CENTRAL AND PERIPHERAL CHEMOREFLEX FUNCTION IN THE SUPINE AND UPRIGHT POSTURES IN WOMEN THROUGHOUT THE MENSTRUAL CYCLE WITH A COMPARISON TO MEN

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ABSTRACT

The primary purpose of the study was to examine sex differences and menstrual cycle time-points on chemoreflex function during supine and 70^{o} upright (HUT) positions during: 1) normoxia, 2) hypercapnia (5% CO_2), or 3) hyperoxia (100% O_2). Women were tested during the early-follicular phase (EF; days 2-5) and the mid-luteal phase (ML; days 18-24). Compared to baseline, men and women had lower cardiac output index (Q_i), mean arterial pressure (MAP), cerebrovascular resistance index, and respiratory rate during HUT. In response to hypercapnia during HUT (compared to supine), men had an augmented increase in MAP, while all groups had an augmented increase in ventilation suggesting sexually dimorphic interactions between the baroreflex and central chemoreflex. In response to hyperoxia during HUT, men and women displayed an attenuated increase of total peripheral resistance index and an attenuated decrease of Q_i suggesting upright posture activated peripheral chemoreceptors.

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Secondly, I would like to recognize my family for their continued motivation and belief that I could not only gaze upon the stars but reach out and grasp them. My ambition and drive to succeed have been cultivated by you all. Mom, Dad, and Cheryl, thank you for your unconditional love and support.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

- BMI → Body mass index
- BSA → Body surface area
- cBRS → Cardiovagal baroreceptor sensitivity
- CPP → Cerebral perfusion pressure
- CVR/CVR_i → Cerebrovascular resistance/cerebrovascular resistance index
- ECG → Electrocardiogram
- EF → Early-follicular
- ET-CO_2 → End-tidal carbon dioxide
- ET-O_2 → End-tidal oxygen
- FEV_1 → Forced expiratory volume in one second test
- FRC → Functional residual capacity
- FVC → Forced vital capacity
- HCO_3^- → Bicarbonate
- HF → High-frequency
- HR → Heart rate
- HRV → Heart rate variability
- HUT → Head-up tilt
- LBNP → Lower body negative pressure
- LF → Low-frequency
- MAP → Mean arterial pressure
- MCA → Middle cerebral artery
- ML → Mid-luteal
- MSNA → Muscle sympathetic nerve activity
- NO → Nitric oxide
- OI → Orthostatic intolerance
- PaCO2 → Arterial partial pressures of carbon dioxide
- PaO2 → Arterial partial pressures of oxygen
- PI → Pulsatility index
- POTS → Postural orthostatic tachycardia syndrome
- PWV → Pulse-wave velocity
- Q/Q_i → Cardiac output/cardiac output index
- RI → Resistance index
- SBP → Systolic blood pressure
- SDRR → Standard deviation of the R-R interval
- SV/SV_i → Stroke volume/stroke volume index
- TCD → Transcranial Doppler
- TPR/TPR_i → Total peripheral resistance/total peripheral resistance index
- V → Velocity
- V_e → Ventilation
- V_t → Tidal volume

INTRODUCTION

Orthostatic intolerance (OI) is characterized by feelings of nausea, light-headedness, syncope and fatigue upon standing or orthostatic stress (128). It has been well established that the prevalence of OI is much higher in young women compared to men (23, 45, 51, 67, 158) possibly due to attenuated hemodynamic responses to orthostatic stress (23, 70, 146). Convertino (1998) showed that women had significantly greater reductions in stroke volume, cardiac output and mean arterial pressure upon orthostatic stress compared to men (23). Similarly, Jarvis et al. (2010) investigated the effects of 70^{o} head-up tilt (HUT) on vasoconstrictor reserve in men and women and ultimately concluded that women have impaired splanchnic vasoconstriction upon assumption of HUT implying reduced venous return (70).

Further, women experience greater light-headedness during the EF phase (low estrogen and low progesterone) of their menstrual cycle compared to the ML phase (high estrogen and high progesterone) (114). Interestingly, Fu et al. (2009) investigated total MSNA during graded orthostatic stress and found that women in the EF phase had an attenuated total MSNA response compared to women in the ML phase (47). Sympathetic output during orthostatic stress can be influenced by many autonomic reflexes such as baroreceptors (65, 149), mechanoreceptors (43), metaboreceptors (96, 154), and chemoreceptors (74, 107, 110, 142, 143, 151), and these reflexes can in turn be influenced by the menstrual cycle. For example, during supine rest, ventilation is higher in the ML phase despite lower $PaCO_2$ compared to women the EF phase suggesting greater central chemoreceptor activity during the ML phase (138). However, there have been no investigations of chemoreflex function through the menstrual cycle in upright posture. Should women in the EF phase retain lower central chemoreceptor reactivity compared the ML phase in the upright posture, this could help explain lower sympathetic activity in the EF phase during tilt due to hypocapnia.

Interactions between autonomic reflexes have previously been studied. For example, the effects of hypoxia and hypercapnia in conjunction with postural changes (i.e. interactions between the chemoreflexes and the baroreflex) have been investigated (41, 46, 137, 147). Somers, Mark, & Abboud (1991) investigated the interaction between baroreflex and chemoreflex control of sympathetic nerve activity in healthy

men and women in a combined group (142). They found that sympathetic nerve activity was attenuated upon stimulation of both the peripheral chemoreceptors (via hypoxia) and baroreflexes (via phenylephrine) compared to hypoxia alone (142). They concluded that activation of baroreceptors by increases of arterial pressure markedly inhibited the sympatho-excitatory response to stimulation of the peripheral chemoreceptors by hypoxia (142). This inhibition of sympathetic activity as a result of baroreflex interaction with the peripheral chemoreceptors was not evident when investigating baroreflex and central chemoreceptor interaction as sympathetic activation was significantly higher after phenylephrine infusion in the presence of hypercapnia (142). This suggests that baroreflex inhibition in the tilted position could influence chemoreflex stimulation. Further, during post-exercise circulatory occlusion to activate the metaboreflex, it was discovered that the activated metaboreflex can stimulate the peripheral chemoreflex in healthy men in the absence of hypoxia (39).

Not only is ventilation and sympathetic activity influenced by CO_2 and O_2, but cerebral vessels maintain brain blood flow in response to changing levels of CO_2 through either vasodilation or vasoconstriction (69). In response to hypercapnia, brain blood flow increases via vasodilation to reduce cerebrovascular resistance (69) and women have also been shown to have increased cerebrovascular CO_2 reactivity to hypercapnia compared to men (75). Previous studies investigating cerebrovascular responses to oxygen have found that hyperoxia reduces brain blood flow (78, 106, 111, 159) likely due to increased cerebrovascular resistance (78, 159) as a result of cerebral vasoconstriction (78, 106, 159).

The purpose of this research was to investigate central chemoreflex activation and peripheral chemoreflex suppression with hypercapnia and hyperoxia, respectively, in the supine or upright postures while investigating the influence of sex and/or menstrual cycle. We hypothesized that in normoxic conditions: 1) During upright tilt, women would have a greater reduction in brain blood velocity compared to men, and 2) Men would have higher ventilation compared to women (in both supine and upright positions) and women during the mid-luteal phase would have higher ventilation compared to the early follicular phase (in both supine and upright positions).

Secondly, we hypothesized that: 1) Central chemoreflex function would be enhanced in the upright posture compared to supine, 2) In both the supine and upright postures, women in ML will have increased cerebrovascular reactivity to hypercapnia compared to men and women in the EF phase; however, women in the EF phase will have greater cerebrovascular reactivity than men, and 3) In both the supine and upright postures, women during the EF phase will have enhanced central chemoreflex activity in response to hypercapnia compared to the ML phase and men.

Lastly, we hypothesized that: 1) In the supine posture, hyperoxia would decrease heart rate and cardiac output index while increasing stroke volume index in all groups (as observed previously), and 2) The peripheral chemoreflex would be activated in the upright posture (i.e. ventilation will decrease during hyperoxia) and this activation would be greatest in the EF phase.

LITERATURE REVIEW

Oxygen

Overwhelming evidence has shown that stimulation of the peripheral chemoreceptors as a result of hypoxia results in increased ventilation and arterial pressure (21, 22, 110, 143). These chemoreceptors are specialized nerve endings located in the carotid and aortic bodies that initiate reflexes in response to fluctuations in the chemical composition of arterial blood to elicit effects upon ventilation and circulation (21, 22). Specifically, they will detect the decreasing arterial partial pressure of oxygen (PaO_2; i.e. hypoxia) and will then communicate with respiratory centres in the brainstem to increase sympathetic activity and ventilation to raise arterial oxygen content. Steinback & Poulin (2008) investigated cardiovascular and cerebrovascular responses to isocapnic (end-tidal CO_2 ($ET\text{-}CO_2$) held constant) and poikilocapnic ($ET\text{-}CO_2$ allowed to vary naturally) hypoxia in humans (144). From baseline measurements to just after the onset of hypoxia, both poikilocapnic and isocapnic conditions saw significant increases in heart rate and ventilation, however, only poikilocapnic hypoxia saw an additional increase in cerebrovascular resistance (CVR) while only isocapnic hypoxia saw additional increases in mean arterial pressure and middle cerebral artery

velocity (144). This coincides with the significant decrease of ET-CO_2 under poikilocapnic conditions since low CO_2 is known to cause cerebral vasoconstriction.

The effects of hyperoxia on cardiovascular, respiratory and cerebral responses have also been investigated; however, the duration of exposure in the literature varies considerably which can change the cardiorespiratory responses. Daly & Bondurant (1962) found that upon exposure to 100% O_2 (unknown duration), healthy men had an increase in mean arterial pressure as well as systemic vascular resistance (31). This was concurrent with a decreased cardiac output and heart rate that was observed with no change seen in stroke volume (SV)(31). In response to 5-30 minutes of hyperoxia, heart rate, cardiac output and stroke volume decrease while mean arterial pressure and systemic vascular resistance increase (29, 36, 78, 148, 157). Indeed, Crawford et al. (1997) investigated forearm vasculature responses to 15 minutes of 100% O_2 and concluded that exposure to hyperoxia increased forearm vascular resistance and mean arterial blood pressure (29). These findings suggest that oxygen can evoke a peripheral vasoconstrictor effect. Potential mechanisms influencing vasoconstriction as a result of hyperoxia include 1) directly affecting vascular smooth muscle cells to depolarize, leading to activation of L-type Ca^{2+} channels resulting in vasoconstriction (160), or 2) reducing the basal release of nitric oxide (NO; vasodilator) leading to a reduction of endothelium-dependent vasodilation as seen in porcine coronary arteries (112). These latter effects are likely elicited through superoxide anion, O_2^-, which is known to inhibit NO, thus, inhibiting vasodilation (55, 166).

Purves (1966) investigated the effects of 100% O_2 on respiratory and circulatory responses in 35 unanaesthetized, new-born lambs (117). Purves (1966) found that within the first minute of hyperoxic stimulus, minute ventilation decreased by an average of 22% and remained 19% lower after 7 minutes (117). Ventilatory responses to hyperoxia have also been widely investigated in healthy humans yet show no changes in ventilation (33, 34, 136). However, only if the peripheral chemoreceptors are activated, can they be suppressed or silenced in response to short periods (e.g. $\leq$2 minutes) of hyperoxia (33, 136). Indeed, Dejours et al. (1958) showed that after a healthy individual was made hyperoxic by continuously breathing 33% O_2, a further exposure to 100% oxygen does not change ventilation further (34). Evidence has shown

that ventilation remains unchanged after exposure to 6 minutes of 100% hyperoxia in healthy men (64, 90), and Hermand et al. (2015) suggested that hyperoxic-inhibition of the peripheral chemoreflex was not sufficient to modulate ventilation in health since the peripheral chemoreceptors were not activated at rest (64). Conversely, one study showed that ventilation increased in response to 30 minutes of 100% hyperoxia in healthy men indicating a time-dependent component to the physiological responses to hyperoxia (8). Becker et al. (1996) discovered that 30 minutes of isocapnic hyperoxia increased ventilation by 10% even if ET-CO_2 is allowed to fall (8). The authors suggest that this increase in ventilation could be as a result of the "Haldane effect" whereby the binding capability for CO_2 is reduced during hyperoxia (44). During hyperoxic administration, carbon dioxide is displaced from hemoglobin by oxygen. This in turn increases the arterial partial pressure of carbon dioxide ($PaCO_2$) resulting in hyperpnea from central chemoreflex activation to subsequently lower arterial carbon dioxide (44).

Johnston et al. (2003) compiled a comprehensive review that looked at the effects of hyperoxia on cerebrovasculature (71). Many studies suggest that hyperoxia exposure attenuates cerebral blood flow in healthy individuals (78, 106, 111, 159). In particular, Kety & Schmidt (1948) investigated hyperoxic effects on calculated cerebral blood flow in young healthy men and found that 100% O_2 reduced cerebral blood flow by 13% (78). An increase in cerebrovascular resistance in response to 100% O_2 was observed indicating vasoconstriction of arteries within the brain reducing brain blood flow (78). Similarly, both Nakajima et al. (1983) and Watson et al. (2000) found a decrease in cerebral blood flow in response to 100% O_2 administration by 16.2% (106) and over 20% at rest (159), respectively. In contrast, Xu et al. (2012) found that hyperoxia exposure left cerebral blood flow unchanged in healthy participants (162). This could be due to the length of exposure to hyperoxia or to a preceding period of hypoxia; their participants first breathed room air (21% O_2) for 8 minutes, followed by hypoxia (14% O_2) for 18 minutes, hyperoxia (50% O_2) for 15 minutes and finally hyperoxia (100% O_2) for 12 minutes (162).

Carbon Dioxide

Much like hypoxia's stimulation of the peripheral chemoreceptors, hypercapnia exerts a powerful stimulatory effect on the central chemoreceptors to promote increases in blood pressure, heart rate (78, 91, 151) and ventilation (8, 33, 68, 78, 91, 143) in healthy individuals through activation of the sympathetic nervous system. As mentioned earlier, the peripheral chemoreceptors detect decreases in partial pressures of arterial oxygen (33, 64, 108); however, they are also able to detect increases in partial pressures of carbon dioxide, but to a lesser extent (74, 102, 110). The location of the central chemoreceptor was the point of some debate until Schlaefke (1981) found that the rostral and caudal chemosensitive areas located on the ventrolateral aspect of the medulla resulted in ventilatory and circulatory changes upon stimulation in cats (127). Further investigation determined that intermediate neurons existed which join these areas to the respiratory and cardiovascular controllers within the medulla (127). This autonomic reflex is one of many able to regulate and influence the specific areas of the brain, such as the medulla, to elicit reflexive responses. Increases in $PaCO_2$ stimulate respiratory centres, resulting in hyperpnea to eliminate excess carbon dioxide. As $PaCO_2$ decreases, this in turn suppresses the central chemoreceptors to decrease ventilation to maintain $PaCO_2$ at homeostatic levels.

Kara et al. (2003) observed that acute hypercapnic stress stimulated the central chemoreceptors to significantly increase sympathetic activity, ventilation and mean arterial pressure in healthy subjects (74). Central chemoreceptor stimulation via hyperoxic hypercapnia in healthy humans was also shown to elicit an acute increase in sympathetic activity resulting in elevated systolic blood pressure and an increase in vascular resistance (103). These results were supported by Usselman et al. (2015) who found significant increases in sympathetic activity, mean arterial pressure, and total peripheral resistance in response to hypoxic hypercapnia (however, both peripheral chemoreceptors and central chemoreceptors were stimulated) (151).

Early investigations in animals sought to isolate chemoreceptor activation through denervation of the peripheral chemoreceptors. However, results suggested that animals continued to respond with a substantial increase in ventilation in response to hypercapnia indicating that the central chemoreceptors are excited by acidity or

changes in carbon dioxide levels irrespective of the peripheral chemoreflex (87, 94, 161). Schwartz, Brackett, Jr., & Cohen (1965) found that canines exhibited linear and marked rises in hydrogen ion concentration as $PaCO_2$ incrementally increased effectively reducing pH and stimulating respiratory centres (129). This was subsequently confirmed by O'Regan & Majcherczyk (1982) who suggested that the central chemoreceptors respond to changes in hydrogen ion concentration in the interstitial fluid of the brain and thus disturbances of acid-base are responsible for ventilatory and circulatory adjustments during hypercapnia (110).

$$CO_2 + H_2O \rightarrow H^+ + HCO_3^-$$

These results were confirmed by Harada et al. (1985) when they investigated the effect of pH and hypercapnia on respiratory drives (59). They found that by keeping $PaCO_2$ constant by continuous equilibration of the solution with 5% CO_2 and lowering bicarbonate (HCO_3^-) to decrease pH, respiratory activity was higher than when pH was high as a result of higher HCO_3^- concentration (59). They concluded that bicarbonate may act independently as a stimulus to the chemoreceptor (59). This study, however, was performed using a rodent model *in vitro*, and must not be attributed to human physiology. Loeschcke (1982) discovered that it was ultimately the extracellular pH that is the main chemical signal determining ventilation in felines and that pH values are dependent on bicarbonate concentration as a result of altered $PaCO_2$ levels (93).

Evidence suggests that hypercapnic stimulus provokes larger increases in ventilation and sympathetic activity compared to hypoxic stimulus (21, 143). Somers et al. (1989) suggested that an inhibitory interaction exists between pulmonary afferents (lung stretch receptors) and peripheral chemoreceptors resulting in an attenuated ventilatory response to hypoxia, but this interaction does not exist with the central chemoreceptors (143). This implies that chemosensitivity in the central chemoreflex is greater than the peripheral chemoreflex. Interestingly, Smith et al. (2006), showed that central chemoreceptors account for approximately 63% of the steady-state ventilatory sensitivity to hypercapnia while the remaining sensitivity, approximately 37%, was due to the peripheral chemoreceptors at rest (139). Similarly, Gelfand & Lambertsen (1973)

showed that approximately 12% of the ventilatory response to hypercapnia in hyperoxic humans (peripheral chemoreceptor suppression) was still a result of peripheral chemoreflexes (53). These findings suggest that while hypoxia has been shown to only affect peripheral chemoreceptors (33, 64, 108), hypercapnia stimulates both the peripheral and the central chemoreceptors (74, 102, 110). However, for the purposes of this thesis, the CO_2 chemoreflex will be referred to as the "central chemoreflex" for clarity. Both additive responses, where the stimulation of one reflex does not influence the other (20, 102) and hyperadditive responses, where the stimulation of one receptor augments the sensitivity of the other (30, 66), have been found to exist in response to a hypercapnic-hypoxic stimulus.

Kontos, Richardson, & Patterson, Jr. (1968) investigated the vasodilatory effects of hypercapnia on healthy human forearm vasculature (81). They observed that hypercapnic acidosis was associated with increases in blood flow with no change in blood pressure and therefore they concluded greater peripheral vasodilation counteracts the increases of sympathetic activity during rest (81) which was confirmed more recently by Lipp et al. (2010). Similarly, cerebral vasculature is known to be reactive to hypercapnia. Initial research conducted by Kety & Schmidt (1948) investigated the effects of altered arterial tensions of carbon dioxide on cerebral blood flow (78). In response to 7% CO_2, they found that brain blood flow increased significantly by 75% (78). They concluded that these responses reflected cerebral vasodilation (78). Furthermore, Reivich (1964) studied the effects of $PaCO_2$ levels on cerebral hemodynamics in the rhesus monkey (118). They observed that when $PaCO_2$ approached low concentrations (10-15 mmHg), cerebral blood flow was diminished and vascular resistance increased via vasoconstriction (118). Further, at higher levels of $PaCO_2$ (150 mmHg), cerebral blood flow increased and vascular resistance decreased via vasodilation (118). More recently, Ito et al. (2003) investigated changes in human cerebral blood flow and volume in response to hypercapnia in healthy subjects under resting conditions (69). They similarly concluded that 7% CO_2 caused significant increases in both cerebral blood flow and volume (69). This evidence suggests that hypercapnia increases cerebral blood flow by directly dilating vessels in the brain and reducing resistance.

