Influence of sex, menstrual cycle, and oral contraceptives on the cerebrovascular response to paced deep breathing.

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Abstract

Purpose: Deep breathing assesses autonomic function; however many researchers/clinicians do not account for hyperventilation, brain blood flow or blood pressure. Methods: Men and women (with/without oral contraceptives) participated. Women participated during low and high hormone phases of the menstrual cycle. Blood pressure, end-tidal carbon dioxide, middle cerebral artery velocity and cerebrovascular resistance were assessed. Results: Deep breathing decreased end-tidal carbon dioxide and middle cerebral artery velocity while increasing cerebrovascular resistance in all participants; blood pressure decreased in men. There were no influences of menstrual cycle or oral contraceptives. Conclusions: Men have different autonomic responses to deep breathing compared to women.

Keywords

Capnography, Blood pressure, Heart rate, Cerebrovascular circulation

Introduction

Paced deep breathing is used as a part of clinical assessment for autonomic dysfunction [8, 9]; however, many clinicians and researchers do not control for hyperventilation, menstrual cycle, or oral contraceptive (OC) use in women which can all affect end-tidal CO_2. Decreases of end-tidal CO_2 can influence autonomic function and decrease respiratory sinus arrhythmia [12] via inhibition of the chemoreflex and reductions in brain blood flow.

In OC users, high progestin has been shown to decrease resting end-tidal CO_2 while increasing the ventilatory response to CO_2 compared to low progestin [13]. Similarly, in women not taking OC, resting end-tidal CO_2 is lower in the luteal phase (high estrogen and progesterone) compared to the follicular phase (low estrogen and progesterone)[13]. However, during maximal exercise, only OC users have a greater ventilatory response to CO_2 in the high hormone (HH; high progesterone and estrogen) phase compared to the low hormone (LH; low progesterone and estrogen) phase, and in the HH phase OC users have an augmented ventilatory response compared to non-OC users [16]. Interestingly, during chemoreflex activation via rebreathe and apnea (hypoxic hypercapnia), sympathetic activation is greater in the LH phase compared to the HH phase in women taking or not taking OC [14, 15]. Usselman et al. also found that women not taking OC in the LH phase had a greater sympathetic response to chemoreflex stress compared to men, indicating both sex and menstrual phase differences in the chemoreflex [14].

Kastrup et al. found that women have greater cerebrovascular reactivity to changes in end-tidal CO_2 compared to men [6]. However, few studies have investigated changes in cerebrovascular reactivity to CO_2 throughout the entire menstrual cycle. Peltonen et al. compared the early follicular phase (low estrogen and progesterone) to the late follicular phase (high estrogen without progesterone) and found no difference in cerebrovascular reactivity to CO_2 [11], yet little is known about responses during the HH phase of the menstrual cycle (high progesterone and estrogen). Similarly, nothing could be found investigating OC use and cerebrovascular reactivity to CO_2.

We hypothesized that the chemoreflex contributes to tonic sympathetic tone and therefore that paced deep breathing at 6 breaths/min would result in 1) lower blood pressure (as observed previously in men [4]), lower end-tidal CO_2 and therefore lower brain blood flow velocity in all groups, 2) a smaller decrease in blood pressure in women during the HH phase compared to the

LH phase (due to reduced chemoreflex sensitivity in HH [14, 15]), 3) a greater drop in blood pressure in the HH phase of OC users compared to non-OC users (due to greater ventilatory response to CO_2 production in the HH phase of OC users [16]), and 4) a greater drop in brain blood flow in women compared to men (due to greater cerebrovascular reactivity in women [6]).

