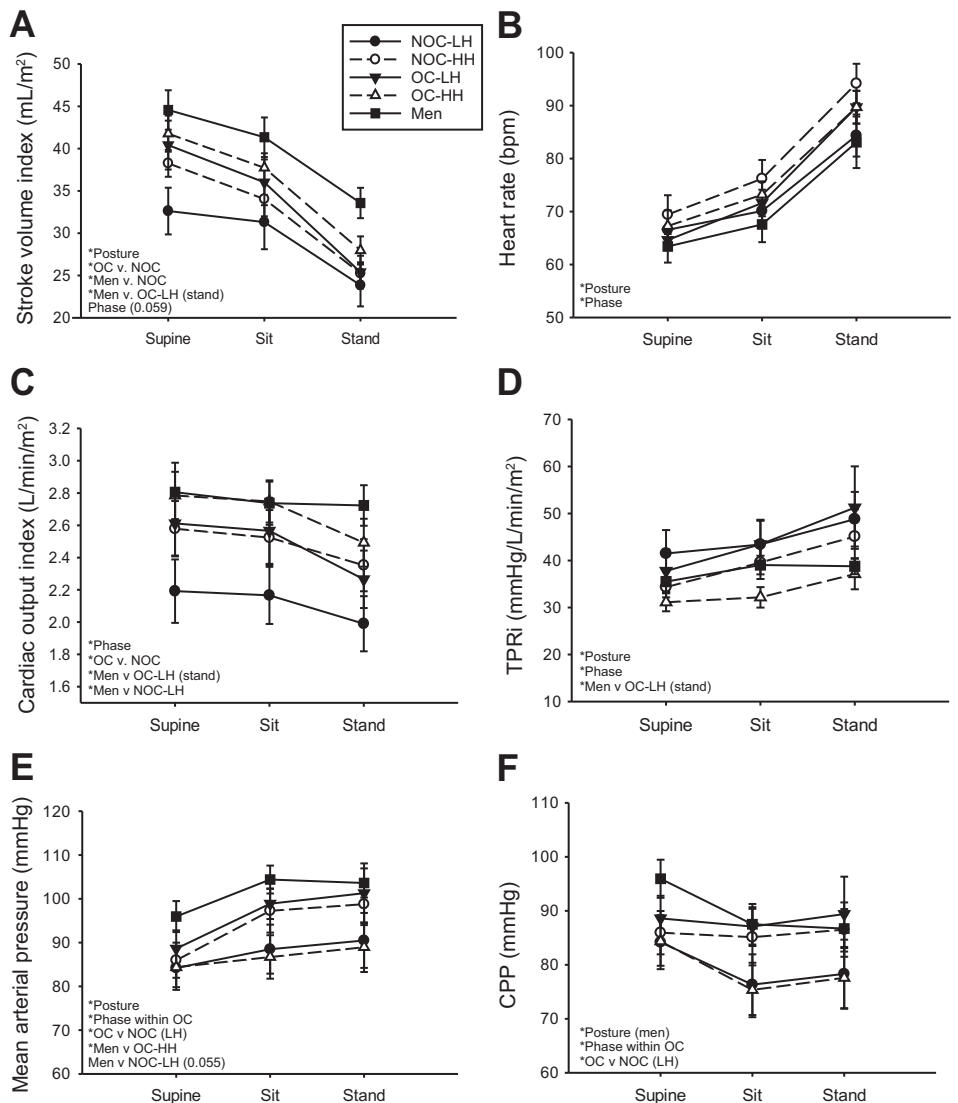


Fig. 3. Change in stroke volume index (A), heart rate (B), cardiac output index (C), total peripheral resistance index (TPRi; D), mean arterial pressure (E), and cerebral perfusion pressure (CPP; F) in response to sitting and standing. *Significant main or interaction effect. Near-significant differences ($P < 0.07$) are noted in parentheses.

peripheral vasodilation, allowing for greater \dot{Q} , and that the presence of pharmaceutical estrogen and progesterone analogs (active pill use in OC users) can lower blood pressure. The results further suggest that the use of OC in women could potentially be leading to cardiac remodeling with hypoventilation and/or changes in resting metabolism; however, this cannot be proven from this cross-sectional study of OC users. Further studies are needed to determine the mechanism(s) behind higher \dot{Q} and lower ETCO_2 in OC users.