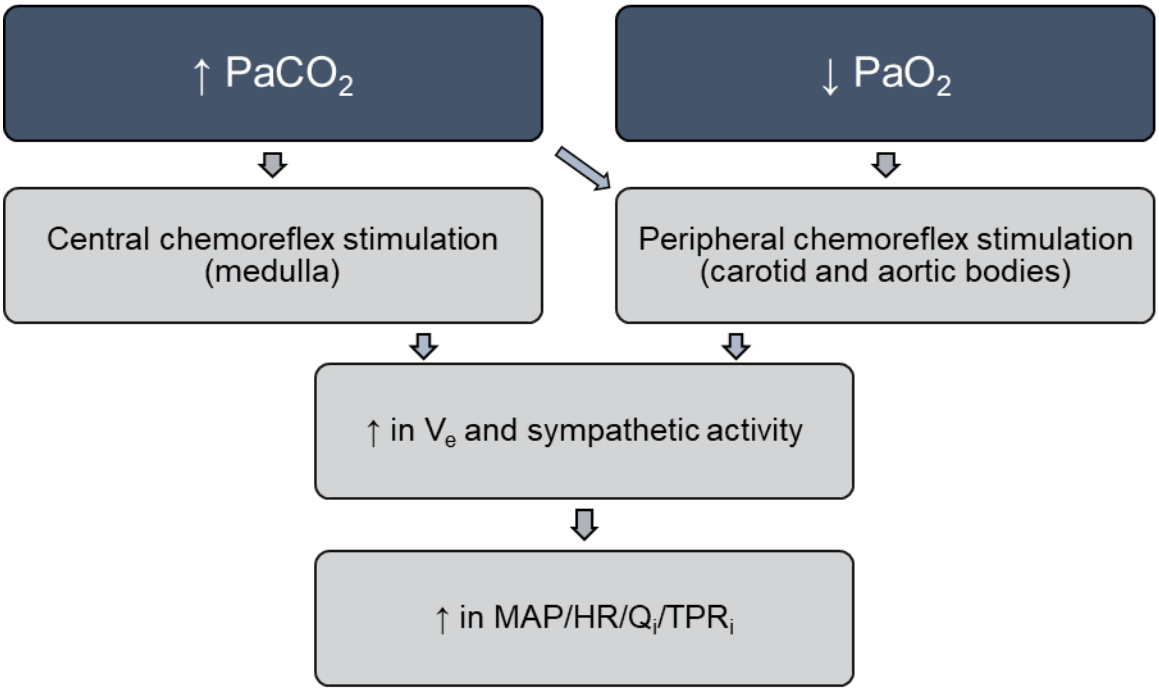


Figure 1: Flow diagram of central and peripheral chemoreflex function

Orthostatic Stress

In general, orthostatic stress elicits increases in ventilation (17, 18, 54, 92, 101, 137, 164), end-tidal oxygen (ET-O_2) (92, 101), heart rate (5, 13, 54, 109, 165), mean arterial blood pressure (27, 141, 165), total peripheral resistance (25, 27, 105, 109, 126, 141), sympathetic activity (26, 87) and cerebrovascular resistance (109, 131) with decreases seen in brain blood flow velocity (109, 131), brain blood flow (89), stroke volume (165), cardiac output (54, 141, 165) and ET-CO_2 (18, 54, 92, 101, 109, 164) in healthy individuals. Immediately after moving into an upright posture, there is an immediate drop in mean arterial pressure and as a result, central venous pressure, venous return, and stroke volume decrease. The immediate fall in blood pressure inactivates baroreceptors leading to an increase of sympathetic activity resulting in an increase of heart rate and total peripheral resistance further leading to a recovery of blood pressure (27, 105).

Upon passive head-up tilt (HUT), pulmonary blood volume is redistributed to the lower half of the lung, and the mechanical descent of the diaphragm into the abdominal

cavity produces an increased functional residual capacity (26, 97) leading to increased ventilation (V_e) and tidal volume (V_t)(18). These findings were further supported by Chadha et al. (1985), Gisolf et al. (2004) and Loeppky & Luft (1975) where an increase in functional residual capacity (FRC) was observed in passive HUT to 60^o in healthy individuals (17, 54, 92). These responses ultimately cause tidal volume to increase (17, 54, 92, 101, 164) without a concurrent decrease in breathing rate (18) leading to greater ventilation and therefore lower ET-CO_2.

Orthostatic stress leads to higher sympathetic nerve activity (27, 57, 88) and heart rate (5, 13, 54, 109, 165) with a concurrent decrease in stroke volume (54, 109, 141, 165) to maintain cardiac output and mean arterial pressure. Interestingly, Convertino (2014) suggests that the integrated control of heart rate requires a rapid response (parasympathetic; i.e. vagal withdrawal) to enhance tachycardia during the onset of orthostatic stress followed by extended (sympathetic) maintenance of elevated heart rate (24). The contribution of peripheral vasoconstriction from sympathetic activity during orthostasis has also proven to be an important regulator of orthostatic tolerance. The increased vascular resistance represents a fundamental compensatory mechanism for maintaining arterial pressure in response to reduced cardiac filling during lower body negative pressure (LBNP)(25, 126).

Interactions between autonomic reflexes have previously been studied. For example, the effects of hypoxia and hypercapnia in conjunction with postural changes (i.e. interactions between the chemoreflexes and the baroreflex) have been investigated (41, 46, 137, 147). Somers, Mark, & Abboud (1991) investigated the interaction between baroreflex and chemoreflex control of sympathetic nerve activity in healthy men and women in a combined group (142). They found that sympathetic nerve activity was attenuated upon stimulation of both the peripheral chemoreceptors (via hypoxia) and baroreflexes (via phenylephrine) as opposed to just hypoxia (142). They concluded that activation of baroreceptors by increases of arterial pressure markedly inhibited the sympatho-excitatory response to stimulation of the peripheral chemoreceptors by hypoxia (142). This inhibition of sympathetic activity as a result of baroreflex interaction with the peripheral chemoreceptors was not evident when investigating baroreflex and central chemoreceptor interaction as sympathetic activation was significantly higher

after phenylephrine infusion in the presence of hypercapnia (142). This suggests that baroreflex inhibition in the tilted position could influence chemoreflex stimulation. Further, during post-exercise circulatory occlusion to activate the metaboreflex, it was discovered that the activated metaboreflex can activate the peripheral chemoreflex in healthy men in the absence of hypoxia (39). This was determined via a suppression of muscle sympathetic nerve activity during hyperoxia administration with occlusion (39).

Richardson et al. (2002) showed that the ventilatory response to hypercapnia in healthy subjects was larger during HUT compared to the supine position suggesting the changes in ventilation reflect augmented central chemoreflex sensitivity in the tilted position; however, it is important to note that neither sex nor menstrual cycle were specifically investigated in this study as the sex of the volunteers were unspecified (119). Taneja et al. (2011) investigated the effects of central and peripheral chemoreceptor activity in the 70^{o} HUT position in healthy individuals (mixed-sex design) (147). In response to hyperoxic hypercapnia during HUT, individuals experienced significant increases in minute ventilation, sympathetic activity, breathing rate, ET-O_2, mean arterial pressure, cardiac output and stroke volume (147). The magnitude of these increases was larger than those seen in the supine position suggesting heightened central chemoreflex sensitivity in the tilted position (147). In contrast, Skow et al.(2010) found no change in ventilation upon graded orthostatic stress (supine to 90^{o}) with or without hyperoxic hypercapnia (via rebreathe) in healthy men and women (combined) concluding that HUT had no effect on the ventilatory response to hypercapnia (137). In response to eucapneic hypoxia in HUT, individuals were seen to have decreases in stroke volume and increases in heart rate compared to the supine position (147). These findings, together, suggest that peripheral chemoreceptor sensitivity is enhanced in the tilted position.

Cerebral autoregulation has been defined as the physiological regulatory mechanism that maintains a constant level of flow over wide ranges of perfusion pressure in the brain (84). Levine et al. (1994) presented two hypotheses whereby syncope, defined as a reduction in cerebral blood flow sufficient enough to result in a loss of consciousness, can occur in the face of orthostatic stress (89). The more commonly accepted hypothesis explains that the fall in cerebral blood flow is secondary

to either an excessive decrease in central blood volume or to inefficient neurohumoral responses to orthostasis (11). The second hypothesis suggests that failure of cerebral autoregulation could compromise cerebral blood flow during orthostatic hypotension (57); however, Levine et al. (1994) argues that subjects used in the latter investigation poorly represented normal physiology as they all had a history of recurrent, unexplained episodes of syncope and most of them received isoproterenol (a beta-adrenergic agonist) during their clinical evaluation (89). Levine et al. (1994) found that upon graded LBNP (orthostatic stress), healthy subjects experienced decreased cerebral blood flow velocity with a greater pulsatility index (PI) suggestive of greater cerebral vasoconstriction (89). These results were supported by Serrador et al. (2006) where healthy subjects were tilted to 85^{o} for 10 minutes (131). During tilt, they observed a significant drop in $PaCO_2$ leading to significant reductions in middle cerebral artery velocity due to increased cerebrovascular resistance (131). Furthermore, these findings were augmented during hyperventilation which resulted in cerebral vasoconstriction (presumably due to a gradual decline seen in ET-CO_2) (131). Similarly, Edwards et al. (2002) investigated the effects of hypercapnia on cerebral hemodynamics in the 45^{o} HUT position in healthy individuals (41). While cerebrovascular resistance index (CVR_i) decreased in HUT under all conditions, CVR_i was significantly higher in hypocapnia and lower in hypercapnia compared to normoxia in both the supine and HUT positions (41).

Sex Differences

It is evident from most research looking at chemoreflex functioning that investigations have primarily either looked at animal models (21, 22) or men (18, 92, 101, 164) which has left a gap in physiological research regarding sex differences. Medical students, comprised of men and women, anonymously self-reported the prevalence, triggers and recurrence rate of syncope which ultimately showed that women experienced a higher prevalence of syncope compared to men (51). Similarly, Convertino (1998) showed that LBNP tolerance was significantly lower in women compared to age and fitness-matched men as greater reductions in stroke volume, cardiac output and mean arterial pressure were seen upon orthostatic stress in women (23). These results have also been seen in other studies investigating hemodynamic

responses to passive orthostatic stress between men and women (47, 98). In order to investigate sex-differences in chemoreflex function in upright posture, Taneja et al. (2010) investigated the effects of a breath-hold in healthy men and women during postural changes (supine and 60º HUT) (146). During breath-holding, men had increases in muscle sympathetic nerve activity (MSNA) burst frequency in both the supine and upright tilted positions, whereas women exhibited an increase only while supine (to a smaller magnitude than men) indicating suppression of chemoreflex induced sympathetic output in upright posture in women (146). The MSNA increases seen in men were greater in tilt compared to the supine position (146). This suggests that combined hypoxic-hypercapnia (breath-hold) and orthostatic stress influence MSNA differently in men and women. Furthermore, the absence of a sympathetic response to hypoxic-hypercapnia in upright posture observed in women may contribute to their greater prevalence of orthostatic intolerance compared to men (146).

Jarvis et al. (2010) found that women had impaired splanchnic vasoconstriction in the 70º HUT position compared to men which could lead to increased orthostatic intolerance via splanchnic blood pooling (70). In the resting, supine position, women had lower splanchnic blood flow and splanchnic vascular conductance compared to men (70). However, splanchnic vascular conductance decreased significantly in men from baseline to tilt, yet it did not decrease in women suggesting blunted vasoconstriction and therefore splanchnic blood pooling during an orthostatic challenge (70). Greater splanchnic pooling would contribute to reduced venous return and therefore lower stroke volume.

Cerebral hemodynamics between healthy men and women have been previously studied (32, 38). Investigating cardiovascular and cerebral hemodynamic responses to posture changes in young, healthy men and women, Edgell et al. (2012) found that men had significantly lower systolic and diastolic middle cerebral artery velocities and higher cerebrovascular resistance index compared to women, regardless of position (38). These results were likely due to the vasodilatory effects of estrogen (women were studied in the late follicular phase when there is estrogen present without progesterone) reducing cerebrovascular resistance index (113).

Very little is currently known about ventilatory and cerebrovascular responses between men and women in upright posture. Sébert & Sanchez (1981) looked at the respiratory effects of a hypoxic-hypercapnic breath-hold between healthy men and women in the supine and seated position (130). Ventilatory responses were found to increase similarly between the sexes following the breath-hold in both the supine and seated positions (130) despite previous observations that MSNA differs between the sexes in an upright breath hold (146). Cerebrovascular CO_2 reactivity differences have been investigated between men and women in the supine position (75). Healthy subjects had their middle cerebral artery reactivity measured while breathing 95% O_2 and 5% CO_2 (75). When plotting mean middle cerebral artery velocity responses as a function of increasing ET-CO_2 levels, women had a steeper mean slope compared to men suggesting they have higher cerebrovascular CO_2 reactivity (i.e. vasodilation) compared to men (75). This implies women have heightened cerebral vasoreactivity compared to men which may lower the hypercapnic stimulus threshold needed to trigger vasodilation. However, it is important to note that in the study by Kastrup et al. (1997), the vasoconstrictive properties of 95% hyperoxia oppose the vasodilatory properties of 5% hypercapnia.

The respiratory muscle pump has been observed to play a critical role in the maintenance of venous return through a pressure gradient that exists between peripheral and thoracic veins (133). As a result of inspiration, the pressure within the chest averages approximately 5 mmHg less than atmospheric pressure (133). Since peripheral venous supply is subjected to normal atmospheric pressures, an externally-applied pressure gradient exists whereby blood is driven back to the heart (133). Thus, increasing respiratory activity promotes increased venous return in both men and women (133). This mechanism of regulating venous return can further be influenced by sexually-dimorphic differences in anatomy. An investigation observing the effects of sex and position on thoraco-abdominal kinematics was conducted on 34 healthy men and women (122). Specifically, they found that women have higher thoracic versus abdominal contribution to tidal volume than men (122). They suggest that this could contribute to the significantly reduced ventilation observed in women compared to men (122).

Menstrual Cycle

To date, very little research has investigated how menstrual cycle differences could affect chemoreflex activation. Peggs et al. (2012) showed that women in the early-follicular (EF) phase of the menstrual cycle (EF; days 2-5) reported significantly higher levels of light-headedness compared to when they were in their mid-luteal phase (ML; days 18-24) (114). These differences could be attributed to sex hormone concentrations since it has been established that the EF phase is when female sex hormones (estrogen and progesterone) are found to be at their lowest while the ML phase is when they are found at high levels, suggesting orthostatic intolerance in women could be associated with an absence of female sex hormones. Slatkovska et al. (2006) investigated the effects of menstrual cycle phase on the control of breathing in healthy women and observed that women during the ML phase had higher ventilation compared to women in the EF phase at rest even though $PaCO_2$ was significantly lower in the ML phase (138). This suggests women in the ML phase have augmented central chemoreflex activity at rest compared to women in the EF phase which could ultimately enhance orthostatic tolerance by increasing sympathetic output.

The effect of circulating sex hormones has been investigated previously to determine their influence on ventilation in women (35, 95). Dombovy et al. (1987) tested the hypoxic and hypercapnic ventilatory response in 8 healthy women throughout their menstrual cycle through a graded exercise challenge protocol (35). They discovered that women during their luteal phase of their cycle had significantly higher ventilation compared to their follicular phase in response to these stimuli throughout the progressive workloads (35). Similarly, Machida (1981) studied the influence of progesterone on arterial blood and cerebrospinal fluid acid-base balances in 36 healthy women during their menstrual cycle under resting conditions (95). They found that cerebrospinal fluid and $PaCO_2$ were significantly lower during the luteal phase compared to the follicular phase (95).

Fu et al. (2009) conducted an important study that investigated the impact of menstrual cycle phase on MSNA in response to graded orthostatic stress (47). Women were tested during the EF and ML phases (47). Women in the EF phase had significantly lower total sympathetic activation during upright tilt compared to the ML

phase (which correlates to greater feelings of light-headedness in this phase (114)) (47). In accordance with these findings, Minson et al. (2000) studied the influence of menstrual cycle on sympathetic activity in young, healthy women and found that resting plasma norepinephrine levels were also significantly higher in the ML phase compared the EF phase (100). Interestingly, it was observed that sympathetic baroreflex sensitivity was higher in women during the ML phase compared to the EF phase implying baroreflex-mediated responses may be influenced by female sex-hormones (100). Therefore, autonomic reflex interactions may play a role in orthostatic intolerance throughout the menstrual cycle.

METHODS

PARTICIPANT DESCRIPTION

All participants were recruited from York University, Keele Campus using online social media, posters, word-of-mouth as well as the KURE database used in the Kinesiology course; Research Methods of Kinesiology (KINE 2049). Inclusion criteria for participation included: 1) Be between 18-30 years of age, and for females, 2) Have a normal menstrual cycle (~28 days). Exclusion criteria for participants included: 1) No previously diagnosed cardiovascular or respiratory disease/dysfunction, and 2) Not be taking any oral contraceptives or have had any for at least one month prior to testing. All subjects were asked to refrain from smoking, heavy exercise, the consumption of fatty/processed foods as well as caffeinated and alcoholic beverages 12 hours prior to testing. Participants were also encouraged to eat a light breakfast and/or lunch before coming in for testing.

Men were tested once (n=13) while women were tested twice (n=14), once during the early-follicular phase (EF; days 2-5) and once during the mid-luteal phase (ML; days 18-24) of their menstrual cycle (Table 1). This assumed day 0 was the first day of menstruation. Female sex hormones (estrogen and progesterone) should be at their lowest concentrations during the EF, compared to the ML when these concentrations are at high levels. Cycle was determined by self-report.

Height (cm) and body mass (kg) were determined by a mechanical beam scale (Health O Meter Professional). These values were used to calculate body surface area (BSA; m^2) using the DuBois and DuBois formula (12);

$$\mathbf{BSA\ (m^2) = 0.007184 \times (weight^{0.425} \times height^{0.725})}$$

BSA was used to normalize hemodynamic variables such as cardiac output (Q_i), stroke volume (SV_i), and total peripheral resistance (TPR_i). The forced expiratory volume in one-second test (FEV_1/FVC) was measured by a heated, linear-pneumotachometer (Hans Rudolph, Series 3813) using spirometry. Self-reported physical activity levels and frequency were recorded and used as a prediction of cardiorespiratory fitness using the Ainsworth equation to obtain an index of $VO_{2\ max}$ (2);

$$\mathbf{VO_{2\ max} = 65 + 1.8(Frequency\ of\ Exercise/Week) - 10(Gender;\ Males = 0,\ Females = 1) - 0.3(Age) - 0.6(BMI)}$$

Table 1: Participant Anthropometrics

	Men	Women	
		EF	ML
n	13	14	
Age (Years)	22.8±1.1	22.8±0.8	
Height (cm)	174.0±1.7$^{\sigma}$	159.6±1.3	
Body mass (kg)	74.9±2.9$^{\alpha\beta}$	60.9±2.8	60.1±2.7
Predicted VO_2 max ($mL \cdot kg \cdot min^{-1}$)	53.0±1.1$^{\sigma}$	41.1±1.3	
BMI (kg/m^2)	24.7±0.7	24.0±1.2	23.6±1.0
FEV_1 (%)	81.7±2.0	84.0±2.2	

EF is the early-follicular phase, ML is the mid-luteal phase, VO_2 max is predicted maximal oxygen consumption, FEV_1 is the forced expiratory volume in one second test and BMI is body mass index. α indicates a main group effect (men vs ML; p=0.001), β indicates a main group effect (men vs EF; p=0.002), σ indicates a main sex effect (men vs women).

MEASUREMENT OF VARIABLES

Cerebral Hemodynamics

A Transcranial Doppler system (TCD; Multigon Industries Inc.) was used to quantify middle cerebral artery (MCA) velocity by using non-invasive ultrasound. A 2 MHz probe was secured in position at the right temporal window by a headband. The cerebrovascular resistance index (CVR_i), resistance index (RI) and pulsatility index (PI) were calculated using the mean, systolic and diastolic values recorded from the Doppler ultrasound (see below). For the supine position, mean arterial pressure (MAP) was used to calculate CVR_i, however, cerebral perfusion pressure (CPP) was used for the tilted positions to account for the height difference (cm) measured from the heart to the ultrasound probe;

$$\mathbf{CVR_i(supine) = MAP/mean\ V_{MCA}, \text{ where } V_{MCA} \text{ is middle cerebral artery velocity}}$$

$$\mathbf{CVR_i(tilt) = CPP/mean\ V_{MCA}, \text{ where } CPP = MAP\ (Distance \times 0.7355\ mmHg/cmH_20)}$$

$$\mathbf{RI = (max\ V_{MCA} - min\ V_{MCA})/max\ V_{MCA}}$$

$$\mathbf{PI = (max\ V_{MCA} - min\ V_{MCA})/mean\ V_{MCA}}$$

These indices are important in determining how cerebral vasculature responds to orthostatic and chemoreflex challenges. The RI, also termed the 'Pourcelot index' (116), and PI were developed to evaluate and compare Doppler waveforms to give an index of vascular resistance in the brain. As downstream impedance increases, the amount of diastolic flow decreases and as a result, RI and PI increase (56, 120).

