THE EFFECT OF ACUTE INTERMITTENT HYPERCAPNIC HYPOXIA ON VENTILATORY PLASTICITY AND CEREBRAL NEUROVASCULAR COUPLING IN HUMANS

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Abstract

Healthy humans exposed to acute intermittent hypoxia (IH) demonstrate comparable physiological responses to those with obstructive sleep apnea (OSA) and chronic exposure to IH. Responses include elevated sympathetic vasomotor outflow, arterial blood pressure, and changes in ventilatory and cerebrovascular sensitivity. Additionally, prolonged elevations in resting ventilation ($\dot{V}_I$) or ventilatory long-term facilitation (vLTF) manifest following IH, although to date only shown when hypercapnia is sustained throughout exposure and recovery. We aimed to determine (1) if acute intermittent hypercapnic hypoxia (IHH) elicits vLTF during isocapnic-normoxic recovery, (2) whether peripheral chemoreceptor drive contributes to vLTF, and (3) if IHH alters cerebral neurovascular coupling (NVC) in healthy men and women. Twenty individuals (age: 22±1; 9 females, tested 0-5 days early follicular phase) were exposed to 40-minutes of IHH comprised of 40-seconds hypercapnic hypoxia (nadir S_PO_2 = 83.3±1.0%, peak $P_{ET}CO_2$ = 3.2±0.3 mmHg above baseline), and 20-seconds of normoxic recovery. Nine males returned for a time-matched room-air control. Beat-by-beat mean arterial blood pressure (MAP; finger-photoplethysmography) was recorded throughout the trial. The peak relative posterior cerebral blood velocity (PCA_V; transcranial Doppler ultrasound) and conductance (PCA_{CVC}; quotient of PCA_V and MAP) response to 5 repeated cycles of 30-seconds eyes-open with standardized visual stimulation determined cerebral NVC before and after IHH. Resting ventilation ($\dot{V}_I$) was recorded for 50-minutes of recovery following IHH and 1-minute bouts of hyperoxia were administered to attenuate peripheral chemoreceptor drive at 5-minute intervals. Resting MAP was elevated from baseline at all time points throughout recovery (main effect; $P<0.01$). Following IHH, peak relative PCA_V response to visual stimulus was not different, however PCA_{CVC} response was increased by 4.5±1.5% ($P<0.01$) and did not changed during time-matched control. Throughout 50-minutes of recovery $\dot{V}_I$ was increased by 4.6±0.1 l/min compared to baseline ($P<0.01$). Hyperoxia depressed $\dot{V}_I$ at baseline and $\dot{V}_I$ depression was augmented throughout recovery ($\Delta\dot{V}_I$ = -0.8±0.2 vs. -1.7±0.3 l/min respectively, $P<0.01$). $\dot{V}_I$ depression was not different during time-matched control. In summary, following IHH (1) vLTF is evident during isocapnic-normoxic recovery, (2) peripheral chemoreceptor inhibition led to greater depression of $\dot{V}_I$ and (3) coupling of cerebral oxygen delivery to metabolic demands was augmented in healthy individuals.

Lay Summary

In obstructive sleep apnea (OSA) brief breathing cessations occur and intermittent exposures to low oxygen and paired high carbon dioxide in circulating blood result in an increased risk for cardiovascular disease. Healthy individuals exposed to similar cycles in oxygen and carbon dioxide develop comparable changes in cardiorespiratory function including elevated respiratory sensitivity and decreased cerebrovascular reactivity to low oxygen and high carbon dioxide. This study exposed healthy individuals to repeated bouts of low oxygen and high carbon dioxide stimuli to characterize functional changes in breathing and brain blood flow control. As evidence of plasticity in respiratory control we found elevated resting breathing during 50-minutes of recovery that was partially inhibited with hyperoxia exposure. Additionally, we observed a higher cerebrovascular response to a standardized stimulus indicating increased brain blood flow coupling. These data advances the understanding of how cardiorespiratory control is altered following stimuli that mimic OSA.