Men had lower systolic and mean MCA blood flow velocity with higher CVRi than all groups of women throughout the posture change protocol. In men only during the posture change protocol, CPP and diastolic MCA blood flow velocity decreased while CVRi, PI, and RI increased, suggesting that, in response to upright posture, only men experienced a reduction of CPP with cerebral vasoconstriction. In women only, during the posture change protocol, respiratory rate decreased, perhaps indicating hypoventilation; however, changes in tidal volume were not measured; therefore, changes in ventilation cannot be concluded from the results of this study. Men had higher SVi than non-OC users (LH and HH phases) and higher

\dot{Q}_i than non-OC users (LH phase) throughout the posture change protocol. Similarly, men had higher SVi and \dot{Q}_i than OC users (LH phase) during standing. These sex differences are likely due to larger left ventricular dimensions in men. Men had lower TPRi (standing), lower diastolic MCA blood flow velocity (all postures), higher PI (standing), and higher RI (all postures) than OC users (LH phase). These results suggest that, during standing, men may have less peripheral vasoconstriction, yet more cerebral vasoconstriction, than women taking OC (LH phase).

Valsalva Maneuver

We hypothesized that women in the LH phase of the menstrual cycle would exhibit a smaller HR response, smaller increase in blood pressure, and smaller increase in brain blood flow velocity during late phase II of the Valsalva maneuver. Although there were no significant differences in HR during late phase II, women in the HH phase (both estrogen and progesterone) tended to have a greater increase in blood pressure than women in the LH phase (neither hormone). Fu et al.