Cardiovascular Hemodynamics

An electrocardiogram (ECG) was collected by a standard II-lead configuration where three disposable electrodes (3M Red Dot) were applied; one inferior to each clavicle on the delto-pectoral grooves and one on the left iliac fossa (left lower

abdomen). Heart rate was continuously calculated from raw ECG data and was used to quantify heart rate variability and cardiovagal baroreceptor sensitivity. Beat-by-beat continuous blood pressure and cardiac output (Q; L/min) were recorded using a non-invasive finger-cuff. Cardiac output was calculated using the Modelflow algorithm (Finometer, Finapres Medical Systems). Stroke volume (SV; ml/beat) was calculated as a quotient of Q and heart rate (HR; bpm). Blood pressure measurements were corrected for finger placement using a standard height correction unit (HCU; ADInstruments, Human NIBP Height Correction Unit – MLT0902) that allowed the Finometer (Finapres Medical Systems) to continually correct for hydrostatic pressure changes if the hand moved below or above heart level. Total peripheral resistance (TPR; mmHg/(L/min)) was calculated as a quotient of mean arterial pressure (MAP; mmHg) and Q. A blood pressure measurement (Return-to-flow function of the Finometer) was taken at the beginning of each test to ensure accurate readings throughout continuous recording and to calibrate recorded values. Further, an automated single blood pressure measurement (BpTRU, BPM-100/200) was taken before each trial to ensure subjects reached baseline pressure between trials and to further calibrate the readings provided by the Finometer. Pulse wave data was collected at the ventral aspect of the left hallux (ADInstruments, Pulse Transducer TN1012/ST) and was used to measure pulse wave velocity (discussed below).

Respiratory Variables

Spirometry was collected by a heated, linear-pneumotachometer (Hans Rudolph, Series 3813). Volumes inspired and expired were calculated as positive and negative integrals of the spirometry channel, respectively. Tidal volume (V_t; L) was calculated as a cyclical measurement of the maximum peaks of the volume inspired while breathing rate was calculated as a cyclical measurement of the rate of inspiration. Lastly, ventilation (V_e; L/min) was recorded as a product of breathing rate (breaths/min) and V_t. The pneumotachometer was calibrated using a 3 Litre calibration syringe (Hans Rudolph, Series 5530) before each test. Oxygen (mmHg) and carbon dioxide (mmHg) levels were measured through O_2 and CO_2 analyzers (Vacumed, Model 17620/17630). End-tidal oxygen levels (ET-O_2; mmHg) were calculated as the minimum O_2 value at the

end of exhalation and end-tidal carbon dioxide levels (ET-CO_2; mmHg) were calculated as the maximum CO_2 values at the end of exhalation. Oxygen and carbon dioxide measurements collected and analyzed by the Vacumed devices were calibrated according to the daily barometric pressure provided by York University's Meteorological Observation station.

All signals obtained were relayed to a Powerlab (ADInstruments, PowerLab 16/35) data acquisition system which compiled analog data signals and converted them into digital signals. This data was then collected in Labchart software (ADInstruments, Version 8.1.3). One-minute averages for all hemodynamic, respiratory and cerebral variables were recorded at specific time-points from every trial. In the supine trials, one minute of data was selected from the last minute of baseline recording and the second minute of gas administration. In the tilted trials, one minute of data was selected from the last minute of tilt (pre-gas administration), and the second minute of gas administration (while in tilt).

PROTOCOL

Subjects were asked to come in approximately 15 minutes before testing to become familiarized with the design protocol, to read and sign the informed consent and to collect anthropometric measurements such as height (cm) and body mass (kg). Each test was comprised of a total of six trials, three of which were in the supine position and three in 70º HUT.

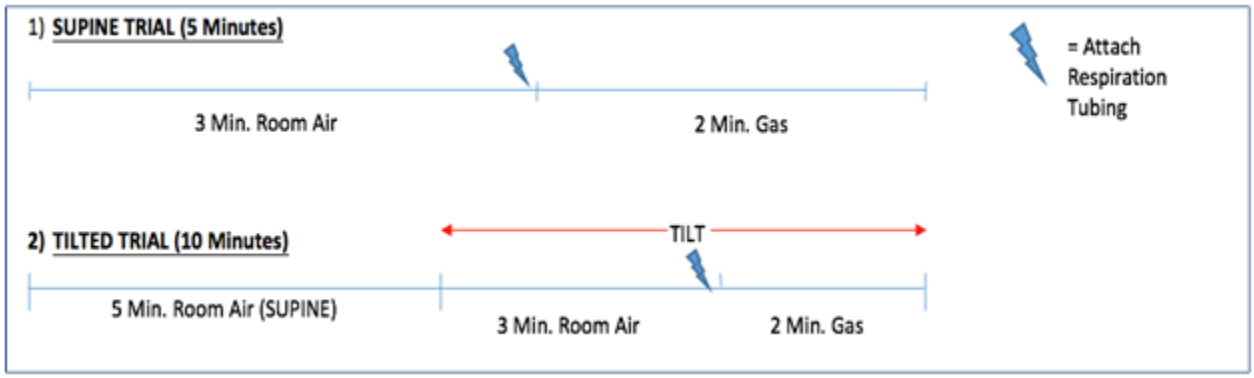


Figure 2: The time-line of the supine and tilted trials

In each position, medical-grade gases were administered for two minutes. These gases included; 1) A hypercapnic mixture (5% CO_2, 21% O_2, nitrogen balance), 2) A hyperoxic mixture (100% O_2), and 3) A normoxic mixture (0.03% CO_2, 21% O_2, nitrogen balance). Gas flowed through a portable humidifier (Fisher & Paykel Healthcare, HC 150 Ambient Tracking) prior to inhalation. Data collection did not exceed 2.5 hours and a washout period of 5 minutes was implemented in between each trial.

At the end of all trials, subjects were asked to perform the FEV_1/FVC to assess their pulmonary function and to validate recruitment of healthy participants. Participants were blinded and randomized to the trials. The trial order was determined using a function that randomly assigns a number to each of the six trials (RAND function) and then subsequently ranking (RANK function) those random numbers (Microsoft Excel, Version 15.26).

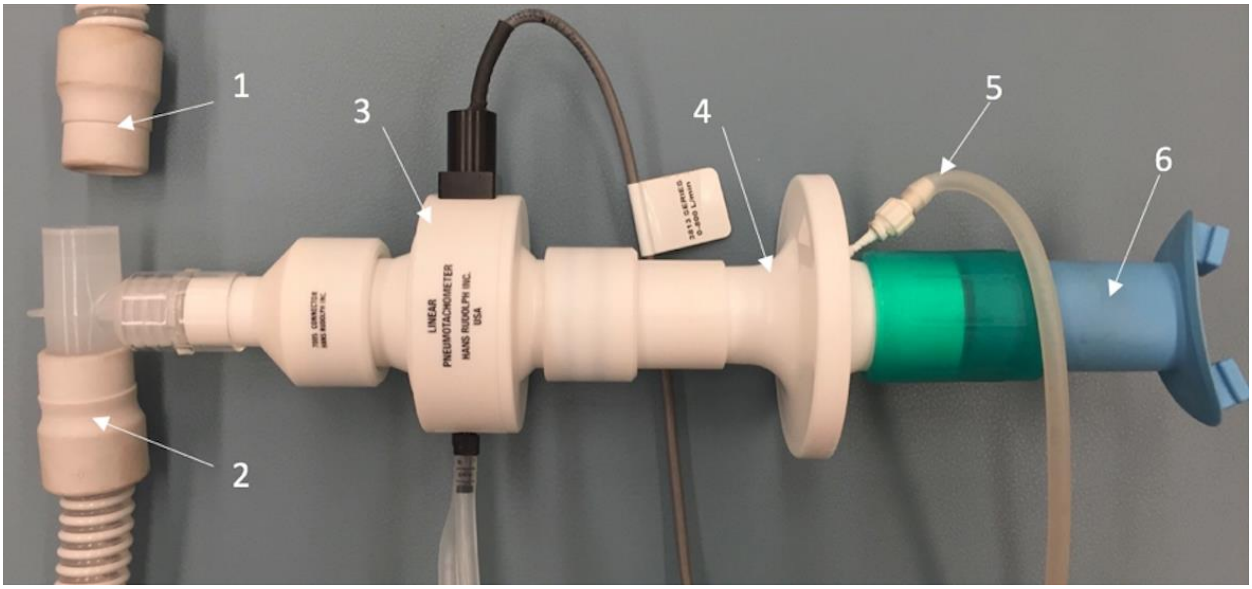


Figure 3: The respiratory apparatus. 1) Detached inhalation tube, 2) Attached exhalation tube, 3) Pneumotachometer, 4) Filter, 5) Gas analyzers, 6) Mouth piece

AUTONOMIC INDICES

Heart Rate Variability (HRV)

Heart rate variability (HRV) is used to assess autonomic function (86) and is determined by vagal and sympathetic influences of the heart (15). Given its validation as a measure of autonomic functioning, HRV was used to help quantify parasympathetic and sympathetic tone in our participants. Five minute sections of ECG data were selected from the supine normoxic trial as well as the entire HUT portion of the tilted normoxic trial in order to compare HRV between the supine and HUT positions. Labchart software (ADInstruments, Version 8.1.3) was used for power-spectral analysis to determine time-domain (SDRR) and frequency-domain measurements. The low-frequency (LF) to high-frequency (HF) ratio (LF/HF) obtained were used to gauge how much of the variability seen was due to either sympathetic (LF) or parasympathetic (HF) tone during the supine and HUT positions.

Cardiovagal Baroreceptor Sensitivity (cBRS)

Five minute sections of ECG and beat-to-beat blood pressure data were selected from the supine normoxic trial and the entire HUT portion of the tilted normoxic trial. We used this data to compare the cBRS in the supine and HUT positions. Sequences of three or more beats in which systolic blood pressure (SBP; mmHg) and the R-R interval (ms) changed in the same direction (either increasing or decreasing) were identified and linear regressions were performed to be averaged that created a single slope expressing cBRS (spontaneous method) (9, 10).

Pulse-Wave Velocity (PWV)

Traditionally, arterial stiffness describes the ability of an artery to expand and contract in response to blood pressure changes (16); however, we used it a surrogate marker for sympathetic activity since vasoconstriction will result in arterial stiffening. Current evidence suggests a correlational relationship exists between central arterial

stiffness (carotid-femoral PWV) and muscle sympathetic nerve activity in healthy men (143).

While carotid-radial and carotid-femoral PWV have been shown to be valid measurements of peripheral and central arterial stiffness, respectively, this research examined finger-toe PWV which has been shown to be significantly correlated with carotid-radial PWV in young adults (40). Although a more common method of measuring arterial stiffness, carotid-femoral (or radial) applanation tonometry has been shown to be both expensive and technically difficult, requiring two technicians and costly equipment (40), thus a more simplified method of measurement (i.e. finger-toe) was used for this study. Both the finger and toe pulse waves were measured continuously using the Finometer (Finapres Medical Systems) and a toe pulse transducer (ADInstruments, Pulse Transducer TN1012/ST), respectively.

PWV = ΔD between two pulse waves (m) / Δt between two pulse waves (s)

The change in distance (ΔD) was calculated as; the distance from the sternoclavicular notch (as an index of the heart) to the Finometer finger cuff subtracted from the distance from the sternoclavicular notch to the toe. The change in time (Δt) was calculated as: the time of the foot of the blood pressure wave form of the finger (i.e. end-diastole) subtracted from the time of the foot of the pulse wave form of the toe using the foot-to-foot method (85). Pulse wave velocity was calculated from at least 20 consecutive heart beats from all supine trials at baseline and the second minute of gas administration.

STATISTICS

All results were analyzed using data analysis software (Sigmaplot, Version 12.0). Data was analyzed at two time-points within each trial, the minute prior to gas administration and the second minute of gas administration. Heart-rate variability and cardiovagal baroreceptor sensitivity was analyzed during five minutes of both supine

data and HUT data during normoxia administration. Responses were compared between the supine and tilted trials within each gas (i.e. supine hyperoxia versus tilted hyperoxia). It is statistically impossible to compare men directly to both phases of the menstrual cycle concurrently since men were only tested once and women were tested twice. Therefore, sex differences were investigated between men and each group of women by using multiple two-way repeated-measures ANOVAs (Sex and Posture (repeated) as factors). Women were analyzed using a two-way repeated-measures ANOVA (Menstrual phase (repeated) and Posture (repeated) as factors). Post-hoc analysis of interaction effects used Tukey's HSD test to determine which groups statistically differed from one another.

RESULTS

Supine Normoxia

Men had a significantly higher stroke volume index compared to women during the early-follicular phase (p=0.004) at baseline and during normoxia and mid-luteal phase (p=0.006) at baseline (Table 2). Women had a significantly higher total peripheral resistance index compared to men in both the early-follicular phase and mid-luteal phase (p<0.001) at baseline and during normoxia (Table 2). There were no significant main effects of time or menstrual phase on heart rate, mean arterial pressure or cardiac output index (Table 2).

Men had a significantly higher cerebrovascular resistance index compared to women during the mid-luteal phase (p=0.007) at baseline and during normoxia (Table 2); however, this difference was not evident compared to women during the early-follicular phase (Table 2). Women during the mid-luteal phase had significantly higher mean middle cerebral artery velocity (MCA) compared to men (p<0.036) at baseline and during normoxia (Table 2). There were no significant time or phase effects of systolic MCA, diastolic MCA, mean MCA, cerebrovascular resistance index, pulsatility index or resistance index at baseline and during normoxia (Table 2).

Men had significantly higher tidal volumes compared to women during both the early-follicular phase (p=0.004) at baseline and during normoxia; however, this

difference was not evident compared to women during the mid-luteal phase (Table 2). Women during their mid-luteal phase had significantly higher respiratory rates compared to their early-follicular phase (p=0.018) and men (p=0.041) (Table 2). Men had lower respiratory rate (p=0.04) and higher end-tidal oxygen levels (p=0.027) during normoxia compared to baseline but only when compared against women during the early-follicular phase (Table 2). There were no significant time or phase effects of tidal volume. Ventilation was significantly lower in women during their early-follicular phase compared to men (p<0.001) and their mid-luteal phase (p=0.008) at baseline and during normoxia (Table 2). During normoxia, women during both phases had significantly lower end-tidal carbon dioxide levels (p=0.05) and higher end-tidal oxygen levels (p =0.009) compared to baseline (Table 2). Women in their mid-luteal phase had higher end-tidal oxygen levels compared to their early-follicular phase (p=0.025) at baseline and normoxia (Table 2).

Normoxic Tilt

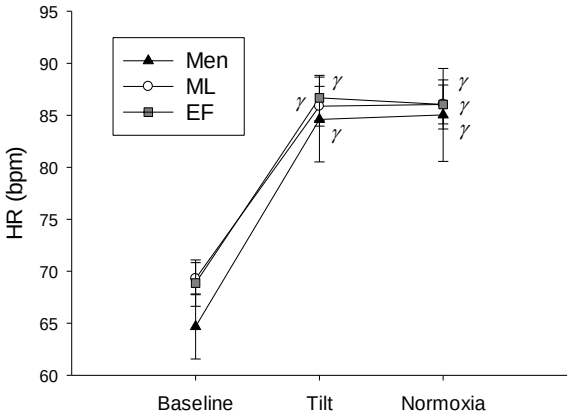
Compared to baseline, all groups had a significant increase in heart rate during tilt (p<0.001) and normoxia (p<0.001) (Figure 4A). Compared to baseline, all groups had significantly lower mean arterial pressure during tilt (p<0.019) and normoxia (p<0.006) compared to baseline (Figure 4B). Men had significantly higher cardiac output index compared to women during the early-follicular phase (p=0.044) (Figure 4C). All groups decreased cardiac output index during tilt (p<0.016) and normoxia (p<0.033) compared to baseline (Figure 4C). Stroke volume index decreased in all groups during tilt (p<0.001) and normoxia (p<0.001) compared to baseline (Figure 4D). Men had significantly higher stroke volume index compared to women during the early-follicular phase (p=0.02) (Figure 4D). Total peripheral resistance index did not change in response to tilt or normoxia compared to baseline in all groups (Figure 4E). Men had significantly lower total peripheral resistance index compared to women during both phases (EF and ML; p<0.001) (Figure 4E). There were no significant effects of phase on heart rate, mean arterial pressure, cardiac output index, stroke volume index, and total peripheral resistance index during tilt or normoxia (Figure 4A-E).

Table 2: Hemodynamic, cerebrovascular, and respiratory responses to normoxia in the supine position.

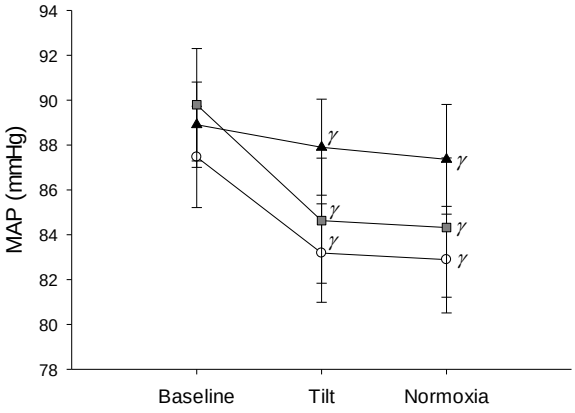
Variables	Men		Women			
			EF		ML	
	Baseline	Normoxia	Baseline	Normoxia	Baseline	Normoxia
Hemodynamics:						
HR (bpm)	65.5±3.3	65.3±3.5	68.4±1.7	67.3±1.8	70.2±1.7	69.5±2.1
MAP (mmHg)	88.8±2.2	88.9±2.2	88.6±2.8	88.9±2.8	89.1±1.8	89.6±1.8
SV_i (mL/m^2)	0.05±0.002$^{\alpha\beta}$	0.05±0.002$^{\alpha}$	0.04±0.002	0.04±0.002	0.04±0.001	0.04±0.002
Q_i (L/min/m^2)	2.9±0.1	2.9±0.1	2.7±0.1	2.6±0.1	2.8±0.1	2.8±0.1
TPR_i (mmHg/L/min/m^2)	8.9±0.7$^{\alpha\beta}$	9.0±0.7$^{\alpha\beta\omega}$	13.1±0.9	13.4±0.9	12.6±0.7	12.7±0.7
Cerebrodynamics:						
Systolic MCA (cm/s)	95.5±5.7	92.2±6.1	97.7±4.2	97.6±4.3	105.4±4.8	105.7±4.9
Diastolic MCA (cm/s)	44.9±2.7	43.1±3.8	46.2±2.7	46.6±2.6	51.3±3.0	50.7±3.0
Mean MCA (cm/s)	62.2±3.6$^{\beta}$	59.5±4.8$^{\beta}$	66.3±3.3	66.1±3.4	72.3±3.6	71.9±3.8
CVR_i (mmHg/cm/s)	1.5±0.1$^{\beta}$	1.6±0.2$^{\beta}$	1.4±0.1	1.4±0.1	1.3±0.1	1.3±0.1
PI	0.81±0.05	0.88±0.11	0.79±0.03	0.78±0.03	0.8±0.03	0.8±0.04
RI	0.53±0.02	0.54±0.03	0.53±0.01	0.52±0.01	0.5±0.01	0.5±0.02
Respiratory Measures:						
V_e (L/min)	12.6±0.3$^{\alpha}$	12.7±0.8$^{\alpha}$	9.8±0.5	10.0±0.4	12.2±0.6$^{\varepsilon}$	12.1±0.7$^{\varepsilon}$
V_t (L)	0.8±0.1$^{\alpha}$	0.9±0.1$^{\alpha}$	0.64±0.04	0.67±0.04	0.69±0.04	0.69±0.04
Respiratory Rate (breaths per minute)	16.7±1.1$^{\beta}$	14.9±1.1$^{\beta\omega}$	16.1±1.1	15.7±1.2	18.4±1.2$^{\varepsilon}$	17.9±1.0$^{\varepsilon}$
ET-O_2 (mmHg)	114.7±1.6	118.7±3.3$^{\omega}$	114.5±1.5	116.8±1.8$^{\gamma}$	118.9±1.7$^{\varepsilon}$	120.1±1.4$^{\varepsilon\gamma}$
ET-CO_2 (mmHg)	39.3±1.2	39.1±1.9	39.2±1.1	38.5±1.3$^{\gamma}$	37.1±1.1	36.7±1.0$^{\gamma}$

HR is heart rate, MAP is mean arterial pressure, SV_i is stroke volume index, Q_i is cardiac output index, TPR_i is total peripheral resistance index, MCA is middle cerebral artery, CVR_i is cerebrovascular resistance index, PI is pulsatility index, RI is resistance index, V_e is ventilation, V_t is tidal volume, ET-O_2 is end-tidal oxygen, and ET-CO_2 is end-tidal carbon dioxide. α indicates main group effect (men vs EF), β indicates main group effect (men vs ML), ε indicates main phase effect (ML vs EF), ω indicates a main time-point effect (against EF), and γ indicates a significant difference from baseline.