Preface

This thesis contains original data collected in the Cardiopulmonary Laboratory for Experimental and Applied Physiology. Tyler Vermeulen and Dr. Glen Foster were responsible for the conception and design of the experiment. Tyler Vermeulen, Jenna Benbaruj, Ryan Niven, Courtney Brown, and Brooke Shafer contributed to the recruitment of participants, and collection of experimental data. Tyler Vermeulen was responsible for complete data analyses with technical guidance by Dr. Glen Foster. The writing in the thesis document was completed by Tyler Vermeulen with the editorial assistance of Dr. Glen Foster. Ethical approval for the project was obtained from the University of British Columbia Clinical Research Ethics Board (CREB# H18-02513).

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List of Abbreviations

5-HT	Serotonin
AHI	Apnea-hypopnea index
ATP	Adenosine triphosphate
BDNF	Brain derived neurotrophic factor
BMI	Body mass index
BTPS	Body temperature, saturated pressure
Ca^{++}	Calcium
CON	Control
CPAP	Continuous positive airway pressure
CSN	Carotid sinus nerve
DBP	Diastolic blood pressure
DEF	Dynamic end-tidal forcing
D_LCO	Diffusion capacity of the lung to carbon monoxide
f_B	Breathing frequency
FCO_2	Fraction of carbon dioxide
FEV_1	Forced expired volume in 1 second
F_ICO_2	Fraction of inspired carbon dioxide
F_IO_2	Fraction of inspired oxygen
FO_2	Fraction of oxygen
FRC	Functional reserve capacity
FVC	Forced vital capacity
H^+	Hydrogen ion
HR	Heart rate
IHH	Intermittent hypercapnic hypoxia
LTF	Long-term facilitation
MAP	Mean arterial pressure
MCA	Middle cerebral artery
MCA_{CVC}	Middle cerebral artery vascular conductance
MCA_V	Middle cerebral artery blood flow velocity

mmHg	Millimeters of mercury
NADPH	Nicotinamide adenine dinucleotide phosphate
NMDAR	N-methyl-D-aspartate receptor
NO	Nitric oxide
NTS	Nucleus tractus solitarii
NVC	Neurovascular Coupling
ODI	Oxygen desaturation index
OSA	Obstructive sleep apnea
PCA	Posterior cerebral artery
PCA_{CVC}	Posterior cerebral artery vascular conductance
PCA_V	Posterior cerebral artery blood flow velocity
$P_{ET}CO_2$	Partial pressure of end-tidal carbon dioxide
$P_{ET}O_2$	Partial pressure of end-tidal oxygen
PVN	Paraventricular nucleus
ROS	Reactive oxygen species
RV	Residual volume
RVLM	Rostral ventrolateral medulla
SBP	Systolic blood pressure
SEM	Standard error of the mean
S_pO_2	Peripheral oxyhemoglobin saturation
TCD	Transcranial doppler ultrasound
TLC	Total lung capacity
$TRPV_1$	Transient receptor potential cation channel subfamily V member 1
V_A	Alveolar volume
$\dot{V}_I$	Minute ventilation
vLTF	Ventilatory long-term facilitation
V_T	Tidal volume

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Chapter 1: Literature Review

1.1 Introduction to the Literature Review

The aim of this literature review is to outline obstructive sleep apnea (OSA) and focus on the changes in cardiorespiratory and cerebrovascular function that occur from exposure to intermittent hypercapnic hypoxia (IHH) similarly experienced during nocturnal apneas. Information from intermittent hypoxia and intermittent hypercapnic hypoxia models in healthy animal and humans will be highlighted with additional details from OSA populations when possible. Briefly the cardiovascular consequences of OSA will be discussed and an introduction of common models of OSA and IHH exposure in healthy individuals will be made. A primary focus will be on the physiological mechanisms for the control of ventilation and the cerebral vasculature in healthy people and knowledge to date will be presented on the influence that IHH (with and without paired hypercapnia) has on the neuroplasticity of ventilatory and cerebrovascular control systems.