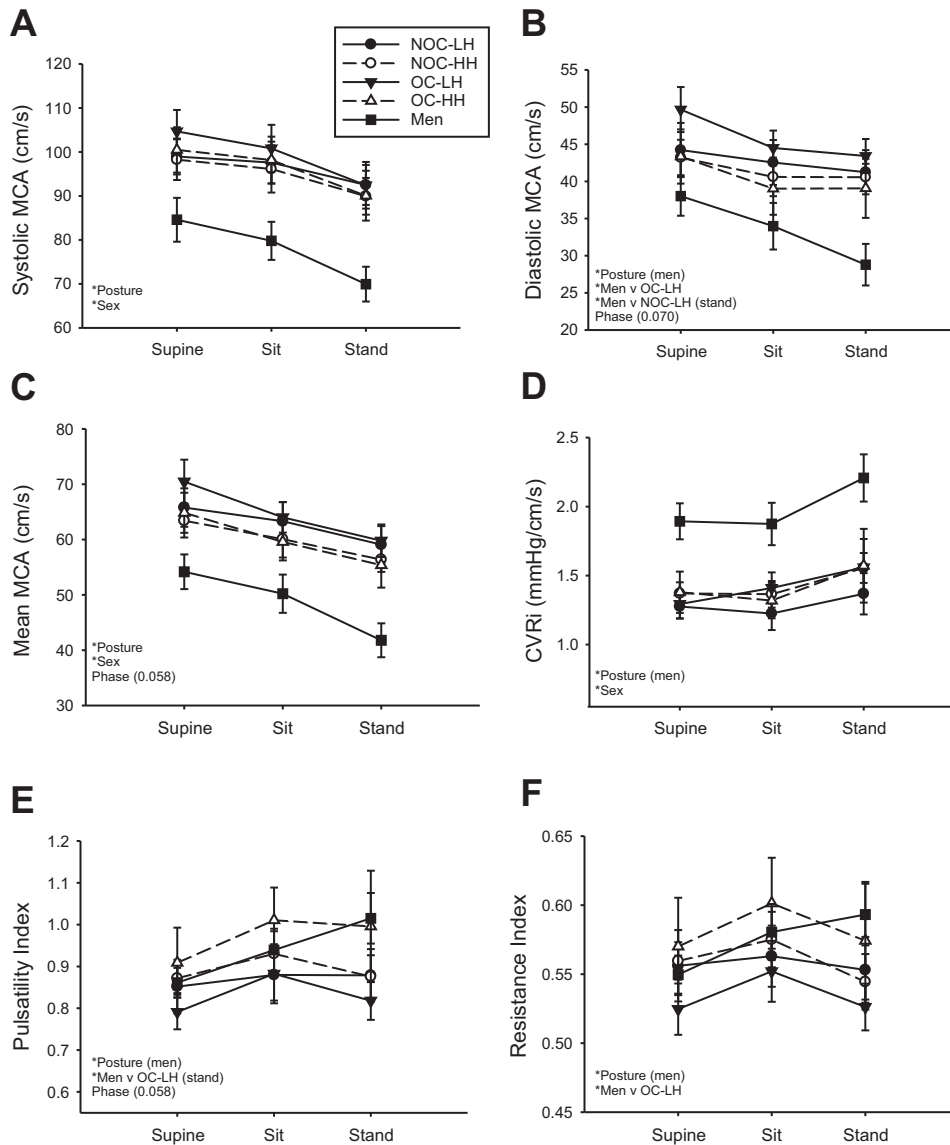


Fig. 4. Change in systolic, diastolic, and mean MCA blood flow velocity (A–C), CVRi (D), and pulsatility and resistance indexes (E and F) in response to sitting and standing. *Significant main or interaction effect. Near-significant differences ($P < 0.07$) are noted in parentheses.

found no differences between menstrual cycle phases in the sympathetic response to the Valsalva maneuver (19). Therefore, the tendency for a greater blood pressure response in the HH than LH phase could be due to somewhat improved peripheral neurovascular transduction (i.e., the ability of the vasculature to constrict in response to an increase in sympathetic activity), as observed by Lawrence et al. in the forearm

Table 3. Slope of MCA blood flow velocity vs. $ETCO_2$ and MCA blood flow velocity vs. CPP relationships

	Men	Women			
		NOC		OC	
		LH	HH	LH	HH
MCA _{mean} vs. $ETCO_2$	1.64 ± 0.24	2.17 ± 0.53	2.17 ± 0.60	2.54 ± 0.55	1.89 ± 0.18
MCA _{mean} vs. CPP	0.52 ± 0.09	0.43 ± 0.19	0.85 ± 0.29	0.43 ± 0.22	0.50 ± 0.19

Values are means ± SE. CPP, cerebral perfusion pressure; MCA, middle cerebral artery; $ETCO_2$, end-tidal CO_2 .

(27). There were no significant differences between sexes or OC users in blood pressure or HR responses to the Valsalva maneuver. This similarity between groups might imply no difference in the autonomic response to an acute fall in blood pressure (i.e., early phase I) between groups; however we did not directly test sympathetic or parasympathetic activity. Despite this similarity between groups, we did observe sex differences in brain blood flow responses to late phase II of the Valsalva maneuver.

Sex differences in the response to increases in $ETCO_2$ have been noted by Kastrup et al., who reported a greater cerebrovasodilatory response to CO_2 in women (24). Similarly, cerebral autoregulation has been shown to be better in adult women than age-matched men (15, 16, 45). Zhang et al. noted that a 15-s Valsalva maneuver was not expected to change $ETCO_2$ significantly (46). Therefore, we suggest that any changes in brain blood flow during the Valsalva maneuver are not due to changes in $ETCO_2$. Indeed, after ganglionic blockade, the increase in brain blood flow during late phase II of the Valsalva maneuver is enhanced (46), implying a restrictive effect of