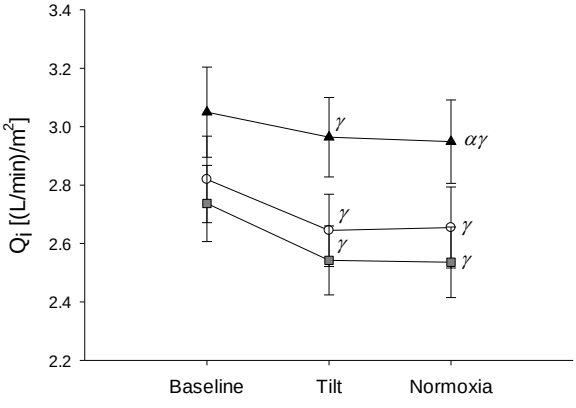
A


Men
ML
EF
HR (bpm)
95
90
85
80
75
70
65
60
Baseline
Tilt
Normoxia
γ

B


MAP (mmHg)
94
92
90
88
86
84
82
80
78
Baseline
Tilt
Normoxia
γ

C


Qi [(L/min)/m2]
3.4
3.2
3.0
2.8
2.6
2.4
2.2
Baseline
Tilt
Normoxia
γ
αγ

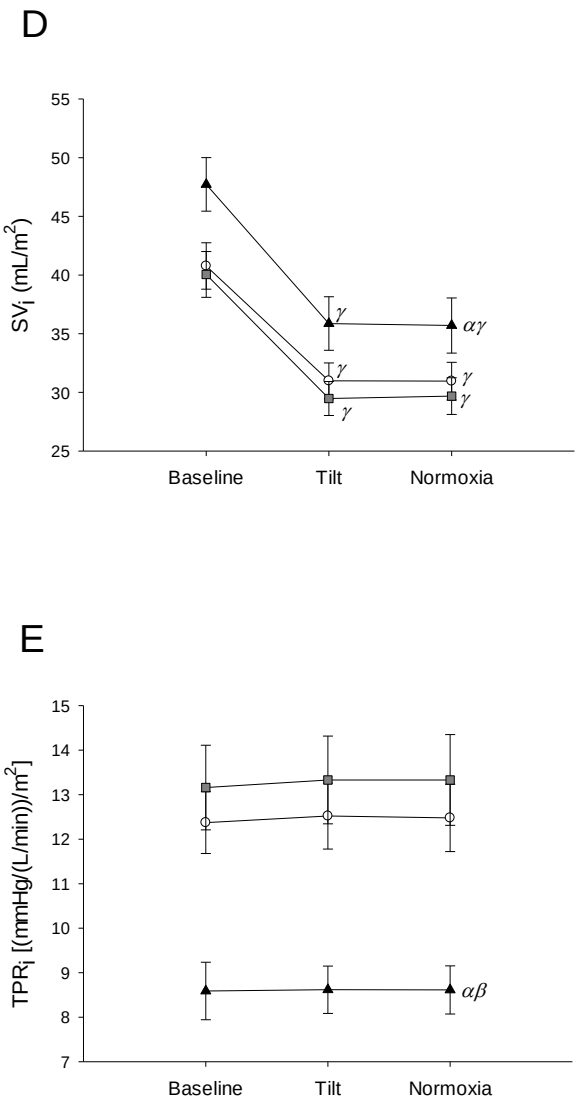
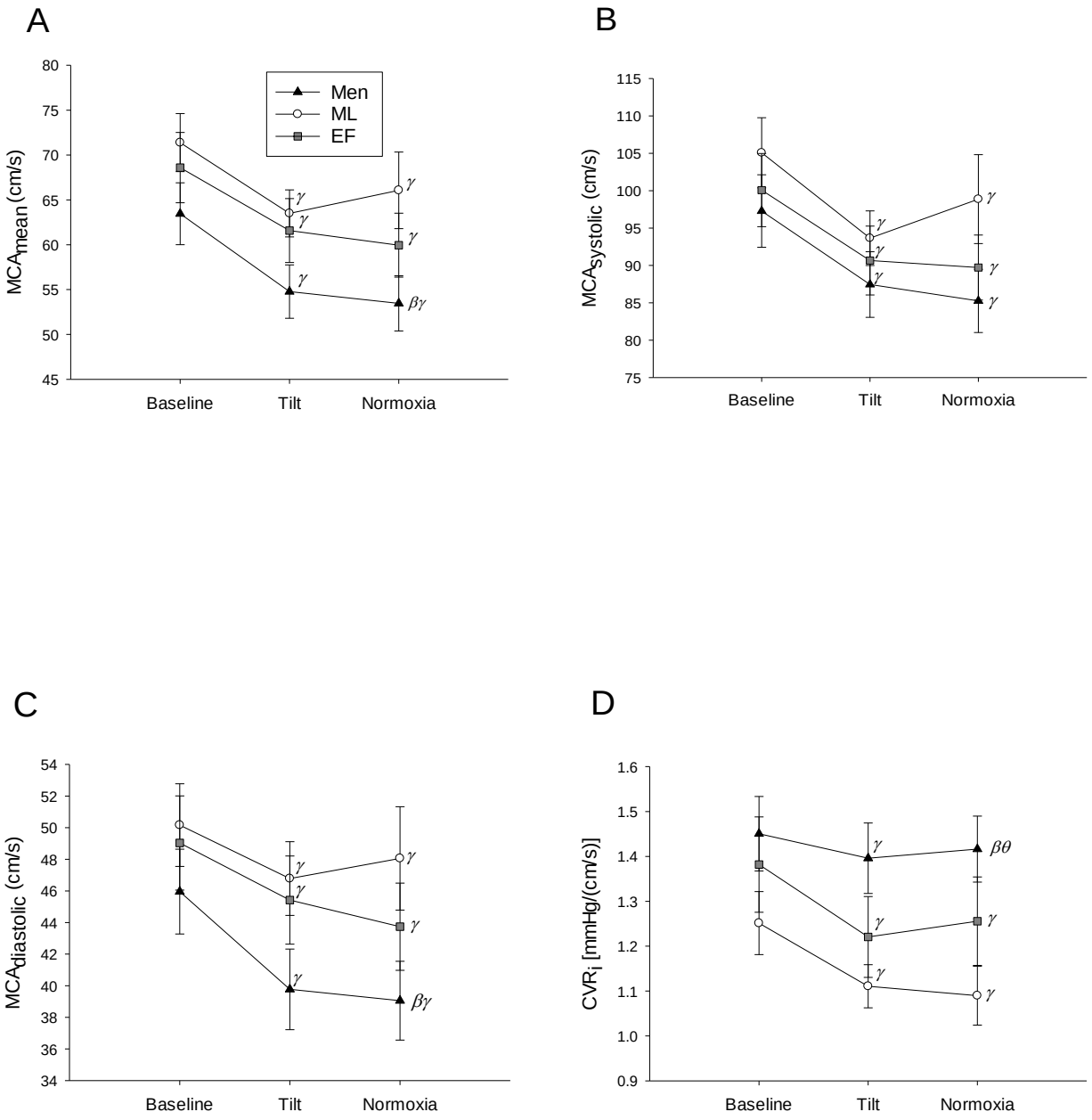


Figure 4: Heart rate (HR; A), mean arterial pressure (MAP; B), cardiac output index (Q_i; C), stroke volume index (SV_i; D), and total peripheral resistance index (TPR_i; E) responses to tilt and normoxia in the tilted position. EF is the early-follicular phase, ML is the mid-luteal phase. α indicates a main group effect (men vs EF), β indicates a main group effect (men vs ML), and γ indicates a significant difference from baseline. Men are black triangles, ML are white circles and EF are grey squares.

All groups had significantly decreased mean, systolic, and diastolic MCA in response to tilt (p<0.036) and normoxia (p<0.002) compared to baseline (Figure 5A-C). Men had significantly lower mean and diastolic MCA velocities compared to women during the mid-luteal phase (p<0.046) (Figure 5A,C). Women during both phases of their cycle decreased their cerebrovascular resistance index during tilt (p<0.001) and normoxia (p<0.001) compared to baseline. Men decreased their cerebrovascular resistance index during tilt (p=0.018), but it was only lower during normoxia when compared to women during the mid-luteal phase (p=0.005) (Figure 5D). Men had significantly higher cerebrovascular resistance index compared to women during their mid-luteal phase at all time-points (p<0.001) (Figure 5D). All groups had significantly decreased cerebral perfusion pressure during tilt (p<0.001) and normoxia (p<0.001) compared to baseline (Figure 5E). There were no significant effects of sex or time on the resistance and pulsatility indices (Figure 5F-G). There were no significant effects of phase on mean MCA, systolic MCA, diastolic MCA, cerebrovascular resistance index, central perfusion pressure, resistance index, and pulsatility index during normoxic tilt (Figure 5A-G).

Men had significantly higher ventilation compared to women during the early-follicular phase (p=0.003) (Figure 6A), with women during their mid-luteal phase having higher ventilation compared to their early-follicular phase (p=0.034) (Figure 6A). Men significantly increased their ventilation during tilt (p<0.016) and normoxia (p<0.034) (Figure 6A). Women during both phases had significantly lower respiratory rates during normoxia (p=0.03) compared to baseline (Figure 6B). Men had significantly lower respiratory rates during tilt (p=0.009) and normoxia (p<0.001) compared to baseline but only when compared to women during the early-follicular phase (Figure 6B). Men had significantly larger tidal volumes compared to women during the early-follicular phase (p=0.035) (Figure 6C). All groups significantly increased their tidal volumes during tilt (p<0.017) and normoxia (p<0.003) compared to baseline (Figure 6C). Men significantly increased end-tidal O_2 levels during tilt (p<0.009) and normoxia (p<0.001) compared to baseline (Figure 6D). Women during both phases also had significantly increased end-tidal O_2 levels but only during normoxia (p=0.019) compared to baseline (Figure 6D), while significantly decreasing end-tidal CO_2 levels during tilt


A
B
C
D
Men
ML
EF
MCAmean (cm/s)
MCAsystolic (cm/s)
MCAdiastolic (cm/s)
CVRI [mmHg/(cm/s)]
Baseline
Tilt
Normoxia

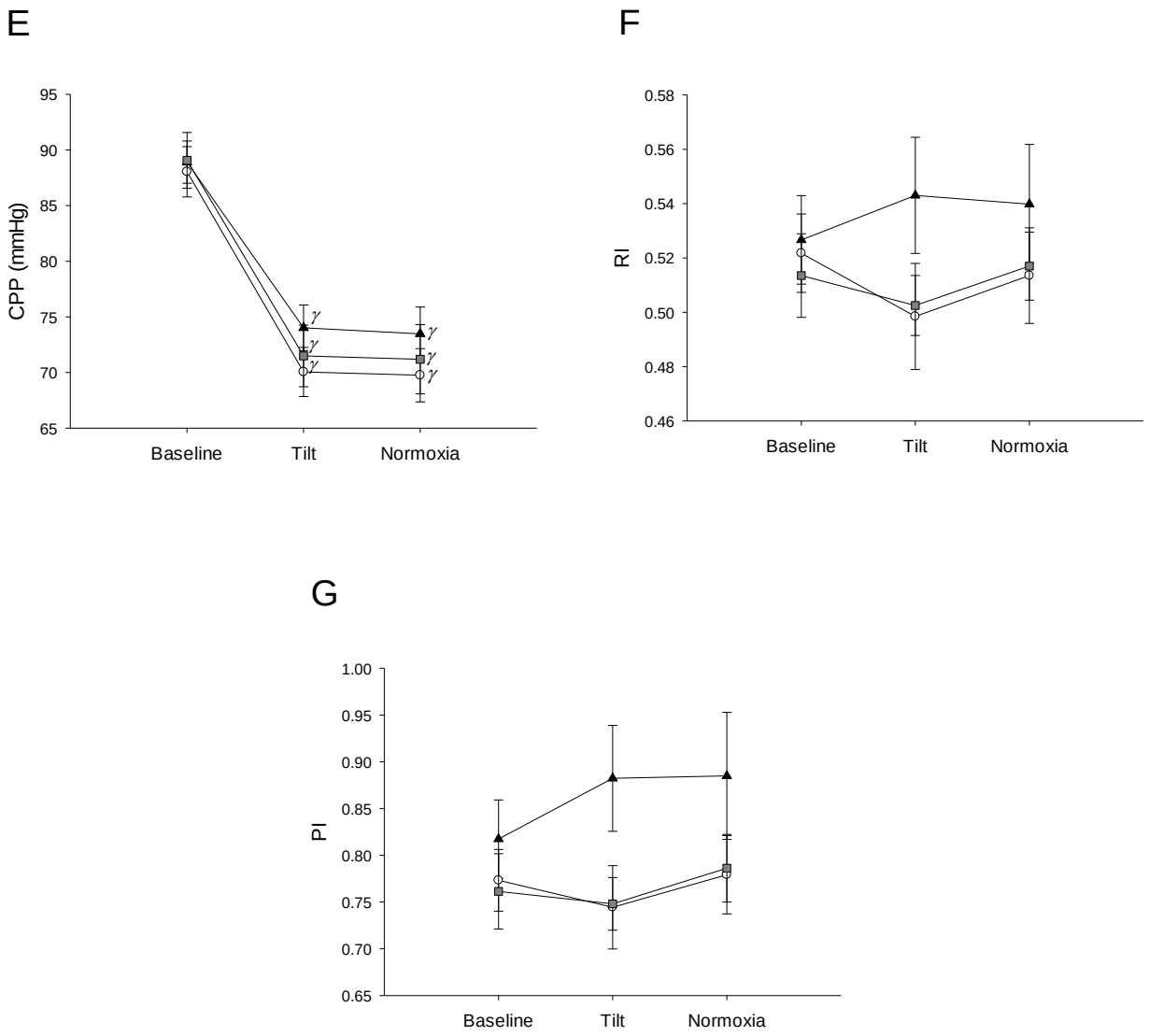
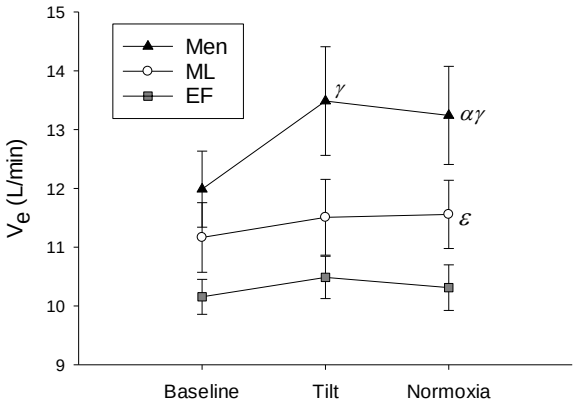
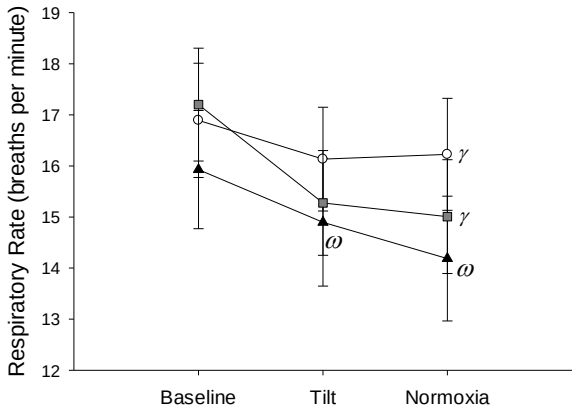


Figure 5: Mean middle cerebral artery velocity (MCA_{mean}; A), systolic middle cerebral artery velocity ($MCA_{systolic}$; B), diastolic middle cerebral artery velocity ($MCA_{diastolic}$; C), cerebrovascular resistance index (CVR_i; D), cerebral perfusion pressure (CPP; E), resistance index (RI; F), and pulsatility index (PI, G) responses to tilt and normoxia in the tilted position. EF is the early-follicular phase, ML is the mid-luteal phase. α indicates a main group effect (men vs EF), β indicates a main group effect (men vs ML), Θ indicates a main time-point effect (against ML in baseline), and γ indicates a significant difference from baseline. Men are black triangles, ML are white circles and EF are grey squares.

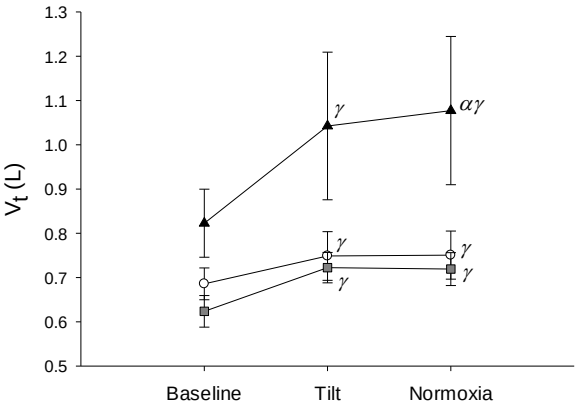
A


Men
ML
EF
V_e (L/min)
15
14
13
12
11
10
9
γ
$\alpha\gamma$
ε
Baseline
Tilt
Normoxia

B


Respiratory Rate (breaths per minute)
19
18
17
16
15
14
13
12
γ
γ
ω
ω
Baseline
Tilt
Normoxia

C


V_t (L)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
γ
$\alpha\gamma$
γ
γ
γ
Baseline
Tilt
Normoxia

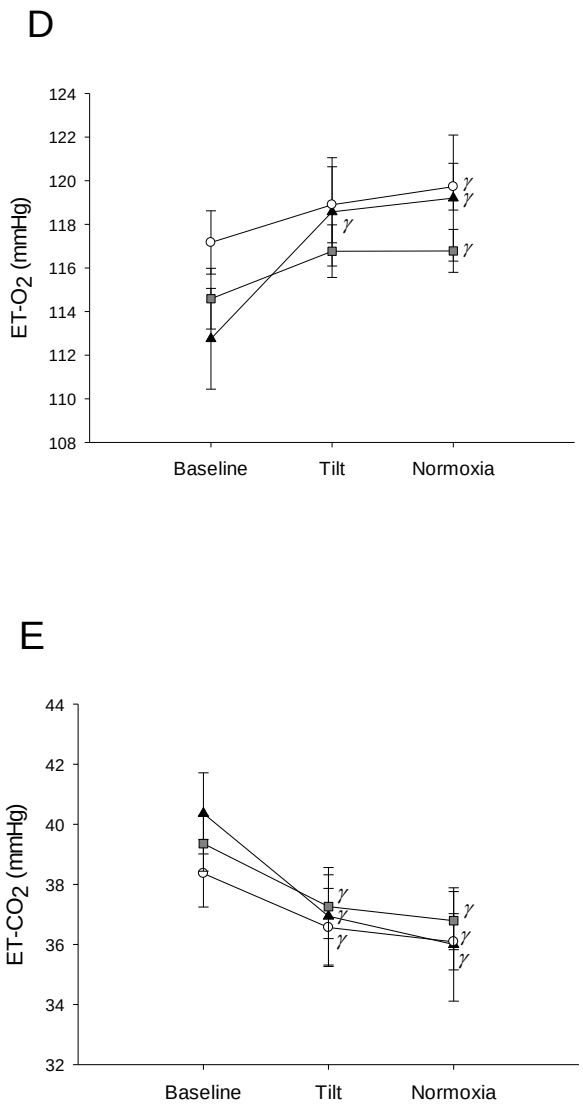


Figure 6: Ventilation (V_e; A), Respiratory Rate (B), Tidal Volume (V_t; C), End-tidal Oxygen (ET-O_2; D), and End-tidal Carbon Dioxide (ET-CO_2; E) responses to tilt and normoxia in the tilted position. EF is the early-follicular phase, ML is the mid-luteal phase. α indicates a main group effect (men vs EF), β indicates a main group effect (men vs ML), ε indicates a main phase effect (ML vs EF), ω indicates a main time-point effect (against EF in baseline), and γ indicates a significant difference from baseline. Men are black triangles, ML are white circles and EF are grey squares.