Materials and Methods

Participants refrained from fatty food, caffeine, alcohol and heavy exercise for at least 12 hours prior to testing; none had previously diagnosed cardiovascular or respiratory disease. Men (n=13, 25.8±1.8years, 26.6±0.6kg/m^2), women not taking OC (NOC; n=12, 21.8±0.5years, 24.5±1.0kg/m^2), and women taking OC (n=14, 22.0±0.7years, 21.8±0.4kg/m^2) participated in this study and for the concurrent study by Abidi et al.[1]. OC types included: Tricyclen (norgestimate 0.18-0.215-0.250mg/ethinyl estradiol 0.035mg, n=6), Tricyclen-lo (norgestimate 0.18-0.215-0.250mg/ethinyl estradiol 0.025mg, n=1), Alesse (levonorgestrel 100μg/ethinyl estradiol 0.020mg, n=5), Marvelon (desogestrel 0.15mg/ethinyl estradiol 0.03mg, n=1), and Novo-Cyprotenone/ethinyl estradiol (cyproterone acetate 2mg/ethinyl estradiol 0.035mg, n=1). Women were tested during the low hormone (LH) phase (day 2-5 in NOC and during placebo in OC) and the high hormone (HH) phase (day 18-24 in NOC and during the highest dose in OC). Phases were determined by self-report. Sex hormone concentrations were not measured; this is a limitation of the study. After recruitment one man had hypertension (158/89), 2 men had pre-hypertension (135/81 & 131/72), one woman taking OC had pre-hypertension in the LH phase (131/90), and one women taking OC had prehypertension in the HH phase (130/67). All participants gave written informed consent approved by the Office of Research Ethics at York University.

Cardiovascular and respiratory measurements

Heart rate was determined using a standard electrocardiogram. Beat-to-beat blood pressure was determined using a non-invasive blood pressure device (Finometer Pro, Finapres Medical Systems, Amsterdam, Netherlands) and was calibrated with manual blood pressure measurements. A 2 MHz ultrasound probe was positioned on the left temple (Multigon Industries Inc., Yonkers, USA) to measure blood flow velocity of the middle cerebral artery (MCA). End-

tidal CO_2 was collected via nasal cannulas for analysis via infrared spectroscopy (VacuMed, Ventura, USA).

Paced deep breathing protocol

Paced deep breathing was done to assess respiratory sinus arrhythmia [9]. Participants were instructed to complete eight breaths at a frequency of 6 breaths/minute. Rate was maintained with a metronome.

Statistics and data analysis

One minute averages were taken before and during the last minute of deep breathing. Exhalation to Inspiration ratio (E:I ratio) was calculated as the ratio of maximum to minimum heart rate within one breath, and 6 breaths were averaged. Cerebrovascular Resistance Index was calculated as mean arterial pressure/mean MCA velocity.

All signals were collected using PowerLab data acquisition and LabChart software (ADInstruments, Colorado, USA). Three-way repeated measures ANOVAs compared responses between OC and NOC groups. Two-way repeated measures ANOVAs compared responses between men and each of the female groups. Where main or interaction effects were significant Holm-Sidak post hoc tests were used. All statistical tests were performed with Sigmaplot 13.0 software (Systat Software, Inc., San Jose, USA). Significance was set at $p<0.05$ and p-values of <0.1 are noted.

Results

There were no effects of OC use ($p=0.66$), menstrual phase ($p=0.34$) or sex ($p\geq0.20$) on E:I ratio during deep breathing (Table 1). Similarly, there were no effects of OC use ($p=0.82$), menstrual phase ($p=0.31$), or sex ($p\geq0.17$) on the change in heart rate during deep breathing (Table 1).

In all participants, deep breathing decreased MCA velocity ($p<0.001$) and end-tidal CO_2 ($p\leq0.014$; Fig. 1a and 1b) with an increase of cerebrovascular resistance index ($p\leq0.020$; Fig1d). Mean arterial pressure decreased only in men during deep breathing ($p<0.001$; Fig. 1c). Men had lower MCA velocity than OC-LH ($p=0.004$) and NOC-LH ($p=0.04$; Fig. 1a). Men had higher end-tidal CO_2 compared to OC-HH ($p=0.047$; Fig. 1b). Men had higher mean arterial pressure

than all groups of women ($p \leq 0.046$; Fig. 1c). Men had greater cerebrovascular resistance index than all groups of women ($p < 0.029$), except for OC-HH at baseline ($p = 0.09$; Fig. 1d).