1.2 Obstructive Sleep Apnea and Intermittent Hypoxia

Obstructive sleep apnea is characterized by repeated cessations in breathing experienced throughout sleep. In OSA, apneas result from a collapse of the upper airway (nasopharynx and hypopharynx) due to lack of structural support and/or loss of motor tone within these structures (Dempsey *et al.*, 2010). Respiratory events in OSA can be classified as apneas, which refer to complete absence in ventilation, or hypopneas, defined as a ≥ 50% reduction in respiratory airflow during sleep. Events lasting a minimum of 10 seconds can be indicated without an associated ≥ 4% reduction in arterial oxygen saturation (S_PO_2), and shorter events scored with an associated desaturation as outlined by recent sleep scoring guidelines (Berry *et al.*, 2012). The respiratory patterns associated with apneas and hypopneas leads to intermittent hypoxia with concomitant hypercapnia and is termed intermittent hypercapnic hypoxia (IHH). The prevalence of OSA in adults is estimated to affect 6-13% of adults between 30-70 years of age and is correlated with increasing severity of obesity (Peppard *et al.*, 2013). Severity of OSA is classified according to the number of apneas and hypopneas observed per hour of sleep, known as the apnea-hypopnea index (AHI). OSA is classified as mild (AHI: 5-15 events/hour), moderate (16-30 events/hour), and severe (> 30 events/hour), although some individuals can experience ≥ 60 events per hour (American Academy of Sleep Medicine, 1999).

The chronic intermittent hypoxia in OSA is associated with overt and robust cardiovascular changes including increases in daytime and nocturnal blood pressure (Somers *et al.*, 1995; Young *et al.*, 1997), elevated muscle sympathetic nerve activity during wakefulness and sleep (Carlson *et al.*, 1993; Somers *et al.*, 1995; Narkiewicz *et al.*, 1999), and impaired neurocognitive function (Lal *et al.*, 2012). Additionally, individuals with OSA have an increase in the prevalence of cardiovascular disease and OSA is recognized as an independent risk factor for hypertension (Young *et al.*, 1997), stroke (Redline *et al.*, 2010), and heart failure (Jean-Louis *et al.*, 2008). It has been postulated that elevated blood pressure in OSA is a result of the concomitant heightened muscle sympathetic nerve activity driven by heightened carotid body chemoreflex activity (Narkiewicz *et al.*, 1998; Prabhakar, 2016). However, the mechanisms responsible for these physiological changes and the consequent development of cardiovascular disease are difficult to discern due to multiple cardiovascular comorbidities frequently present in OSA.

Experimental models involving healthy animals and humans exposed to either acute or chronic intermittent hypoxia offer unique experimental designs to interrogate the underlying physiological changes that occur in OSA in the absence of concomitant cardiovascular and respiratory consequences. The results from both acute and chronic exposures mimicking the intermittent hypoxia paradigm of OSA in animal and human models demonstrate similar changes in muscle sympathetic nerve activity (Fletcher, 2000, 2003; Xie *et al.*, 2000; Cutler, 2004), arterial blood pressure (Fletcher *et al.*, 1999; Foster *et al.*, 2009*a*, 2010; Tamisier *et al.*, 2009; Gilmartin *et al.*, 2010), ventilatory and cerebrovascular chemosensitivity (Foster *et al.*, 2005; Tamisier *et al.*, 2009; Marcus *et al.*, 2010), and respiratory neuroplasticity (Baker & Mitchell, 2000; Olson *et al.*, 2001; Harris *et al.*, 2006; Griffin *et al.*, 2012).

1.3 Overview of the Control of Ventilation

A close temporal control of ventilation is necessary to maintain adequate O_2 and CO_2 within the arterial blood. This control is achieved through sensory afferent information from chemoreceptors propagated to the respiratory control center within the brainstem. Integration of the afferent signal takes place throughout multiple brainstem nuclei and results in adjustment of efferent activity to respiratory muscles altering ventilatory rate and depth. The key muscle of respiration being the diaphragm controlled through the phrenic motor neuron. Additional accessory muscles of respiration include the external and internal intercostal muscles, and muscles of the upper airway

including the genioglossus tongue muscles which maintain patency of the upper airway tract. Changes in arterial blood gases are detected by two chemo-sensitive bodies, the peripheral chemoreceptors located in aortic and carotid bodies which are sensitive primarily to changes in O_2 and CO_2, and central chemoreceptors in the brainstem primarily sensitive to changes in CO_2 though changes in $[H^+]$.