(p<0.001) and normoxia (p<0.001) compared to baseline (Figure 6E). Men had significantly decreased end-tidal CO_2 levels during tilt (p<0.001) and normoxia (p<0.001) compared to baseline (Figure 6E). There were no significant effects of phase on respiratory rate, tidal volume, end-tidal O_2 and end-tidal CO_2 levels (Figure 6B-E).

All groups had significantly increased LF power with decreased total power and HF power in HUT compared to baseline (Men; p≤0.001, EF; p≤0.001, ML; p≤0.004) (Table 3). Men had significantly higher LF/HF power and lower HF power compared to women during their early-follicular phase (p≤0.032) (Table 3). This difference was also observed compared to women during their mid-luteal phase during HUT (p≤0.002) (Table 3). Men had significantly higher LF power compared to women during the mid-luteal phase during HUT (p=0.003) (Table 3). Women during their mid-luteal phase had significantly higher LF power and lower HF power compared to their early-follicular phase, but only in supine (p≤0.038) (Table 3). Men and women during their early-follicular phase were observed to have a significant increase in their LF/HF ratio during HUT compared to supine (Men; p<0.001, EF; p<0.001) (Table 3). In HUT only, women during their early-follicular phase had significantly higher LF/HF ratios compared to women during their mid-luteal phase (p=0.013) (Table 3). All groups had a significantly decreased cardiovagal baroreceptor sensitivity slope in HUT compared to supine (Men; p<0.001, EF; p<0.001, ML; p<0.001) (Table 3). All groups significantly decreased their cardiovagal baroreceptor sensitivity slopes in HUT compared to supine (p<0.001). Men had significantly lower cardiovagal baroreceptor sensitivity slopes compared to women during the early-follicular phase (p=0.026) (Table 3). Men had significantly lower standard deviation of the R-R interval during HUT compared to supine (p<0.022) (Table 3). There were no significant effects of phase on the standard deviation of R-R intervals, total power and cardiovagal baroreceptor sensitivity slopes (Table 3).

Table 3: Heart-rate variability and cardiovagal baroreceptor sensitivity during normoxia in the supine and HUT positions

Variable	Men		Women			
			EF		ML	
	Supine	HUT	Supine	HUT	Supine	HUT
HRV:						
SDRR (ms)	78.7±10.2	68.6±6.2$^{\gamma}$	74.4±7.1	62.2±4.6	68.6±6.5	59.2±4.8
LF Power (nu)	37.6±5.3	75.5±4.6$^{\beta\gamma}$	30.3±3.3	61.0±4.6$^{\gamma}$	38.4±3.9$^{\varepsilon}$	55.6±4.3$^{\gamma}$
HF Power (nu)	59.2±4.5$^{\alpha}$	24.9±4.5$^{\alpha\beta\gamma}$	67.9±3.6	39.5±4.5$^{\gamma}$	59.6±3.9$^{\varepsilon}$	44.9±4.2$^{\gamma}$
Total [Power(μs^2)]	7511.1±1685.9	3612.0±737.6$^{\gamma}$	7307.5±1440.5	3026.2±609.1$^{\gamma}$	5442.5±963.0	2805.6±6±598.5$^{\gamma}$
LF/HF [Power(%)]	0.8±0.2$^{\alpha}$	5.9±1.8$^{\alpha\beta\gamma}$	0.5±0.1	2.2±0.5$^{\gamma\varepsilon}$	0.7±0.1	1.6±0.3
cBRS:						
Mean Slope (ms/mmHg)	25.8±4.9$^{\alpha}$	6.9±0.9$^{\alpha\gamma}$	34.6±3.4	11.9±1.4$^{\gamma}$	35.21±4.2	11.9±1.7$^{\gamma}$

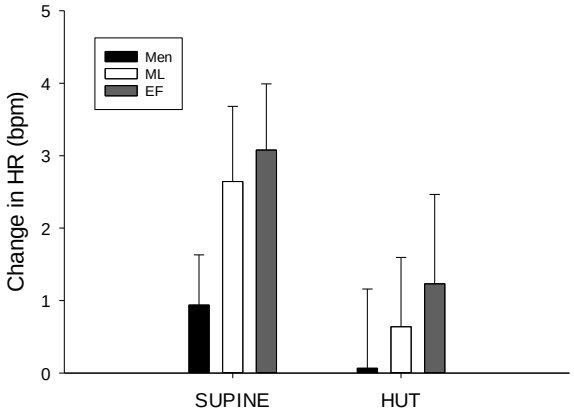
EF is the early-follicular phase, ML is the mid-luteal phase, HUT is head-up tilt, SDRR is the standard deviation between R-R intervals, LF is low-frequency, HF is high-frequency, LF/HF is the ratio between LF and HF, cBRS is cardiovagal baroreceptor sensitivity, and nu are normalized units. α indicates main group effect (men vs EF), β indicates main group effect (men vs ML), ε indicates main phase effect (ML vs EF), and γ indicates a significant difference from supine.

Hypercapnia

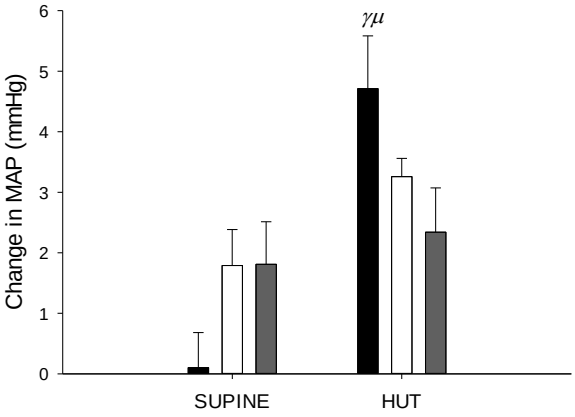
There were no significant effects of sex, phase, or position on changes in heart rate in response to hypercapnia (Figure 7A). Men had a significantly augmented increase in mean arterial pressure in response to CO_2 during HUT compared to supine (p<0.001) and during HUT compared to women in the early-follicular phase (p=0.026) (Figure 7B). Women tended to have significantly augmented increases in mean arterial pressure in response to CO_2 during HUT compared to supine (p=0.054). All groups had a significantly augmented increase in cardiac output index in response to CO_2 during HUT compared to supine (p<0.012) (Figure 7C). All groups had a significantly augmented increase in stroke volume index in response to CO_2 during HUT compared to supine (p<0.001) (Figure 7D). Women during both phases had a significantly augmented decrease in total peripheral resistance index in response to CO_2 during HUT compared to supine (p=0.012) (Figure 7E). Men also had a significantly augmented decrease in total peripheral resistance index in response to CO_2 during HUT compared to supine, but only when compared with women during their mid-luteal phase (p=0.016) (Figure 7E). Men had significantly attenuated changes in total peripheral resistance index compared to women only during their early-follicular phase (p=0.004) (Figure 7E). There were no significant effects of phase on changes in mean arterial pressure, cardiac output index, stroke volume index or total peripheral resistance index (Figure 7A-E).

In response to CO2 (in both postures), men had significantly attenuated increases in diastolic MCA velocity compared to women during their mid-luteal phase (p=0.039) (Figure 8C). Men had a significantly augmented increase in central perfusion pressure in response to CO2 during HUT (p<0.001), which was not observed in women (Figure 8E). Men had a significantly augmented increase in central perfusion pressure in response to CO2 compared to women during the early-follicular phase during HUT (p=0.026) (Figure 8E). Women tended to have significantly augmented increases in cerebral perfusion pressure in response to CO2 during HUT compared to supine (p=0.054) (Figure 8E). There were no significant effects of phase on changes in mean MCA velocity, systolic MCA velocity, diastolic MCA velocity, cerebrovascular resistance index, central perfusion pressure, resistance index or pulsatility index (Figure 8A-G).

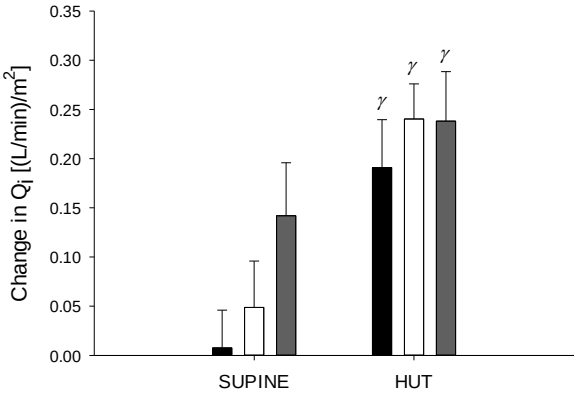
A


Men
ML
EF
Change in HR (bpm)
0
1
2
3
4
5
SUPINE
HUT

B


γμ
Change in MAP (mmHg)
0
1
2
3
4
5
6
SUPINE
HUT

C


γ
γ
γ
Change in Qi [(L/min)/m2]
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
SUPINE
HUT

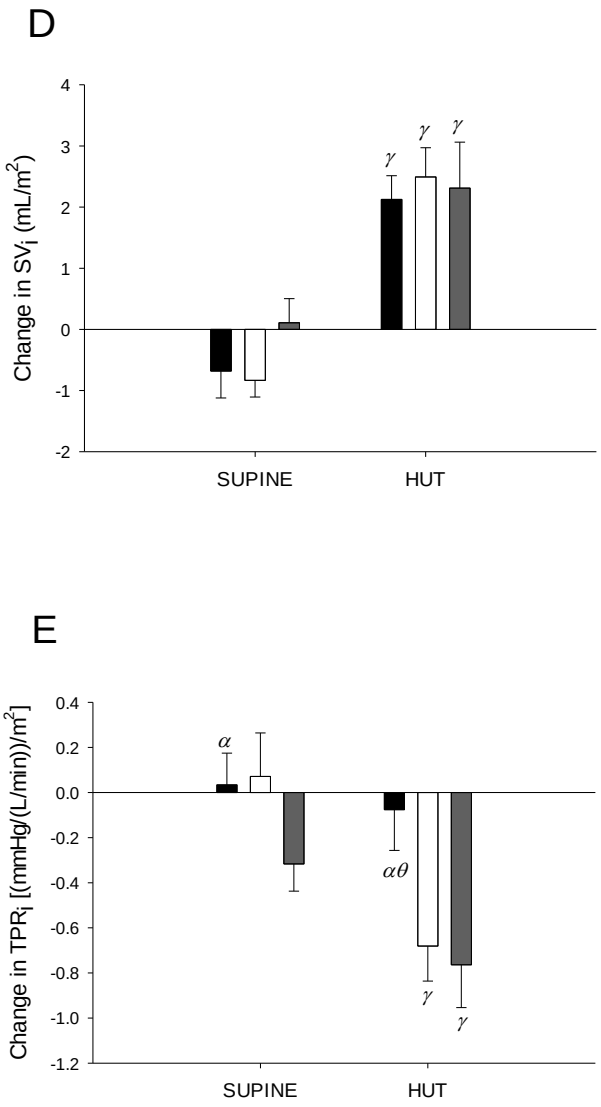
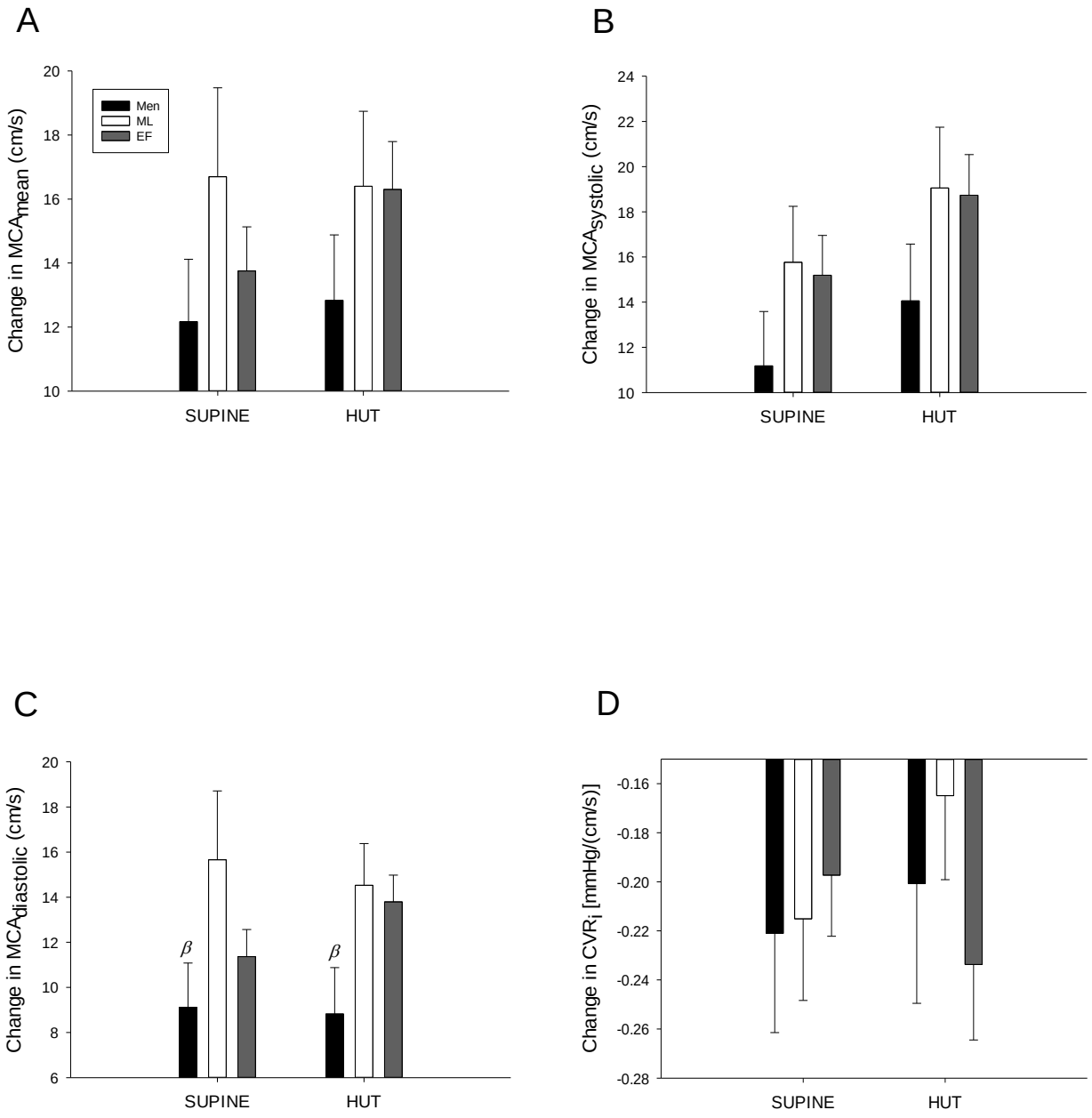


Figure 7: Changes in heart rate (HR; A), mean arterial pressure (MAP; B), cardiac output index (Q_i; C), stroke volume index (SV_i; D), and total peripheral resistance index (TPR_i; E) in response to hypercapnia in the supine and 70º head-up tilted (HUT) positions. EF is the early-follicular phase, ML is the mid-luteal phase. α indicates a main group effect (men vs EF), Θ indicates main time-point effect (against ML in supine), µ indicates main group effect within HUT (men vs EF), and γ indicates a significant difference from baseline. Men are black bars, ML are white bars and EF are grey bars.


A
B
C
D
Men
ML
EF
Change in MCAmean (cm/s)
Change in MCAsystolic (cm/s)
Change in MCAdiastolic (cm/s)
Change in CVRI [mmHg/(cm/s)]
SUPINE
HUT
β
β

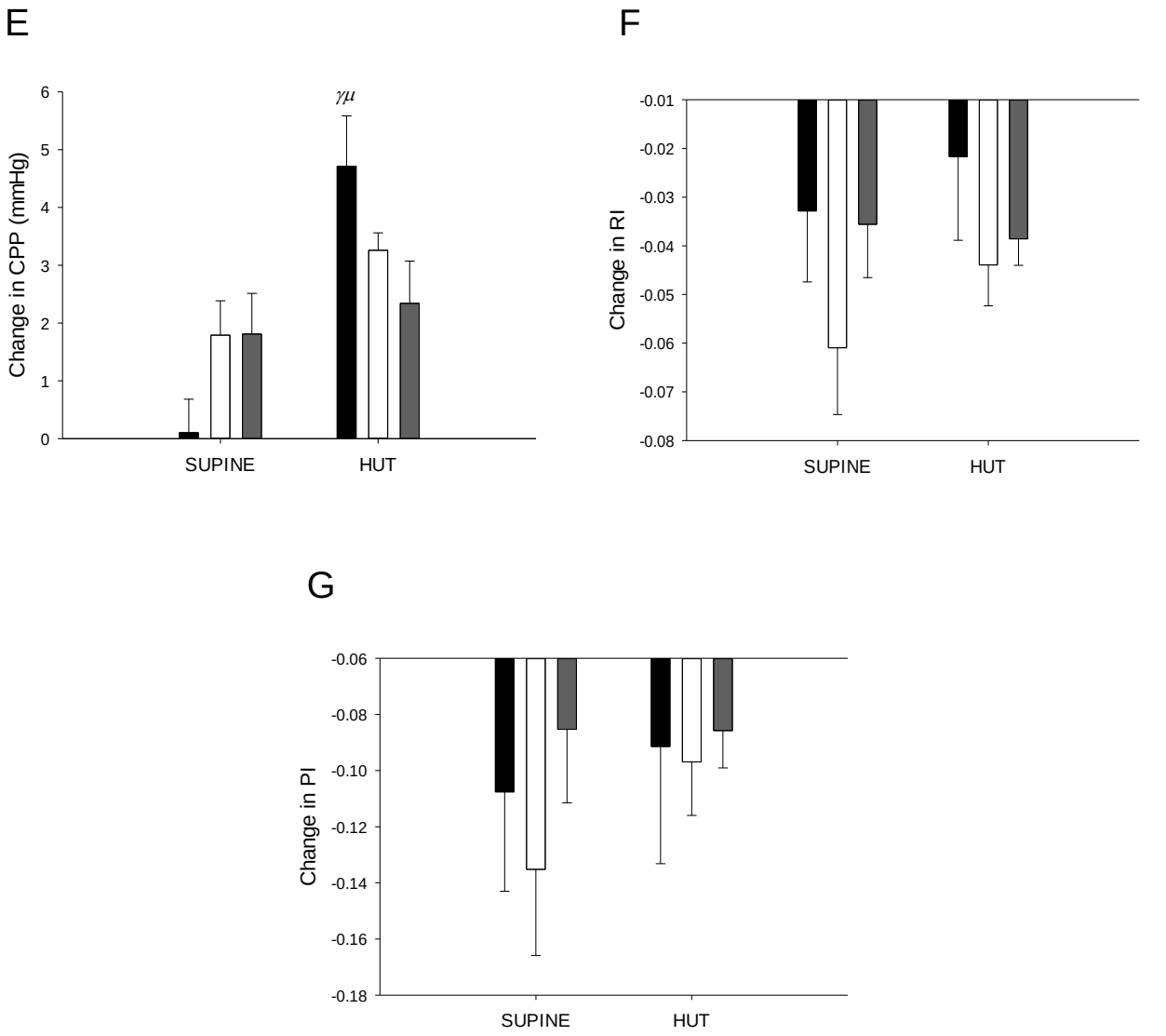


Figure 8: Changes in mean middle cerebral artery velocity (MCA_{mean}; A), systolic middle cerebral artery velocity ($MCA_{systolic}$; B), diastolic middle cerebral artery velocity ($MCA_{diastolic}$; C), cerebrovascular resistance index (CVR_i; D), cerebral perfusion pressure (CPP; E), resistance index (RI; F), and pulsatility index (PI; G) in response to hypercapnia in the supine and 70° head-up tilted (HUT) position. EF is the early-follicular phase, ML is the mid-luteal phase. β indicates a main group effect (men vs ML), μ indicates a main group effect within HUT (men vs EF), and γ indicates a significant difference from baseline. Men are black bars, ML are white bars and EF are grey bars.