Discussion

In support of our first hypothesis, deep breathing decreased end-tidal CO_2 and MCA velocity while increasing cerebrovascular resistance index in all participants; however, only men decreased mean arterial pressure. Contrary to our other hypotheses, there were no effects of menstrual cycle or OC use on heart rate, MCA velocity, end-tidal CO_2, or mean arterial pressure responses to deep breathing.

In a mixed sex population, Limberg et al. found that deep breathing at 7 breaths/min did not change muscle sympathetic nerve activity or blood pressure compared to spontaneous breathing [7]. At this breathing frequency participants had greater tidal volume, yet end-tidal CO_2 was not different, indicating no change in ventilation. We suggest that deep breathing at 6 breaths/min, as used in the current study and during clinical assessments [8, 9], results in an augmented increase of tidal volume and ventilation lowering end-tidal CO_2 in all participants. The lack of tidal volume measurement is a limitation of the current study and should be investigated in future work. We further suggest that clinical autonomic assessments use a deep breathing rate of 7 breaths/min to remove hyperventilation and hypocapnia as confounding factors.

While there are no sex differences in the blood pressure response to chemoreflex stress via rebreathe and apnea (i.e. hypoxic hypercapnia) [14], we found that blood pressure decreased only in men during deep breathing (i.e. hypocapnia). We suggest that men experience a reduction of sympathetic tone due to either suppression of tonic chemoreflex activity or due to reduced respiratory rate as shown by Wallin et al.[17] This reduction of sympathetic tone would result in lower blood pressure in men due to their greater neurovascular transduction [3, 14]. We did not observe any influence of menstrual cycle on the cardiovascular responses to deep breathing; however, women's responses may change after menopause [10] Similarly, Usselman et al. found no effect of menstrual cycle on the blood pressure response to hypoxic hypercapnia in both OC users and non-OC users [14, 15] despite the fact that there was a greater sympathetic response in the LH phase. Usselman et al. suggested that neurovascular transduction is higher in the LH phase compared to the HH phase during chemoreflex stress [14]. Future studies isolating the

effects of hypercapnia from hypoxia while concurrently measuring sympathetic nerve activity and peripheral blood flow are needed.

Since end-tidal CO_2 decreased similarly in men and women, we expected to see a greater decrease in MCA velocity due to greater cerebrovascular reactivity in women [6], yet this was not the case. However, Kastrup et al. provided 5% CO_2 with 95% O_2 which increased ET-CO_2 from ~30 to ~50mmHg (~4-5x greater change than the current study) and which would enhance cerebrovascular reactivity due to hyperoxia as observed during hyperventilation in a mixed sex group by Johnston et al.[5]. Therefore, we suggest that the greater cerebrovascular reactivity previously observed in women could partially be due to their responses to 95% inhaled hyperoxia. Lastly, while changes in end-tidal CO_2 of <5mmHg (as observed in this study) have been noted not to change MCA diameter [2] (which would have influenced brain blood flow), sex differences have not been investigated. Further studies on cerebrovascular reactivity and changes in MCA diameter between the sexes are needed.

Conclusions

While there was no influence of menstrual cycle or oral contraceptive use on the cardiovascular responses to deep breathing, there were sex differences. It is notable that this standard clinical assessment resulted in lower end-tidal CO_2 and brain blood flow velocity in all participants with a decrease in mean arterial pressure only in men. We therefore suggest that autonomic responses differ between the sexes during deep breathing. Future investigations of sex differences should include direct measurements of sympathetic output and tidal volume during deep breathing, and these measurements should be repeated in clinical populations that may experience impaired chemoreflexes or cerebrovascular function.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Table 1: Heart rate (HR) responses to paced deep breathing

	Men	NOC		OC	
		LH	HH	LH	HH
E:I ratio	1.40±0.05	1.44±0.06	1.48±0.05	1.43±0.04	1.45±0.04
Change in HR (bpm)	20.9±1.9	24.2±3.4	26.3±3.1	24.0±2.1	24.9±2.1

NOC is no oral contraceptive use; OC is oral contraceptive use; LH is low hormone phase; HH is high hormone phase; E:I is expiratory to inspiratory ratio