1.3.1 Peripheral Control of Ventilation

The main peripheral chemoreceptors are the carotid bodies located at the bifurcation of the common carotid artery as it branches to the internal and external carotid arteries. The carotid bodies communicate with the brain stem through the carotid sinus nerve (CSN), which is a branch of the glossopharyngeal nerve (cranial nerve IX). Type I or glomus cells within the carotid bodies are the primary oxygen sensors. These cells are highly sensitive to hypoxemia, such that when reductions in arterial PO_2 are detected, depolarizations occur from increased currents through outward rectifying potassium channels and influx of Ca^{2+} through voltage-gated channels resulting in neurotransmitter release and afferent signals emitted along the CSN. In isolated preparations, the CSN discharge is shown to be exponentially increased when PO_2 levels reach 45-55 mmHg (Black *et al.*, 1971; Vidruk *et al.*, 2001). Importantly, hyperoxia (> 200 mmHg) can substantially attenuate the discharge rate of carotid bodies, although higher hyperoxic levels of ~400 mmHg cannot completely abolish the discharge of carotid body afferents (Biscoe *et al.*, 1970; Lahiri *et al.*, 1983). Hypercapnia is also detected by the carotid bodies and leads to similar increases in CSN activity (Black *et al.*, 1971; Bisgard *et al.*, 1986). During respective decreases and increases in PO_2 and PCO_2, a synergistic effect is noted leading to enhanced afferent activity along the CSN (Lahiri & DeLaney, 1975; Lahiri *et al.*, 1983; Wilson & Teppema, 2016).

A series of signal communications occur within the respiratory control centers of the medulla to integrate the ventilatory response. Afferent signals from the CSN synapse initially onto the nucleus solitary tract (NTS) located at the base of the medulla. Synaptic information is then transmitted to the rostral ventrolateral medulla (RVLM) which has been implicated as the primary regulatory control center in the brain for the coordinated cardiorespiratory and autonomic response to hypoxia and hypercapnia (Warren Cottle & Calaresu, 1975; Guyenet, 2006; Kline *et al.*, 2010). Additional communication between the NTS and paraventricular nucleus of the hypothalamus (PVN) occur before integration to the RVLM (King *et al.*, 2012). Moreover, the PVN is shown to

be sensitive to hypoxia and hypercapnia and is also implicated in the cardiorespiratory response to alterations in arterial blood gas status (King *et al.*, 2012; Blackburn *et al.*, 2018). Input from the PVN is sent to the RVLM and the efferent response is propagated to modulate ventilation. Efferent signals from the RVLM are sent along the phrenic motor neuron which innervates the diaphragm, one of the main respiratory effector muscles (Lee & Fuller, 2011). Multiple neurotransmitters including glutamate, dopamine, angiotensin II, and ATP among others are responsible for the neuronal transmission between the afferent integration sites in the medulla and the coordinated respiratory response (Pamenter & Powell, 2016; Zera *et al.*, 2019).

1.3.2 Central Control of Ventilation

Central chemoreceptors are located amongst varying regions within the medulla and are inherently sensitive to changes in $[H^+]$ which is dependent on local tissue CO_2 status (Dempsey & Forster, 1982; Nattie & Li, 2012). Additionally, the location of central chemoreceptors and their ability to respond to changes in oxygen has been proposed, although definitive evidence on their role and mechanism of action remain unknown (Teppema & Dahan, 2010; Wilson & Teppema, 2016; Funk & Gourine, 2018; Teppema, 2018). The central brainstem structures responsible for the communication of peripheral chemosensory afferent signals have also been noted as responsible for the central sensing of CO_2. Putative central chemoreceptors include the retrotrapezoid nucleus located in the RVLM (Guyenet & Mulkey, 2010), NTS which project information to the pre-Botzinger complex (Dean *et al.*, 1989, 1990), and medullary Raphe neurons which project to respiratory control centers and the phrenic motor neuron (Iceman *et al.*, 2013; Iceman & Harris, 2014).