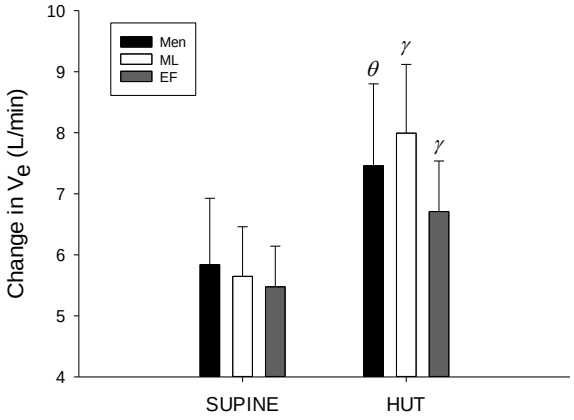
There were no significant effects of sex or position on changes in mean MCA velocity, systolic MCA velocity, cerebrovascular resistance index, resistance index or pulsatility index (Figure 8A-B, D, F-G).

Women during both phases experienced a significantly augmented increase in ventilation in response to CO_2 during HUT compared to supine (p=0.002) while men had a significantly augmented increase in ventilation in response to CO_2 during HUT compared to supine but only when compared to women during their mid-luteal phase (p=0.028) (Figure 9A). All groups had a significantly augmented increase in respiratory rate in response to CO_2 during HUT compared to supine (p<0.019) (Figure 9B). There were no significant effects of sex, phase, or posture on changes in tidal volume (Figure 9C). All groups experienced a significantly attenuated increase in end-tidal oxygen levels in response to CO_2 during HUT compared to supine (p<0.04) (Figure 9D). All groups had a significantly augmented increase in end-tidal carbon dioxide levels in response to CO_2 during HUT compared to supine (p<0.004) (Figure 9E). There were no significant effects of sex or phase on changes in ventilation, respiratory rate, tidal volume, end-tidal oxygen levels or end-tidal carbon dioxide levels (Figure 9B-E).

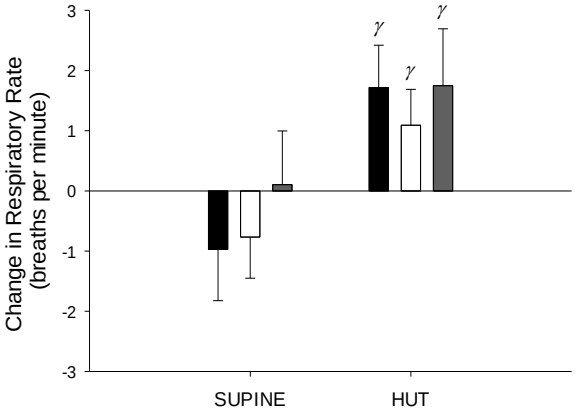
Hyperoxia

Men had significantly attenuated decreases in heart rate in response to O_2 compared to women during their early-follicular phase (p=0.007) (Figure 10A). Men had an increase of mean arterial pressure in response to O_2 during HUT compared to supine but only when compared to women during the mid-luteal phase (p=0.029) (Figure 10B). All groups experienced a significantly attenuated decrease in cardiac output index in response to O_2 during HUT compared to supine (p<0.004) (Figure 10C). Women during the early-follicular phase tended to have a smaller decrease of cardiac output index in response to O_2 compared to the mid-luteal phase regardless of position(p=0.066) (Figure 10C). There were no significant effects of sex on changes in cardiac output index to O_2 (Figure 10C). Men had significantly attenuated increases in stroke volume index in response to O_2 compared to women during their mid-luteal phase (p=0.009) (Figure 10D), regardless of posture.

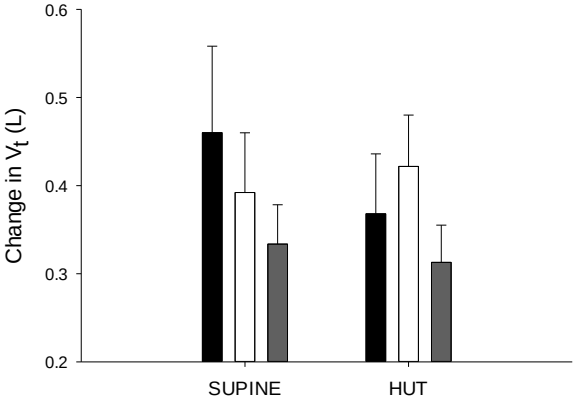
A


Men
ML
EF
Change in V_e (L/min)
10
9
8
7
6
5
4
θ
γ
γ
γ
SUPINE
HUT

B


Change in Respiratory Rate (breaths per minute)
3
2
1
0
-1
-2
-3
γ
γ
γ
SUPINE
HUT

C


Change in V_t (L)
0.6
0.5
0.4
0.3
0.2
SUPINE
HUT

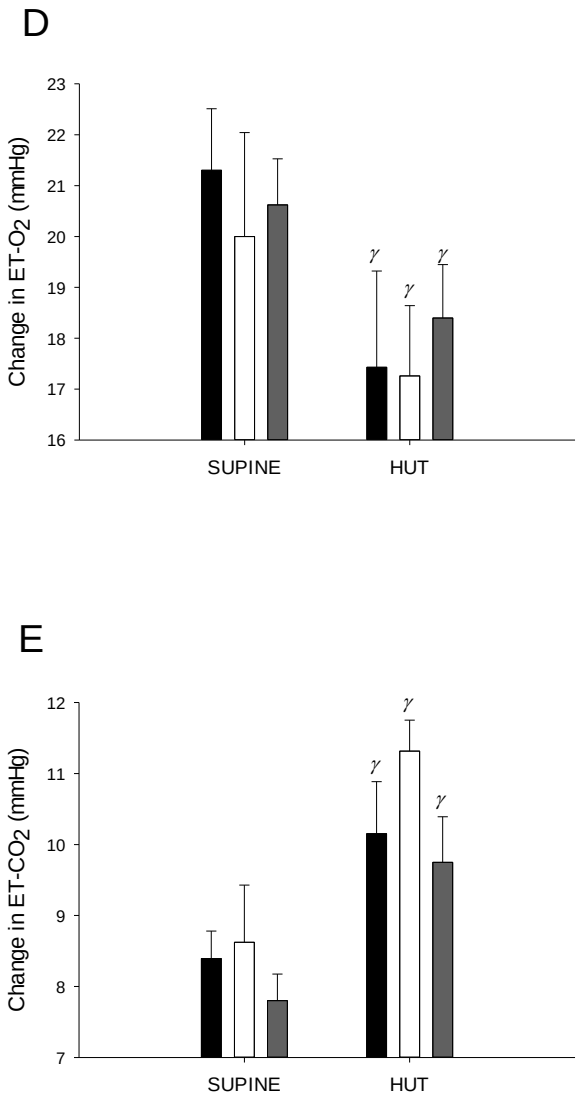
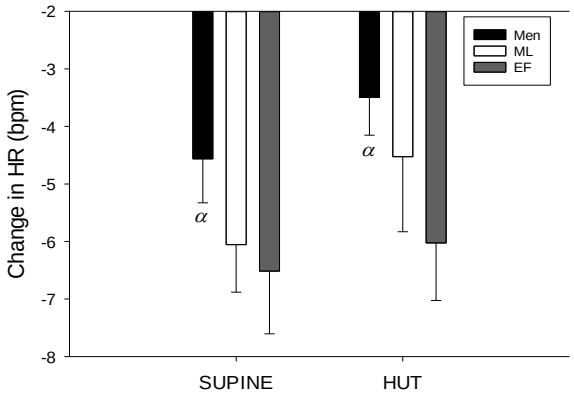
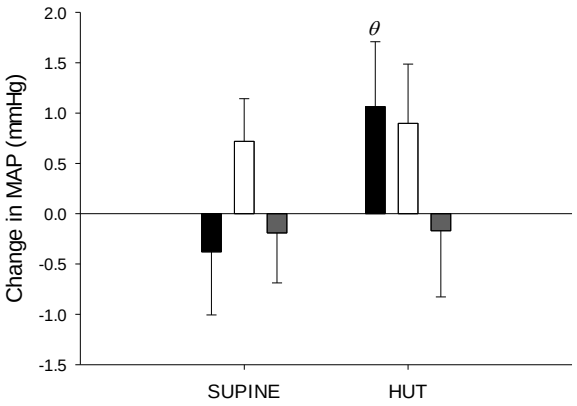


Figure 9: Changes in ventilation (V_e; A), respiratory rate (B), tidal volume (V_t; C), end-tidal oxygen (ET-O_2; D), and end-tidal carbon dioxide (ET-CO_2; E) in response to hypercapnia in the supine and 70^o head-up tilted (HUT) position. EF is the early-follicular phase, ML is the mid-luteal phase. Θ indicates a main time-point effect (against ML in supine) and γ indicates a significant difference from baseline. Men are black bars, ML are white bars and EF are grey bars.

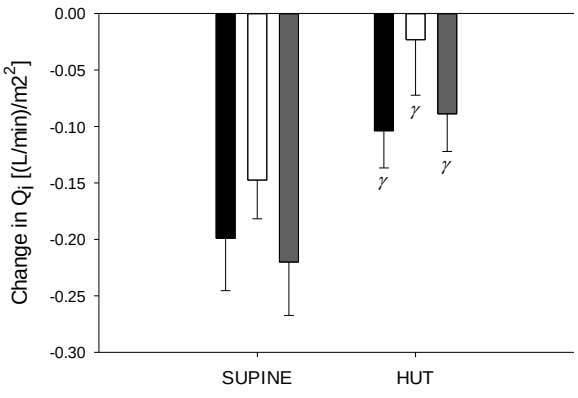
A


Change in HR (bpm)
-2
-3
-4
-5
-6
-7
-8
Men
ML
EF
α
α
SUPINE
HUT

B


Change in MAP (mmHg)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
θ
SUPINE
HUT

C


Change in Qi [(L/min)/m2]
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.30
γ
γ
γ
SUPINE
HUT

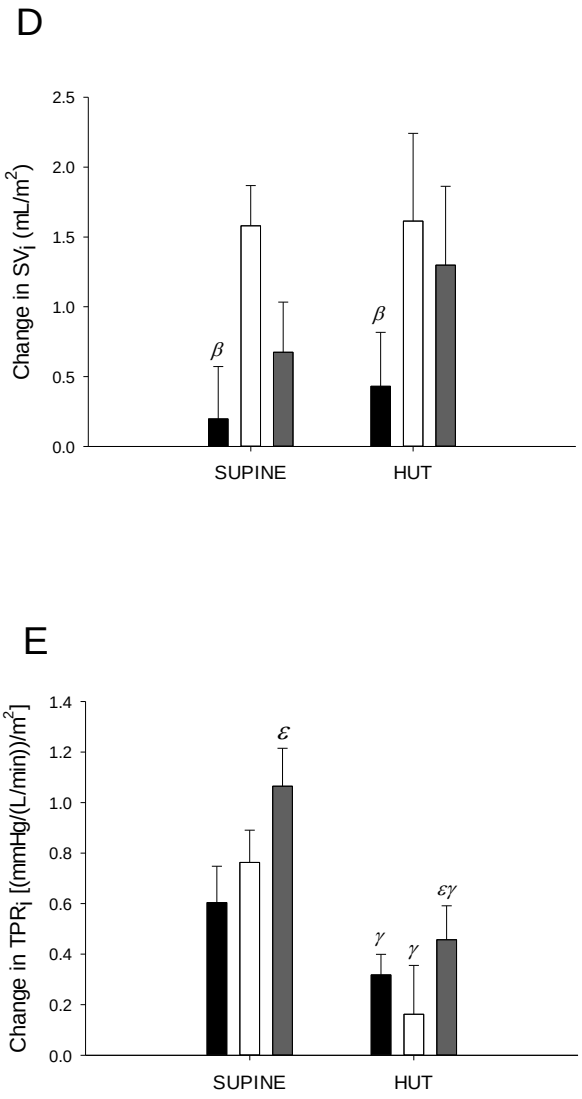
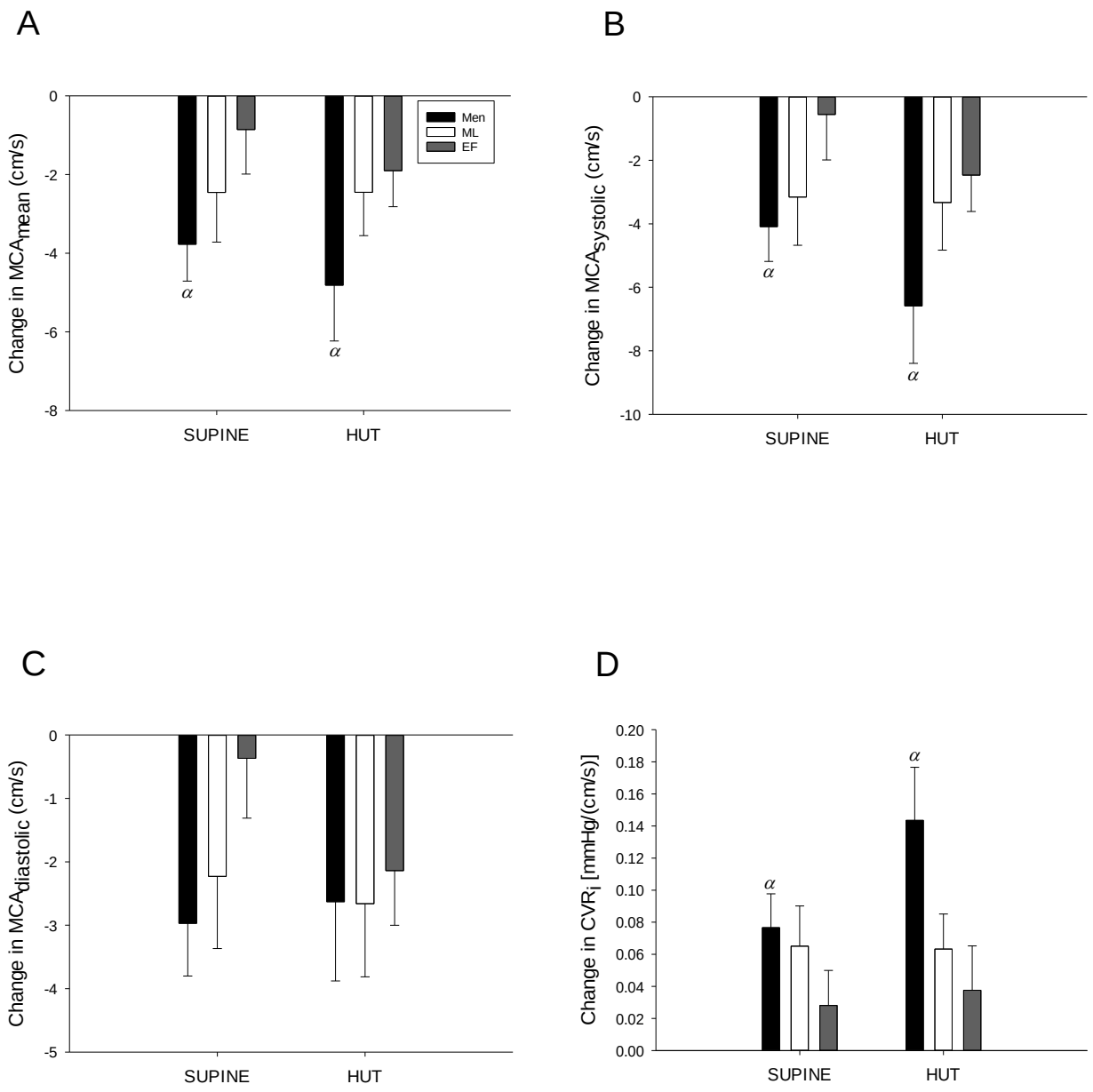


Figure 10: Changes in heart rate (HR; A), mean arterial pressure (MAP; B), cardiac output index (Q_i; C), stroke volume index (SV_i; D), and total peripheral resistance index (TPR_i; E) in response to hyperoxia in the supine and 70° head-up tilted (HUT) positions. EF is the early-follicular phase, ML is the mid-luteal phase. α indicates a main group effect (men vs EF), β indicates a main group effect (men vs ML), ε indicates a main phase effect (ML vs EF), Θ indicates a main time-point effect (against ML in supine), and γ indicates a significant difference from baseline. Men are black bars, ML are white bars and EF are grey bars.

All groups had significantly attenuated increases in total peripheral resistance index in response to O_2 during HUT compared to supine (p<0.006) (Figure 10E). Women during the mid-luteal phase had significantly augmented increases in total peripheral resistance index in response to O_2 compared to their early-follicular phase (p=0.044) (Figure 10E). There were no significant effects of posture or phase on changes in heart rate or stroke volume index (Figure 10A, D).

Men had significantly augmented decreases of mean and systolic MCA velocity in response to O_2 when compared to women during their early-follicular phase (p=0.017 and p=0.006, respectively) (Figure 11A-B). There were no significant effects of posture, sex, or phase on diastolic MCA velocity (Figure 11C). Men had significantly augmented increases in cerebrovascular resistance index in response to O_2 when compared to women during their early-follicular phase (p=0.006) (Figure 11D). Men had an increase of cerebral perfusion pressure in response to O_2 during HUT compared to supine but only when compared to women during the mid-luteal phase (p=0.029) (Figure 11E). There were no significant effects of posture or phase on changes in mean MCA velocity, systolic MCA velocity, diastolic MCA velocity, cerebrovascular resistance index, resistance index, or pulsatility index (Figures 11A-D, F, G). There were no significant effects of sex on changes in resistance index and pulsatility index (Figure 11F-G).

There were no significant effects of sex or phase on changes in ventilation, respiratory rate or tidal volume (Figure 12A-C). Men had a significantly attenuated increase in end-tidal oxygen levels in response to O_2 during HUT compared to supine but only when compared to women during the mid-luteal phase (p<0.001) (Figure 12D). There were no significant effects of posture on changes in ventilation, respiratory rate, tidal volume, and end-tidal carbon dioxide levels (Figure 12A-C, E). Women during their mid-luteal phase experienced a significantly attenuated increase in end-tidal oxygen levels in response to O_2 during HUT compared to supine (p=0.004) (Figure 12D). Men had significantly augmented decreases in end-tidal carbon dioxide levels when compared to women during the mid-luteal phase (p=0.015) in response to O_2 (Figure 12E).