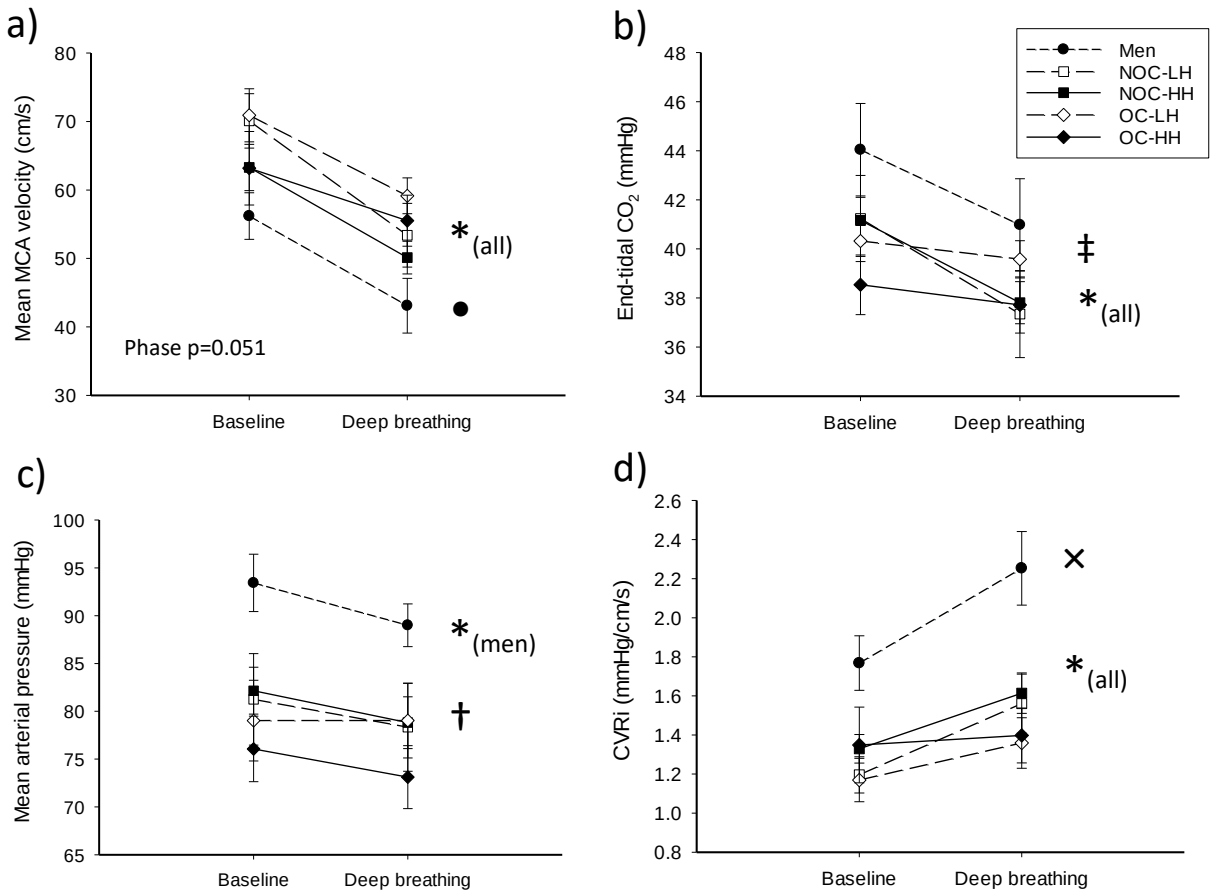

Fig. 1 a) Middle cerebral artery (MCA) velocity, b) End-tidal CO_2, c) Mean arterial pressure, and d) Cerebrovascular resistance index (CVRi) responses to paced deep breathing in men (black) circles), women not taking oral contraceptives (NOC) in the low hormone (LH; white squares) and high hormone (HH; black squares) phases, and women taking oral contraceptives (OC) in the low hormone (white diamonds) and high hormone (black diamonds) phases. *indicates a significant effect of deep breathing; • indicates a significant difference between men and OC-LH and NOC-LH; † indicates a significant effect of sex; ‡ indicates a significant difference between men and OC-HH; × indicates a significant effect of sex except for men v. OC-HH at baseline (p=0.09)