1.3.3 Sex Differences in Ventilatory Control

Compared to women, men typically have larger conducting airways, and greater lung volumes and these differences persist in comparisons between height matched men and women (Dominelli *et al.*, 2018). Differences between men and women become relevant during whole body exercise where it has been demonstrated that women are more likely to develop expiratory flow limitations compared to men (Guenette *et al.*, 2007; Dominelli *et al.*, 2015*a*). Additionally, women demonstrate a greater work of breathing during exercise ventilatory rates greater than 50 l/min due to a greater inspiratory resistive work of breathing in women (Guenette *et al.*, 2007; Dominelli *et*

al., 2015*a*). The higher work of breathing in women is concomitant with a higher oxygen uptake of respiratory muscles compared to men at matched ventilatory rates and can impose further constraints during exercise as a greater proportion of cardiac output is directed away from the locomotor muscles to the respiratory muscles (Dominelli *et al.*, 2015*b*; Sheel *et al.*, 2016). Importantly, differences between men and women are only demonstrated during whole body exercise approaching near maximal workloads and to date these differences have not been shown at lower workloads.

The influence of fluctuating hormone levels is an important considering when investigating sex differences in the control of ventilation (Behan *et al.*, 2003). Differences in ventilatory control between men and women may originate from influences occurring peripherally at the carotid body, or the medulla where integration of respiratory afferent signals and coordination of efferent response occurs (Behan & Wenninger, 2008). Whether the ventilatory chemoreflex response to hypoxia and hypercapnia is different between males and females has demonstrated conflicting results. With respect to hypoxic sensitivity, prior repots have shown males having an augmented (White *et al.*, 1983), attenuated (Tatsumi *et al.*, 1991) or no difference in sensitivity compared with women (Loeppky *et al.*, 2001; Guenette *et al.*, 2004; Jensen *et al.*, 2005). Similarly, hypercapnic sensitivity in males has been shown to be higher (White *et al.*, 1983) or not different compared to females (Rebuck *et al.*, 1973). Inconsistent findings may be impacted by the method used for inducing the chemoreflex response (i.e. re-breath method, fixed fraction, end-tidal forcing), and inconsistencies in the experimental control for menstrual cycle. Macnutt and colleagues (2012) recently measured chemoreflex responses between men and women measured at multiple time points throughout the menstrual cycle with experimental measures of hormones. Findings showed similar hypoxic and reduced hypercapnic chemosensitivities in women across all phases of menstruation compared to men. Notably, an effect of menstrual cycle on resting ventilation in women was evident, such that resting ventilation was elevated during the mid-luteal phase compared to other phases, although resting ventilation was not different compared to males at all phases (Macnutt *et al.*, 2012).

1.3.4 Effect of Intermittent Hypoxia on Ventilatory Chemoreflex and Sensory Neuroplasticity

The influence of intermittent hypoxia on ventilatory control and sensitivity is of interest as changes in ventilatory control can reflect alterations in ventilatory instability and lead to an increased severity of OSA. To characterize changes in ventilatory control, studies utilizing intermittent hypoxia models in humans and animals have shown increases in resting ventilation during recovery and heightened ventilatory responses to chemoreflex stress following exposure. These changes in ventilatory sensitivity occur from alterations in plant gain (the change in arterial PCO_2 for a given change in ventilation) and controller gain (the ventilatory response to a given change in arterial PCO_2) the relationship of which is referred to as loop gain or the overall stability in the control of ventilation (White, 2005). The integrative control of ventilation can demonstrate long-term facilitation (LTF) or plasticity in neural control of an integrative system based on previous experience (Mitchell & Johnson, 2003). Sites of neuroplasticity and long-term facilitation within a neural system typically occur at neural synapses. For example, ventilatory long-term facilitation may occur at the peripheral chemoreceptors or within the CSN as increased afferent activity indicating sensory long-term facilitation, within central respiratory nuclei (e.g. NTS, RVLM), or the phrenic motor nuclei, leading to long-term changes in rhythm generation and the consequent pattern of ventilation (Mitchell & Johnson, 2003).