A
B
C
D
Men
ML
EF
Change in MCA$_{mean}$ (cm/s)
Change in MCA$_{systolic}$ (cm/s)
Change in MCA$_{diastolic}$ (cm/s)
Change in CVR$_i$ [mmHg/(cm/s)]
SUPINE
HUT
α

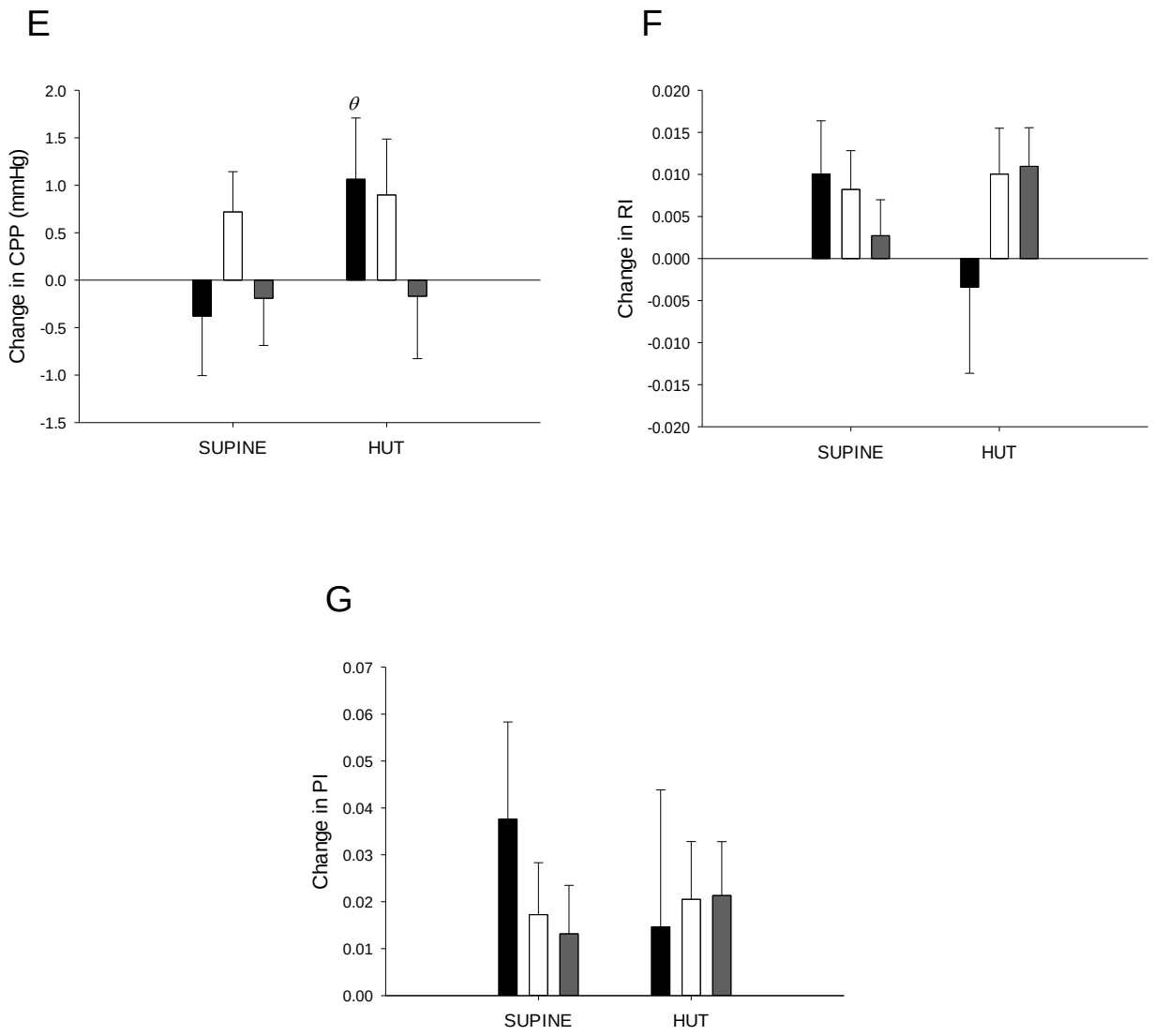
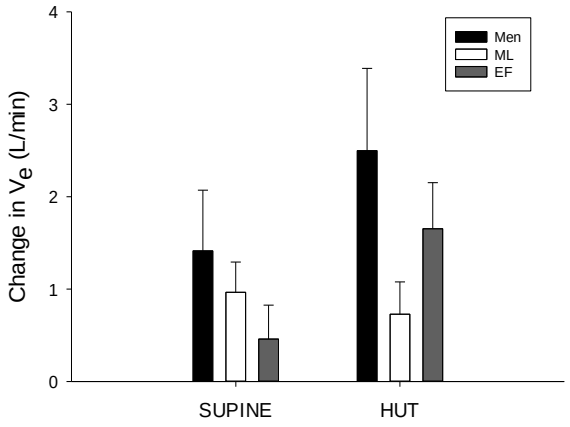
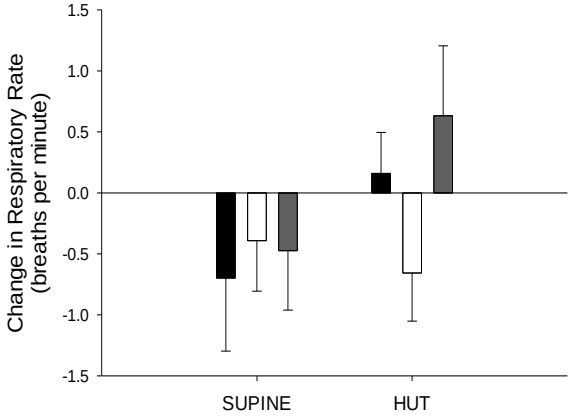


Figure 11: Changes in mean middle cerebral artery velocity (MCA_{mean}; A), systolic middle cerebral artery velocity ($MCA_{systolic}$; B), diastolic middle cerebral artery velocity ($MCA_{diastolic}$; C), cerebrovascular resistance index (CVR_i; D), cerebral perfusion pressure (CPP; E), resistance index (RI; F), and pulsatility index (PI; G) in response to hyperoxia in the supine and 70º head-up tilted (HUT) position. EF is the early-follicular phase, ML is the mid-luteal phase. α indicates a main group effect (men vs EF) and Θ a main time-point effect (against ML in supine). Men are black bars, ML are white bars and EF are grey bars.

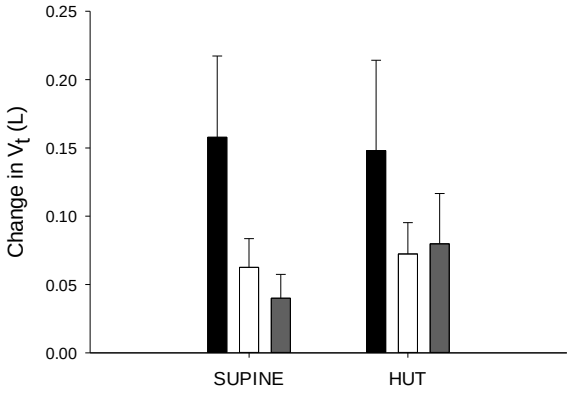
A


Men
ML
EF
Change in V_e (L/min)
4
3
2
1
0
SUPINE
HUT

B


Change in Respiratory Rate (breaths per minute)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
SUPINE
HUT

C


Change in V_t (L)
0.25
0.20
0.15
0.10
0.05
0.00
SUPINE
HUT

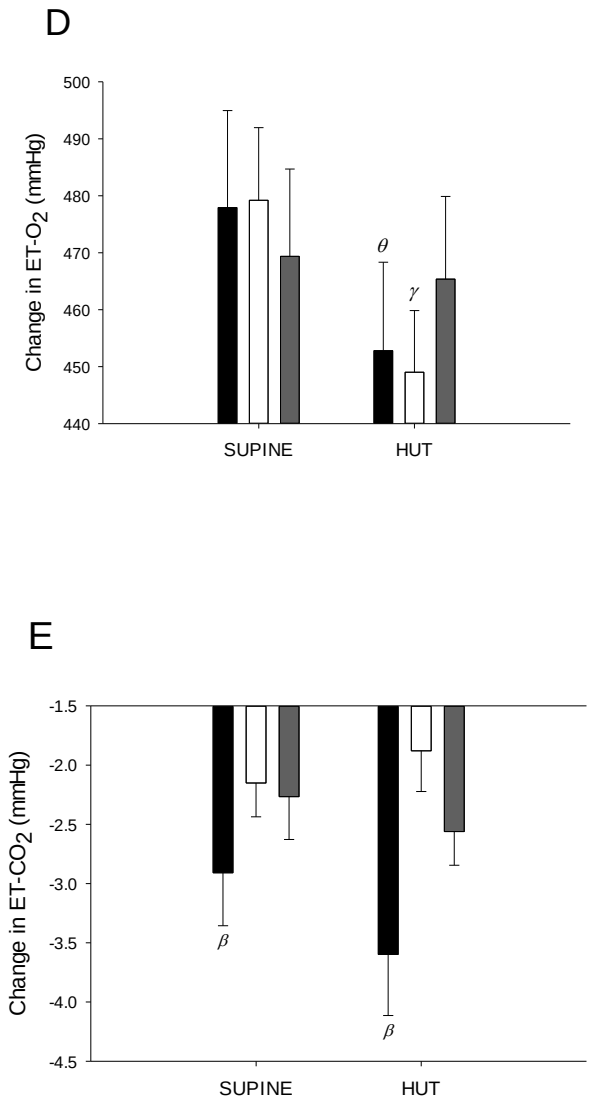


Figure 12: Changes in ventilation (V_e; A), respiratory rate (B), tidal volume (V_t; C), end-tidal oxygen (ET-O_2; D), and end-tidal carbon dioxide (ET-CO_2; E) in response to hyperoxia in the supine and 70° head-up tilted (HUT) position. EF is the early-follicular phase, ML is the mid-luteal phase. α indicates a main group effect (men vs EF), β indicates a main group effect (men vs ML), Θ indicates a main time-point effect (against ML in supine), and γ indicates a significant difference from baseline. Men are black bars, ML are white bars and EF are grey bars.

Table 4: Pulse-wave velocity during hypercapnia, hyperoxia and normoxia in the supine position

Trial	Men		Women			
			EF		ML	
	Baseline	Gas	Baseline	Gas	Baseline	Gas
Normoxia	6.9±0.6	7.0±0.6	6.4±0.4	6.3±0.4	6.6±0.3	6.9±0.4
Hypercapnia	7.0±0.6	7.8±0.8 γ	6.2±0.4	6.6±0.5γ	6.6±0.3	7.0±0.3γ
Hyperoxia	7.0±0.6	6.7±0.6	7.0±0.8	7.0±0.5	6.4±0.4	6.8±0.4

Where EF is the early-follicular phase and ML is the mid-luteal phase. γ indicates a significant difference from baseline; $p<0.001$).

Pulse-Wave Velocity

In the supine position, all groups experienced significantly higher pulse-wave velocity in response to carbon dioxide compared to baseline (p<0.001) (Table 4). There were no significant effects of time on pulse-wave velocity in response to normoxia or hyperoxia (Table 4). There were no significant effects of sex or phase on pulse-wave velocity in response to normoxia, hypercapnia or hyperoxia (Table 4).

DISCUSSION

Summary

In response to HUT, all groups had significant increases in heart rate and end-tidal O_2 with decreases in stroke volume index and end-tidal CO_2. All groups had a significant decrease in mean arterial pressure due to significantly reduced cardiac output index with no concurrent decrease in total peripheral resistance. Men had significantly higher cardiac output index compared to women during the early-follicular phase (likely due to significantly larger stroke volumes) and lower total peripheral resistance index compared to women during both phases. Men had lower brain blood flow velocity compared to women during the mid-luteal phase while all groups had a significant decrease of cerebrovascular resistance index in response to HUT indicative of increased cerebral vasodilation. During HUT, women experienced a significant decrease in respiratory rate with a concurrent increase in tidal volume to maintain ventilation at supine levels. On the other hand, men also increased their tidal volume and decreased their respiratory rate yet this resulted in significantly higher ventilation during HUT and normoxia. These results suggest that men have desensitized pulmonary stretch receptors. Together, these results suggest the lack of hyperventilation observed in women during HUT could result in attenuation of the respiratory pump action causing diminished venous return in women.

All groups experienced a significantly augmented increase in cardiac output index in response to CO_2 in the HUT position likely due to an augmented increase in

stroke volume index with no concurrent changes in heart rate (possibly due to the greater respiratory rate and therefore greater respiratory pump in HUT). Only men had a significantly augmented increase in mean arterial pressure in response to CO_2 during HUT suggestive of increased sympathetic nerve activity or greater neurovascular transduction (i.e. the ability of blood vessels to constrict during sympathetic activation). Indeed, men had a smaller reduction of TPR_i during HUT compared to the other groups further suggesting increased sympathetic output or greater neurovascular transduction in men. In response to CO_2, men had significantly smaller increases in diastolic middle cerebral artery velocity compared to women during the mid-luteal phase indicating that women in the mid-luteal phase may experience greater cerebrovascular reactivity to CO_2. However, there were no changes seen in cerebrovascular resistance index, resistance index or pulsatility index. During hypercapnic tilt, both men and women were observed to have significantly augmented increases in end-tidal CO_2 and respiratory rate with a decrease in end-tidal O_2. All groups had significantly augmented increases in ventilation during hypercapnic tilt. These results suggest that while the central chemoreflex is enhanced in HUT in both sexes, the autonomic or vascular responses to CO_2 in HUT are different between men and women. We suggest that in HUT during hypercapnia, men have greater sympathetic output (or greater neurovascular transduction) compared to women.

In response to hyperoxia during HUT, all groups were observed to have significantly attenuated increases in total peripheral resistance index (and attenuated decreases in cardiac output index from lower cardiac afterload) suggesting attenuated sympathetic tone indicating that the peripheral chemoreflex was inhibited in the upright posture. During hyperoxia, women during the early-follicular phase had a greater increase in total peripheral resistance index compared to the mid-luteal phase indicating that the presence of female sex hormones attenuates peripheral vasoconstriction. Compared to women, men had a smaller decrease in heart rate (early-follicular) and a smaller increase in stroke volume index (mid-luteal) in response to hyperoxia suggesting greater pulmonary vasodilation in men. During hyperoxia, men also had augmented reductions in systolic and mean middle cerebral artery velocities and an

augmented increase in cerebrovascular resistance index compared to women during their early-follicular phase implying greater cerebrovascular constriction.

Head-up Tilt in Normoxia

We hypothesized that in normoxic conditions: 1) During upright tilt, women would have a greater reduction of brain blood velocity compared to men, and 2) Men would have higher ventilation compared to women (in both supine and upright positions) and women during the mid-luteal phase would have higher ventilation compared to the early follicular phase (in both supine and upright positions). The first hypothesis was not supported yet the second hypothesis was partially supported.

Hemodynamic sex-differences have been previously noted where men have larger stroke volumes compared to women regardless of position (47, 60, 61, 98), and this was also observed in the current investigation. Yamada et al. (2007) studied 181 healthy subjects and discovered that women have significantly smaller ventricular volumes compared to age-matched men indicative of smaller stroke volumes contributing to a significantly smaller cardiac output index (163). This was supported by other studies finding cardiac output to be higher in men than women (47, 60, 61, 98). In response to varying lengths of orthostatic challenge (5 minutes to 45 minutes), both men and women have been observed to experience decreases in cardiac output as a result of a simultaneous decrease in stroke volume and increase in heart rate compared to supine (47–49, 82, 98, 105, 134). Our results agree with these previous findings as all groups were observed to experience a significant decrease in cardiac output index during HUT. This is likely due to the significant decrease in mean arterial pressure observed in men and women due to HUT. Interestingly, Fu et al. (2004) observed that only women experienced gradual decreases in both systolic and diastolic pressure due to 45 minutes of 30^{o} and 60^{o} HUT, respectively (47). Therefore, extending the trial length may have resulted in a maintenance of mean arterial pressure and cardiac output in men and not women during HUT which could be as a result of increased sympathetic activity or greater neurovascular transduction in men (47, 48, 134).

While we did not directly measure sympathetic activity, heart rate variability in men was significantly higher in the low-frequency domain (primarily sympathetic activity)

compared to the mid-luteal phase during HUT and significantly lower in the high-frequency domain (parasympathetic activity) compared to both phases during HUT indicating higher sympathetic and lower parasympathetic output in men compared to women. Similarly, cardiovagal baroreceptor sensitivity, another indicator of parasympathetic nerve activity, was also observed to be significantly lower in men compared to women during the early-follicular phase in both positions. Higher sympathetic activity in men would lead to higher blood pressure and peripheral vasoconstriction. However, Hart et al. (2011) observed that no relationship exists between muscle sympathetic nerve activity and total peripheral resistance in young women, contrary to what is seen in young men (60), and suggests that increased beta$_2$-adrenergic vasodilatory responses in women, may offset alpha-adrenergic-mediated vasoconstriction during a sympathetic stimulus (i.e. HUT) (60). Therefore, they conclude that in young women, sympathetic nerve activity does not determine peripheral vasculature resistance, cardiac output, or blood pressure (60). This could help to explain why men may be better able to maintain cardiac output index and mean arterial pressure during orthostatic stress.

Men were found to have significantly lower mean and diastolic middle cerebral artery velocities compared to women during the mid-luteal phase throughout the normoxic tilt trial. These results have been confirmed by previous studies showing significantly lower middle cerebral artery velocity in men (32, 38, 150, 153). This is likely explained by the significantly larger middle cerebral artery diameters in men compared to women (104). Further, estrogen's potent endothelium-dependent vasodilation (76, 113) likely resulted in the significantly lower cerebrovascular resistance index observed in women during the mid-luteal phase compared to men.

Contrary to our first hypothesis, women did not have a greater reduction of mean brain blood flow velocity during HUT compared to men. All groups experienced a reduction in mean, systolic, and diastolic brain blood flow velocity and cerebrovascular resistance index during tilt implying cerebrovascular vasodilation during HUT likely caused by the significant reduction in cerebral perfusion pressure during HUT. Contrary to our results, Abidi et al. (2017) found that men significantly increased cerebrovascular resistance index in response to 10 minutes of standing indicating cerebral

vasoconstriction in men during orthostatic stress (1). However, Abidi et al. (2017) used 10 minutes of active standing rather than 5 minutes of passive tilt, as used in the current study.

Ventilation was significantly increased in men during tilt and normoxia which was not observed in women. Confirming our results, previous studies also found increased ventilation in response to orthostatic stress (18, 54, 97, 101, 156, 164) due to concurrent increases in tidal volume (18, 54, 164). In the current study, all groups were observed to have significant increases in tidal volume with concurrent decreases in respiratory rate but only women did not increase ventilation during orthostatic stress. This could potentially be explained by sex differences in the Hering-Breuer reflex, a negative feedback loop where inflation of the lungs activates slowly adapting receptors to initiate a signal cascade resulting in termination of inspiration (133). Since men have anatomically larger lungs, the Hering-Breuer reflex may be less sensitive allowing for over-inflation of the lungs leading to increased ventilation compared to women. Romei et al. (2010) found that women have a lower abdominal contribution to ventilation compared to men (122) potentially allowing men to maintain mean arterial pressure through displacement of blood volume from the abdomen, presumably from the splanchnic vascular bed, increasing venous return (3). This increase in ventilation in response to orthostatic stress observed in men could result in an enhanced respiratory pump action compared to women leading to the maintenance of mean arterial pressure through greater venous return. Despite this sex difference in the ventilatory response to tilt, end-tidal CO_2 and end-tidal O_2 change similarly between groups (as previously observed by Serrador et al. (131)). Increased ventilation in men would normally be expected to decrease end-tidal CO_2 and increase end-tidal O_2; however, the movement of hypercapnic and hypoxic blood pools from the abdomen may obscure changes in gas exchange.

Partially confirming the second hypothesis, it was observed that men had significantly higher ventilation than women but only during their early-follicular phase, and it was also observed that women during the mid-luteal phase had higher ventilation than women in the early-follicular phase throughout the normoxic tilt trial. The greater ventilation in men is likely due to significantly greater tidal volume and lung sizes

compared to women. Dombovy et al. (1987) also found higher ventilation in women during their luteal phase compared to their follicular phase and attributed it to higher progesterone levels in the luteal phase (35). Indeed, the increase in ventilation due to progesterone can be mediated by upregulation of progesterone-receptors in hypothalamic neurons (7). Further, female cats injected with progesterone display increased phrenic nerve activity compared to males allowing the lungs to inspire quicker and deeper resulting in hyperventilation (7). The enhancement of ventilation by progesterone could also help to explain why no ventilatory difference was seen between men and women during the mid-luteal phase.

Although sympathetic activity was not measured directly, heart rate variability was used as an indicator of sympathetic nerve activity and showed that women have lower sympathetic output compared to men in both positions suggesting that men might be better able to maintain cardiac output and blood pressure through higher sympathetic nerve activity and/or greater neurovascular transduction. Further, men were observed to have higher ventilation in response to orthostatic stress leading to greater respiratory muscle pump action compared to women. Our results indicate that sexually dimorphic differences in anatomy and circulating sex hormones throughout the menstrual cycle can influence ventilation in men and women regardless of position.

Head-up Tilt in Hypercapnia

We hypothesized that: 1) Central chemoreflex function would be enhanced in the upright posture compared to supine, 2) In both the supine and upright postures, women in ML will have increased cerebrovascular reactivity to hypercapnia compared to men and women in the EF phase; however, women in the EF phase will have greater cerebrovascular reactivity than men, and 3) In both the supine and upright postures, women during the EF phase will have enhanced central chemoreflex activity in response to hypercapnia compared to the ML phase and men. Our first hypothesis was supported by the evidence, our second hypothesis was partially supported, but the third hypothesis was not supported by the evidence. Although men were observed to have a significantly augmented increase in mean arterial pressure in response to CO_2 during HUT compared to the supine position, all groups displayed a greater ventilatory

response in hypercapnic tilt compared to supine. These results support our first hypothesis that the central chemoreflex is enhanced in upright posture compared to the supine position in all participants likely due to interactions with the baroreflex.