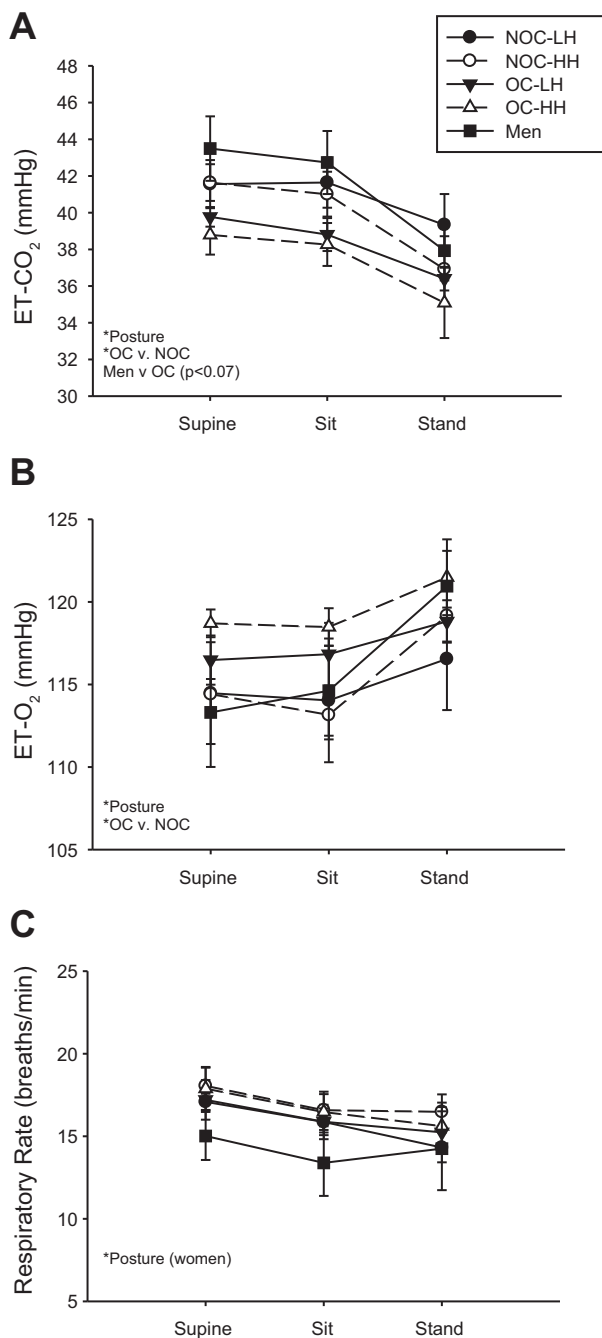


Fig. 5. Change in end-tidal CO₂ (ET-CO₂; A), end-tidal O₂ (ET-O₂; B), and respiratory rate (C) in response to sitting and standing. *Significant main or interaction effect. Near-significant differences ($P < 0.07$) are noted in parentheses.

sympathetic activity. Fu et al. found no differences between sexes in the sympathetic response to falling diastolic blood pressure during phase II of the Valsalva maneuver (19), yet we observed a greater increase in diastolic MCA blood flow velocity in women. Therefore, we suggest greater neurovascular transduction of cerebral arteries in men, as seen by Hart et al. in the forearm (22). Indeed, during the posture change protocol, we observed increased CVRi, PI, and RI in men only. Investigations into neurovascular transduction (cerebral and peripheral) throughout the menstrual cycle are needed.

Considering the tendency toward a greater increase in blood pressure during the Valsalva maneuver with similar increases in brain blood flow velocity in the HH phase of the menstrual cycle, we investigated CVR indexes during the Valsalva maneuver. There is currently no information about changes in MCA diameter during the Valsalva maneuver; however, there is growing evidence that the MCA constricts during sympathetic activation. For example, using MRI, Verbree et al. found that, at constant ET-CO₂, the MCA vasoconstricts by 2% during 5 min of rhythmic handgrip exercise in a mixed-sex population (43). In the current study, CVR indexes were calculated during the Valsalva maneuver in an attempt to determine cerebrovascular dilation/constriction, yet we did not observe differences between sexes, menstrual cycle phases, or OC use in CVRi, RI, or PI. During the Valsalva maneuver, the RI values were based on single heartbeats at the beginning and end of late phase II and, therefore, may be underpowered. This is a major limitation of this analysis, and we suggest the use of MRI for measurement of MCA diameter and flow during the Valsalva maneuver in future studies. However, when we used 1-min averages during the posture change protocol, we found tendencies toward higher PI and lower mean and diastolic MCA blood flow velocity in women in the HH phase of the menstrual cycle, implying greater vasoconstriction of the cerebral vessels, both at rest and during an orthostatic challenge. Indeed, women in the HH phase have been shown to have greater sympathetic activity at rest and during orthostasis than women in the LH phase (8, 19).

In the current study we did not find evidence of hemodynamic differences in the response to the Valsalva maneuver between sexes or with OC use. We did, however, find a strong tendency for a larger blood pressure response in the HH than LH phase of the menstrual cycle, possibly due to greater peripheral neurovascular transduction. Men had a smaller increase in diastolic MCA blood flow velocity than women during late phase II of the Valsalva maneuver, implying a smaller increase in brain blood flow, which we suggest could be due to enhanced neurovascular transduction in the cerebral vessels of men.