In response to hypercapnia in the supine position, all participants increased heart rate, mean arterial pressure, and cardiac output index. These results confirm previous studies in healthy humans (28, 73, 78, 79, 91) and are likely due to increased sympathetic activation (28, 79). Middle cerebral artery velocity increased and cerebrovascular resistance indices decreased in response to hypercapnia confirming results of Ito et al. (2003) and Kety & Schmidt (1948) who also reported increased brain blood flow with a concurrent decrease in cerebrovascular resistance during hypercapnia (69, 78). All subjects increased ventilation, tidal volume, end-tidal O_2, and end-tidal CO_2 in response to hypercapnia. These results are consistent with previous findings that showed healthy humans respond to hypercapnia by increasing ventilation (14, 21, 33, 68, 143), tidal volume (14), end-tidal O_2 (28), and end-tidal CO_2 (28, 68, 79).

Compared to women, men displayed a greater increase in mean arterial pressure (and a corresponding attenuated decrease in total peripheral resistance index) in hypercapnic tilt indicating either greater sympathetic nerve activity or greater neurovascular conductance (as seen previously in men (61)) reflecting significant effects of sex on the autonomic and/or vascular responses to hypercapnia during HUT. Beyond the effects of CO_2 on autonomic function via the central chemoreflex, CO_2 also has strong vasodilatory effects on peripheral vasculature. By increasing end-tidal CO_2 to 9 mmHg above baseline, Simmons et al. (2007) observed vasodilation in cutaneous skin blood vessels in healthy men and women (135). Similarly, Kontos (1971) observed vasodilation and increased forearm vascular conductance in skeletal muscle of healthy men in response to inhalation of CO_2 (80). However, Viswanathan et al. (1976) administered 5% CO_2 to 20 healthy men which resulted in significantly increased pulmonary vascular resistance and cardiac output perhaps reflecting pulmonary vasoconstriction (155). Similarly, Balanos et al. (2003) studied 12 healthy subjects (mixed-sex) and found that pulmonary vascular resistance rose significantly in response to hypercapnia, suggesting pulmonary vasoconstriction (6). Therefore, we suggest that in the current study, the higher end-tidal CO_2 observed in hypercapnic tilt compared to

supine hypercapnia is responsible for the augmented increases in both stroke volume index and cardiac output index via pulmonary vasoconstriction. This increase of end-tidal CO_2 during tilt could be due to enhanced respiratory pump action increasing the venous return of hypercapnic blood from the periphery as suggested by Serrador et al. (2006).

Compared to supine hypercapnia, both men and women experienced augmented decreases in total peripheral resistance during hypercapnic tilt; however, the decrease was greater in women. This occurs in spite of presumed pulmonary vasoconstriction due to the greater end-tidal CO_2 observed in hypercapnic tilt in all subjects. Since we do not see an augmented decrease of cerebral vascular resistance during hypercapnic tilt, we hypothesize that the reduction in total peripheral resistance index in hypercapnic tilt is driven by greater peripheral blood flow in unmeasured vascular beds such as those in the muscles, liver, kidneys, and splanchnia. Interestingly, all groups had significantly increased pulse-wave velocity during supine hypercapnia suggesting increased peripheral vasoconstriction from increased sympathetic nerve activity.

The results of this study partially confirm those of Kastrup et al. (1997) who found greater cerebrovascular reactivity to CO_2 in women compared to men (75). In the current study, men had a significantly attenuated increase in diastolic middle cerebral artery velocity in response to CO_2 compared to women during the mid-luteal phase. This suggests diminished cerebral vasodilation in the presence of hypercapnia compared to women during the mid-luteal phase; however, there were no significant sex differences found within any of the measured resistance indices (pulsatility index, resistance index and cerebrovascular resistance index) or significant effects of HUT on the cerebrovascular responses to CO_2. Other unmeasured resistance indices, such as critical closing pressure and resistance area product (as described in 121), could display changes in cerebral vasculature responses. In order to make firm conclusions about cerebral vasoconstriction or dilation, future investigations using magnetic-resonance imaging to measure cerebral blood flow are important. Although we hypothesized that women during the ML phase would have increased cerebrovascular reactivity to CO_2 in both positions compared to the EF phase, there were no significant changes observed in cerebral hemodynamics in either position within the menstrual

cycle. This suggests that female sex hormone concentration does not influence cerebrovascular reactivity to CO_2 between the early-follicular and mid-luteal phases of the menstrual cycle. Similarly, we did not find any evidence that suggested cerebrovascular reactivity difference between the supine and upright posture. Peltonen et al. (2016) also investigated the cerebrovascular response to hypercapnia and found no difference between the early-follicular and late-follicular phases of the menstrual cycle (115). We did not find any evidence that suggested cerebrovascular reactivity differed between the supine and upright postures.

All groups had significantly augmented increases in ventilation during HUT in response to CO_2. This suggests that women also experience interactions between autonomic reflexes in upright posture which could enhance the central chemoreflex (63) and/or attenuate the pulmonary stretch reflex (146) resulting in the observed augmented increase in ventilation during hypercapnic tilt. All participants experienced a significantly augmented increase of respiratory rate in hypercapnic tilt which would lead to greater respiratory pump action contributing to the observed augmented increases in stroke volume and cardiac output. Similarly, all participants experienced attenuated increases in end-tidal CO_2 and augmented increases in end-tidal O_2 during hypercapnic tilt which is likely due to augmented increases in venous return from the enhanced respiratory pump action (as suggested by 133). There were no significant effects of menstrual cycle phase on the respiratory responses to hypercapnic tilt. This does not support out third hypothesis that women during the early-follicular phase would have enhanced central chemoreflex activity during hypercapnic tilt compared to the mid-luteal phase and men.

In the current study, only men had an augmented increase in mean arterial pressure during hypercapnic tilt suggesting sex-differentiated effects of the interactions between the baroreflex and central chemoreflex. These sex differences suggest one potential mechanism for greater orthostatic intolerance in women. All groups have enhanced central chemoreflex functioning during HUT in response to CO_2; however, the augmented increases in mean arterial pressure in response to hypercapnia in HUT observed in men suggests divergent autonomic responses between the sexes (but not between menstrual cycle phases).

Head-up Tilt in Hyperoxia

We hypothesized that: 1) In the supine posture, hyperoxia would decrease heart rate and cardiac output index while increasing stroke volume index in all groups (as observed previously), and 2) The peripheral chemoreflex would be activated in the upright posture (i.e. ventilation will decrease during hyperoxia) and this would be greatest in the EF phase.

Consistent with our first hypothesis; all participants had a decrease in heart rate in response to hyperoxia which has been confirmed in previous studies (31, 50, 77, 86, 148, 157), likely caused by greater parasympathetic activity as shown by the significant increases in the high-frequency domain of heart-rate variability analysis. Further, in response to hyperoxia, men and women had increases in stroke volume index, yet decreases in cardiac output index which has been reported previously (31, 50, 77, 148, 157). These decreases in cardiac output are likely due to increased cardiac afterload since men and women also increased total peripheral resistance in response to hyperoxia, as observed by others (31, 77, 148, 157), indicating peripheral vasoconstriction. Indeed, Ganz et al. (1972) showed that healthy subjects had reduced coronary sinus blood flow due to increased left-ventricular coronary resistance in response to 90-95% O_2 (50). In the current study, pulse-wave velocity was not significantly altered due to hyperoxia in the supine position which appears to conflict with the increase of total peripheral resistance. However, finger-toe pulse wave velocity is a measurement of arterial stiffness in the limbs and central arteries only and does not consider potential vasoconstriction in other vascular beds.

In response to hyperoxia during HUT, there was a significantly attenuated increase in total peripheral resistance and an attenuated decrease in cardiac output index in all groups suggesting attenuated peripheral vasoconstriction and sympathetic output, partially supporting our second hypothesis that the peripheral chemoreceptors are activated during orthostatic stress. This could be explained by interactions with other reflexes during upright posture such as the baroreflex and/or the metaboreflex. For example, during HUT, baroreflexes are deactivated due to an immediate decrease in blood pressure and a lack of distension in artery walls (48, 49, 58), and interactions between the baroreflex and the chemoreflex have previously been observed in dogs

such that the ventilatory responses to chemoreceptor stimulation are augmented by baroreceptor unloading and attenuated by baroreceptor activation (62). Similarly, in humans, stimulation of the metaboreflex via post-exercise circulatory occlusion activates the peripheral chemoreflex (39). Therefore, our observation that the peripheral chemoreflex is activated in upright posture could be due to activation of the metaboreflex via muscle activation. Interestingly, women during the early-follicular phase had augmented increases in total peripheral resistance index compared to the mid-luteal phase in response to hyperoxia, regardless of position. Therefore, higher levels of circulating sex hormones could be responsible for less peripheral vasoconstriction as observed by previous studies (76, 113). However, contrary to our hypothesis, there was no evidence of a menstrual cycle phase effect on the activation of the peripheral chemoreflex in upright posture.

All participants responded to hyperoxia with lower middle cerebral artery velocity (mean, systolic, and diastolic) and higher cerebrovascular resistance index in all groups indicating cerebrovascular vasoconstriction. The cerebral arteries are known to constrict due to hyperoxia in healthy individuals (possibly due to the generation of superoxide anions, thus resulting in inactivation of nitric oxide as observed by Zhilyaev et al. (2003)). This has been widely studied and confirmed by others who saw consistent decreases in brain blood flow velocity and increased cerebrovascular resistance in healthy subjects (4, 78, 106, 111, 123, 159). However, previous investigations studied only men (78, 159), looked at older populations (4, 106) or did not differentiate the cerebrovascular responses by sex-differences (111). Interestingly, in the current study, men had greater decreases in mean and systolic middle cerebral artery velocities and a greater increase of cerebrovascular resistance index in response to hyperoxia regardless of position compared to women in the early-follicular phase, thus indicating greater cerebral vasoconstriction during hyperoxia. This difference was not observed in women during the mid-luteal phase. Although we observed cerebral vasoconstriction in all participants in response to hyperoxia, we did not find any evidence of HUT influencing cerebral vasoconstriction during hyperoxia.

Hyperoxia resulted in higher ventilation and tidal volume leading to higher end-tidal O_2 and lower end-tidal CO_2. However, no changes in the ventilatory responses to

hyperoxia were observed during HUT except for an attenuated increase of end-tidal O_2 in women during the mid-luteal phase and men when compared to women in the mid-luteal phase. These attenuations were only approximately 6.3% in women during the mid-luteal phase and 5.3% in men and the final end-tidal O_2 values during hyperoxic tilt were still considerably higher than what would lead to 100% hemoglobin saturation. Previous studies have shown increases in ventilation in young healthy subjects in response to 75%-99.4% hyperoxia (8, 83); however, these studies administered hyperoxia for 30 minutes (8) and 50 minutes (83). The proposed mechanism for this increase in ventilation was due to the "Haldane effect" where oxygenation of pulmonary blood in the lungs readily displaces CO_2 from hemoglobin resulting in hyperventilation (19). Men had significantly augmented decreases in end-tidal CO_2 levels in response to hyperoxia possibly due to greater pulmonary vasodilation (as a result of the vasodilatory effects of testosterone on pulmonary vasculature (42, 72, 124)) and therefore improved gas exchange. Greater pulmonary vasodilation in men during hyperoxia is further evidenced by the smaller increase of stroke volume index. The augmented reduction in end-tidal CO_2 could also help to explain the greater vasoconstriction in the cerebrovasculature of men. The suppression of the peripheral chemoreflex in hyperoxic tilt should have produced attenuated ventilation in all subjects, yet this was not observed. We suggest that the autonomic interactions occurring in upright posture indeed result in lower sympathetic output during hyperoxia as expected due to inhibition of the peripheral chemoreceptor; however, due to the changes in mechanical forces on the chest cavity (i.e. gravity pulling the diaphragm downwards) and therefore, pulmonary stretch, the expected reduction in ventilation was obscured.

These results suggest that in hyperoxic tilt there is an overall reduction in sympathetic tone indicated by the attenuated increase of total peripheral resistance index and attenuated decrease of cardiac output index; however, this is not reflected in cerebrovascular, respiratory, or mean arterial pressure responses. Future investigations utilizing direct measurements of muscle sympathetic nerve activity responses to hyperoxia during HUT are warranted to firmly conclude that the peripheral chemoreflex is indeed activated during upright posture.

CONCLUSIONS

The purpose of this research was to investigate central chemoreflex activation and peripheral chemoreflex suppression with hypercapnia and hyperoxia, respectively, while supine or upright while also investigating sex and/or menstrual cycle differences. This investigation confirmed that; 1) the central chemoreflex is augmented in men and women during HUT, and 2) the peripheral chemoreflex is activated in men and women during HUT. Under normoxic conditions regardless of position, men were observed to have lower middle cerebral artery velocities compared to women in the ML phase. Ventilation was significantly increased in men in tilt which was not observed in women, and women in the mid-luteal phase were observed to have higher ventilation compared to the early-follicular phase in both positions. Men and women experienced lower brain blood velocity, orthostatic hypotension and decreased cardiac output index during HUT. In women, the absence of an increase in respiratory pump activity (i.e. no increase in ventilation) likely plays a role in the higher prevalence of orthostatic hypotension.

In response to hypercapnia during tilt, both men and women exhibited significant but divergent responses compared to supine hypercapnia. While all participants had greater ventilation during hypercapnic tilt, only men had augmented increases in mean arterial pressure. These results suggest that central chemoreflex activation is augmented in both men and women during HUT likely due to interactions with other autonomic reflexes; however, this augmentation results in divergent autonomic responses between the sexes. Although no menstrual cycle differences were observed in the cerebrovascular reactivity to CO_2 in response to hypercapnia, women in the ML phase were exhibited to have greater increases in diastolic MCA velocity during hypercapnia compared to men likely due to the presence of female sex hormones causing more vasodilation. However, no sex differences were observed in resistance indices of cerebrovasculature responses to hypercapnia, thus, further investigation into other measurements of resistance may support the observed differences. Both men and women exhibited peripheral chemoreflex activation during HUT as evidenced by the attenuated increase of total peripheral resistance index and attenuated decrease of cardiac output index during HUT. This is suggestive of autonomic reflex interactions (i.e. peripheral chemoreflex and baroreflex) during HUT in both men and women.

There were very few significant main effects of menstrual cycle throughout this investigation other than reduced ventilation in the mid-luteal phase compared to the early-follicular phase; however, that had already been well documented. The importance of testing women throughout the menstrual cycle should not be underestimated. When comparing each phase to men, sex differences were not always evident. Therefore, when investigating sexually dimorphic responses, menstrual cycle should be taken into consideration to avoid false negatives or false positives.

The respiratory pump plays a crucial role in the maintenance of venous return, cardiac output index and mean arterial pressure during orthostatic stress. However, by not increasing ventilation in HUT, women displayed attenuated respiratory pump action in HUT compared to men which may precipitate syncope, lightheadedness, orthostatic hypotension and fainting observed in women. Further, increased sympathetic nerve activity, greater neurovascular transduction, improved pulmonary gas exchange, and/or reliance on abdominal breathing could allow men to maintain mean arterial blood pressure during orthostatic stress better than women. Refer to Appendix A for summary of results.

LIMITATIONS AND FUTURE STUDIES

This study collected pulse-wave velocity using pulse waves from the finger and toe. Although not considered the “gold standard” of measuring central arterial stiffness (carotid-femoral pulse-wave velocity is the “gold standard”) (85), this measurement has been found to be closely correlated with carotid-radial pulse-wave velocity in young adults (40). This simplified method of collection is not a direct measurement of central arterial stiffness as is carotid-femoral pulse-wave velocity. Rather, it is a measure of peripheral arterial stiffness. Although pulse-wave velocity is not a direct measurement of muscle sympathetic nerve activity, it has been shown that there is a strong correlation between the stiffening of arteries (reflected by high pulse-wave velocity) and sympathetic activation (145), thus, it was used in this study as an indirect, non-invasive, surrogate marker for sympathetic activity. In future studies, implementation of microneurography to directly assess muscle sympathetic activation will strengthen

conclusions regarding sympathetic activation. Lastly, since this method required a pulse transducer to be placed on the toe, signal quality was insufficient in most participants during HUT, thus, we were unable to determine arterial stiffness during HUT.

In response to normoxia in the supine position, women had increases in end-tidal O_2 with concurrent decreases in end-tidal CO_2 compared to baseline. This could be due to the constant flow of the normoxic mixture from the tanks into the respiratory apparatus (Figure 3) during gas administration, thus, increasing end-tidal O_2 and reducing end-tidal CO_2 by approximately 1-2% (Table 2).

All female participants in this study self-reported menstrual cycle phase defining day 0 as the first day of menstruation. Unfortunately, we did not confirm plasma hormone concentrations of estrogen or progesterone in the mid-luteal phase of the menstrual cycle (days 18-24). Future studies should include this or urinary testing of luteinizing hormone to confirm ovulation. Similarly, we did not collect blood samples for plasma hormone measurements of vasoactive hormones such as norepinephrine, epinephrine, renin, and arginine vasopressin. The concentrations of these hormones have been observed to change in men and women during postural changes (supine to 70^{o} HUT), and found to be influenced by sex and position (52). Measurement of these circulating hormones would be beneficial in accounting for vasoactive influences on cardiovascular and cerebrovascular responses between men and women and during posture changes.

True cerebral blood flow, measured as the product of mean blood flow velocity and cross-sectional area of the vessel, was unable to be quantified due to the inability of measuring the cross-sectional area of the middle cerebral artery. However, previous studies have found no significant changes in middle cerebral artery diameter during either 10 minutes of orthostatic stress (132) or 3 minutes of hyperventilation (152) in mixed-sex groups. Direct measurement of cerebral vessel diameter through magnetic resonance imaging will allow an accurate calculation of flow to investigate cerebrovascular reactivity with or without orthostatic stress.

Peripheral blood flow was not measured in this study. This limitation in conjunction with no direct measurements of muscle sympathetic nerve activity omits an accurate indication of neurovascular transduction in our participants. Future studies

should implement ultrasonography to measure cross-sectional areas of peripheral blood vessels to determine vasoconstriction or vasodilation and, as mentioned previously, microneurography to directly measure muscle sympathetic nerve activity. The combination of measurements will help to accurately describe changes in neurovascular transduction.

Blood oxygen saturation levels in our participants were not monitored through pulse-oximetry although we expected it to rise to 100% in response to hyperoxia. Recently, Ceylan et al. (2016) showed that blood oxygen saturation did not differ between 5 different positions in 235 young, healthy men and women and that they remained in a normal range (94-98%) (140) throughout testing. Since our protocol did not include the effects of hypoxia on cardiovascular, respiratory, or cerebral variables, pulse-oximetry was not necessary for the health and safety of our participants. In future studies, we plan to implement a hypoxic protocol to assess peripheral chemoreflex activation in the supine and upright postures. This will require the use of pulse-oximetry.

The relationship between respiration and hemodynamics is closely linked by the respiratory muscle pump and is an important factor in maintaining venous return during orthostatic stress (99, 125). As seen previously, inferior vena cava diameter and central venous pressure measurements have been used as indices of venous return (37); however, these measurements were not used in the current study. We were therefore unable to make firm conclusions about changes in venous return since we were unable to directly measure it.

There were no direct measurements of participant fitness (i.e. VO_{2max}). However, we implemented the use of the Ainsworth equation which has been shown to represent 78% ($R^2 = 0.78$) of the variance observed in measured VO_{2max} thus proving to be a valid predicted measure of VO_{2max} in young healthy individuals (2).

Future studies could incorporate elite athletes to determine their responses to chemoreflex and orthostatic stress to determine if highly trained individuals are less able to maintain orthostatic tolerance compared to untrained individuals. Further, our lab hopes to use this data to expand our research to investigate clinical populations such as patients with postural orthostatic tachycardia syndrome (POTS) and orthostatic hypotension, conditions that are highly prevalent in women.

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Appendix A: Summary of results

Condition	Major Results
Normoxic Tilt	• Men have increased respiratory pump action compared to women during HUT.
Hypercapnia	• The central chemoreflex is augmented in HUT in all groups; however, only men have augmented increases in MAP.
Hyperoxia	• The peripheral chemoreflex is activated in HUT in all groups.