Standing

Cardiorespiratory response. We hypothesized that, in the upright posture, 1) women would exhibit lower SVi and lower Qi than men, 2) women in the LH phase of the menstrual cycle would have lower SVi and lower Qi than women in the HH phase, and 3) OC users would have higher SVi and higher Qi than non-OC users. These hypotheses are supported by the results. As expected, SVi and Qi were higher in men than most groups of women. However, SVi and Qi were not higher in men than in OC users in the HH phase due to augmentation from both menstrual cycle phase and OC use.

Estrogen is well known to have vasodilatory effects on peripheral vasculature (23, 26, 32). This vasodilation could contribute to the lower TPRi in the HH phase of the menstrual cycle, in turn, leading to the increase in Qi and SVi due to reduced cardiac afterload. Indeed, Minson et al. found greater calf blood flow in the HH than LH phase of OC users (33). Furthermore, the OC users experienced lower MAP in the HH than LH phase throughout the posture change protocol, as seen previously at rest (33). This reduction of MAP could be due to

Table 4. Heart rate variability and cardiovascular baroreceptor sensitivity

	Women					Significance
	Men	NOC		OC		
		LH	HH	LH	HH	
SDRR, ms						
Supine	83.9 ± 12.1	70.7 ± 12.1	76.8 ± 12.2	70.9 ± 8.6	85.0 ± 8.4	*Posture; *OC vs. NOC
Seated	91.2 ± 11.3	78.6 ± 8.9	69.6 ± 7.6	90.9 ± 9.1	95.2 ± 10.8	
Standing	56.0 ± 5.1	48.2 ± 6.7	42.0 ± 3.5	48.6 ± 3.6	55.4 ± 4.6	
LF, nu						
Supine	41.4 ± 5.1	31.1 ± 3.8	39.6 ± 5.7	35.8 ± 4.8	42.3 ± 4.4	*Posture
Seated	70.8 ± 4.8	51.8 ± 8.7	57.5 ± 4.7	55.8 ± 6.8	52.8 ± 5.8	
Standing	76.2 ± 5.3	70.4 ± 5.2	75.7 ± 4.5	70.6 ± 6.0	74.9 ± 4.3	
HF, nu						
Supine	55.7 ± 4.5	67.9 ± 3.5	58.0 ± 4.7	62.2 ± 4.4	56.3 ± 4.3	*Posture; Men vs. NOC-LH ($P = 0.056$)
Seated	28.6 ± 4.4	48.0 ± 8.4	42.1 ± 4.7	43.9 ± 6.5	46.9 ± 5.6	
Standing	23.1 ± 4.8	29.9 ± 5.0	24.1 ± 4.3	30.2 ± 5.9	25.6 ± 4.2	
LF/HF						
Supine	0.9 ± 0.2	0.5 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	*Posture
Seated	4.4 ± 1.9	2.3 ± 1.3	2.4 ± 1.2	1.9 ± 0.5	1.5 ± 0.3	
Standing	6.6 ± 1.8	3.8 ± 1.3	5.1 ± 1.3	4.1 ± 1.0	4.5 ± 1.2	
cBRS slope, ms/mmHg						
Supine	36.0 ± 5.3	44.7 ± 10.7	33.1 ± 5.4	42.4 ± 11.3	37.6 ± 4.8	*Posture
Seated	21.1 ± 3.1	23.4 ± 2.9	23.2 ± 6.1	30.2 ± 8.1	21.6 ± 2.3	
Standing	12.6 ± 3.1	12.2 ± 2.4	8.0 ± 1.0	10.5 ± 1.8	12.7 ± 1.9	

Values are means ± SE. SDRR, SD between R-R intervals; LF, low frequency; HF, high frequency; cBRS, cardiovascular baroreceptor sensitivity; nu, normalized units. * $P < 0.05$.

a combination of factors, including 1) greater β -adrenergic-mediated vasodilation in OC users than non-OC users (28) combined with the vasodilatory effects of estradiol in the HH phase, 2) reduced sympathetic and cardiovascular baroreflex sensitivity in the HH phase of OC users (33), 3) impaired postural stability/greater postural sway in OC users, as similarly observed by Mokosakova et al. (34), leading to greater muscle activation and, therefore, vasodilation, or 4) the slight, but significant, hypocapnia and hyperoxia experienced by OC users compared with non-OC users throughout the posture change protocol. This reduction of $ETCO_2$ and increase in ETO_2 could be due to greater ventilation, leading to attenuation of the central and peripheral chemoreflexes, respectively, which would, in turn, lead to reductions in sympathetic output. Indeed, Usselman et al. found attenuated chemoreflex function in the HH phase of OC users (42). We did not observe changes in respiratory rate due to OC use or menstrual cycle phase; however, we did not measure tidal volume/ventilation in this study.

Cerebrovascular resistance. We hypothesized that, in the upright posture, 1) women would exhibit higher CVR than men, 2) women in the LH phase of the menstrual cycle would have higher CVR than women in the HH phase, and 3) OC users would have lower CVR than non-OC users. Interestingly, these hypotheses were not supported by the results. CVR is dependent on both CPP and arterial CO_2 pressure (measured by $ETCO_2$). In the upright posture, we observed lower CPP and higher CVR_i in men only, suggesting greater cerebrovascular vasoconstriction in men than women (regardless of menstrual cycle phase or OC use). There were no sex differences in $ETCO_2$ responses to standing.

Very few studies have investigated sex differences in CVR during orthostatic stress. Edgell et al. previously investigated CVR in the upright posture in men and in women taking cyclic OC and in the “late follicular phase” of the menstrual cycle

(i.e., 1st wk of hormone pill) (17). Throughout the posture change protocol, they found lower CVR indexes (CVR_i, RI, and PI) in women than men, whereas in the current study, only the CVR_i was lower in women. This likely stems from an increase in MAP in women after 10 min of standing in the current study (from 84 ± 5 to 89 ± 6 mmHg in the OC-HH group) compared with the finding of Edgell et al. that MAP did not change in women after 5 min of standing (from 84 ± 2 to 82 ± 3 mmHg). These differences could be due to the duration of standing or different amounts of estradiol/progesterone analog (i.e., 1st wk of pill vs. last week of pill), highlighting the importance of controlling for OC use and menstrual cycle phase.

There were no significant effects of menstrual cycle phase or OC use on CVR indexes during the posture change protocol. However, there were trends for lower diastolic ($P = 0.07$) and mean MCA blood flow velocity ($P = 0.06$) with higher PI ($P = 0.06$) in women in the HH than LH phase, implying reduced brain blood flow due to cerebral vasoconstriction. Brackley et al. similarly observed higher PI and RI of the MCA in the luteal than midfollicular phase of the menstrual cycle (7). To our knowledge, this is the first study to investigate the effect of OC use on CVR indexes.

The current study has provided evidence indicating that both menstrual cycle phase and OC use can affect cardiorespiratory responses to standing. We have found that cycling female sex hormones are responsible for peripheral vasodilation, leading to reduced cardiac afterload and, therefore, higher SV_i and Qi. Furthermore, we have found that the use of pharmaceutical female sex hormones lowers MAP. While there was no statistically significant evidence for an effect of menstrual cycle phase or OC use on cerebrovascular responses during the standing trial, there was a trend for greater cerebrovascular constriction in the HH than LH phase. Lastly, we have provided significant evidence of greater cerebrovascular constrict-

tion in men than women (regardless of menstrual cycle phase) during standing.

Limitations

We did not measure ventilation or resting fitness levels in our participants; nor did we investigate differences between groups in lower-body and core strength, muscle fatigability, or mitochondrial activity, all of which could lead to differences in postural sway and metabolite buildup, in turn leading to changes in vasodilation and TPR. OC users in the HH phase of the menstrual cycle experienced lower blood pressure throughout the posture change protocol. We suggested that this could be due to greater peripheral vasodilation, reduced sympathetic reflex function (not directly tested in this study), or postural sway. The increase in postural sway in OC users could be due to 1) increased ventilation, 2) greater height, or 3) greater fatigue in core and/or leg skeletal muscles, perhaps due to changes in mitochondrial activity or a reduction of strength. Further studies are needed to elucidate these possibilities.

Transcranial Doppler measurements of blood flow velocity as an index of brain blood flow are limited because of the unknown diameter of the vessel. In 2000, using a 1.5-T MRI scanner, Serrador et al. found no change in MCA diameter during 10 min of orthostatic stress (41). However, more recently, using a 3-T MRI scanner, Coverdale et al. found that hypocapnia (-13 mmHg) resulted in MCA vasoconstriction (14), yet using a 7-T MRI scanner, Verbree et al. found that milder hypocapnia (still greater than that seen during orthostatic stress; -7.5 mmHg) elicited no significant change in MCA diameter (44). All the aforementioned investigations were carried out in a mixed-sex population, and there is no information on differences according to sex, menstrual cycle phase, or OC use.

The current study was a cross-sectional investigation of OC use. In future investigations, women should be studied before and during OC use to strengthen our conclusions. Furthermore, we did not have plasma concentrations of estrogen and progesterone (or analogs) during this study. Therefore, the veracity of data from the HH phase of the menstrual cycle of non-OC users could be in doubt if participants did not ovulate in the month of testing.

Participants were not tested in the fasted state. While women were tested at the same time of day to control for changes in circadian rhythm, we did not control for diet beyond our request that the subjects avoid fatty foods, caffeine, and alcohol. Diet can acutely affect the cardiovascular system. For example, in young healthy participants, consumption of ω -3 fatty acids can improve postprandial deteriorations in endothelial function and arterial stiffness (12, 36), and hypohydration can reduce endothelial function and lower plasma volume (2). Indeed, any food consumption can alter hemodynamics via changes in splanchnic blood flow (21) and sympathetic activity (18).

Conclusions

Compared with the LH phase of the menstrual cycle, there were strong tendencies for women in the HH phase to exhibit a greater increase in MAP during the Valsalva maneuver, potentially indicating greater peripheral neurovascular trans-

duction. Men exhibited a smaller increase in brain blood flow velocity than women toward the end of the Valsalva maneuver, suggesting that 1) men had greater cerebral vasoconstriction or 2) women had greater cerebral vasodilation. However, the RI values used in the current study (calculated using single heartbeats of data) did not support these suggestions. Future studies using MRI to measure both total cerebral blood flow and changes in MCA diameter throughout the Valsalva maneuver are needed for clarification. Similarly, only men experienced an increase in CVR indexes during the posture change protocol, indicating greater cerebral vasoconstriction. There were no statistically significant effects of menstrual cycle phase or OC use on cerebral responses to the Valsalva maneuver or standing; however, women in the HH phase showed strong tendencies toward greater cerebral vasoconstriction than women in the LH phase throughout the posture change protocol.

Women in the HH phase of the menstrual cycle had significantly higher HR and Qi with lower TPRi than women in the LH phase, suggesting an acute vasodilatory effect of female sex hormones (endogenous or pharmaceutical). OC users had significantly higher SVi and Qi than non-OC users with no effect on TPR or HR. Lastly, OC users exhibited reduced MAP in the HH phase compared with the LH phase, which could be from ventilatory changes, greater postural sway, enhanced vasodilatory capacity, or reduced sympathetic reflex control. Interestingly, these hemodynamic differences due to menstrual cycle phase or OC use are primarily due to baseline differences that persist throughout the posture change protocol.

Very few physiological research studies control for sex difference, menstrual cycle phase, and/or OC use, yet the results of the current study highlight their importance. Furthermore, many clinicians use the cardiovascular responses to the Valsalva maneuver and orthostatic stress as diagnostic tools for conditions such as orthostatic hypotension, postural orthostatic tachycardia syndrome, vasovagal syncope, and dysautonomia (29, 37), yet they do not control for menstrual cycle phase or OC use. These results suggest that controlling for (or at least recording) menstrual cycle phase and/or OC use could improve clinical diagnoses. Future investigations will expand into clinical populations with the goal of improving diagnosis in women.

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No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.A., M.N., and H.E. conceived and designed research; S.A., M.N., S.S., S.K., and C.H. performed experiments; S.A., M.N., S.S., S.K., C.H., and H.E. analyzed data; S.A., M.N., and H.E. interpreted results of experiments; S.A., M.N., S.S., S.K., C.H., and H.E. edited and revised manuscript; S.A., M.N., S.S., S.K., C.H., and H.E. approved final version of manuscript; H.E. prepared figures; H.E. drafted manuscript.

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