The human ventilatory chemoreflex to acute hypoxia following intermittent hypoxia exposure has been widely characterized (Foster *et al.*, 2005; Lusina *et al.*, 2006; Ainslie *et al.*, 2007; Pialoux *et al.*, 2009; Tamisier *et al.*, 2009; Gerst *et al.*, 2010). The consensus across prior reports summate to heightened ventilatory sensitivity to graded hypoxic stress following both acute (< 24 hours of a single exposure) or chronic (> 1 day of repeated exposure) intermittent hypoxia exposure. This increase in ventilatory sensitivity to hypoxia following intermittent hypoxia is related to the prolonged elevation in resting muscle sympathetic nerve activity, termed sympathetic LTF, in healthy humans suggesting plasticity in these systems share similar control centers (Cutler *et al.*, 2004; Lusina *et al.*, 2006). However, the effect of intermittent hypoxia on the ventilatory chemoreflex to hypercapnia in humans has been inconsistent across investigations with some showing increased ventilatory response to isolated CO_2 challenges (Katayama *et al.*, 2005; Tamisier *et al.*, 2009) and CO_2 challenges under a hypoxic background (Mateika *et al.*, 2004) and others showing no change (Katayama *et al.*, 2002; Foster *et al.*, 2005; Pialoux *et al.*, 2009; Beaudin

et al., 2015) in response to acute hypercapnia. The hypercapnic sensitivity may be related to the severity of chronic intermittent hypoxia exposure as Katayama et al., (2005) showed an enhanced ventilatory response to hypercapnia following two weeks of daily 1-hour exposures, which was not altered following just one week of exposure. Although the paradigm of 1-hour continuous daily hypoxic exposure does not match to the higher frequency transient changes in arterial blood gases seen in OSA, Katayama et al., (2005) highlights the severity dependent potential of intermittent hypoxia models. The discrepant findings between investigations is potentially due differences in intermittent hypoxia models utilized as evidence suggests the severity and length of the intermittent exposure and inter-apnea recovery period has implications on the magnitude of the functional response.

Exposure to intermittent hypoxia in animals provides further understanding of the integrative mechanisms responsible for altered ventilatory control that occur following intermittent hypoxia. Peng et al., (2003) established in rats previously conditioned with chronic intermittent hypoxia that isolated CSN recordings show sustained elevations in the afferent activity from the carotid bodies lasting up to an hour following an acute bout of intermittent hypoxia that was not evident following a continuous hypoxia exposure. This sustained elevation in carotid body activity represents a form of neural plasticity of the carotid body known as sensory long-term facilitation. As noted previously, differences in severity of intermittent hypoxia may have implications on the neuroplasticity observed. Peng and Prabhakar (2004) followed up on this initial investigation and administered chronic intermittent hypoxia in short (multiple hypoxic episodes an hour) versus long (single 4-hour exposure of hypoxia per day) duration cycles over a 10-day period and observed elevated carotid body sensitivity to hypoxia but not hypercapnia exposure and this plasticity was evident only in the group exposed to short duration intermittent hypoxia. Additionally, the CSN afferent activity demonstrates progressive augmentation, another form of neuroplasticity, that manifests as greater afferent activity in response to successive bouts of hypoxia (Cummings & Wilson, 2005). Though carotid body sensitivity to hypercapnia does not appear to change following intermittent hypoxia, when the intermittent exposures are paired with elevations in CO_2 (i.e. hypercapnia) sensory long-term facilitation is evident (Roy *et al.*, 2018), noting an important role of CO_2 in the neuroplasticity of respiratory control. The sustained elevations of the CSN afferent activity may be responsible for the observed changes in ventilatory sensitivity following

intermittent hypoxia in humans due to concomitant enhancements in phrenic motor neuron output observed in rat models (Peng & Prabhakar, 2003; Peng *et al.*, 2006).

The mechanisms responsible for evoking sensory long-term facilitation are not completely understood. Sensory long-term facilitation may involve activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase through a serotonin (5-HT) dependent mechanism leading to generation of reactive oxygen species (ROS) within the carotid bodies (Peng *et al.*, 2006, 2009). This pathway is further supported in rats, when circulating ROS was suppressed with prior antioxidant treatment, 21 days of intermittent hypoxia attenuated the expected increase in chemosensory afferent activity to hypoxia (Del Rio *et al.*, 2010). Interestingly, the increase in ventilatory sensitivity to graded hypoxia observed in humans is related to increases in oxidative stress markers produced during intermittent hypoxia (Pialoux *et al.*, 2009). Although, it remains unknown whether prior treatment with antioxidants influences the change in ventilatory sensitivity to hypoxia following intermittent hypoxia in humans. Angiotensin II, which is increased during intermittent hypoxia (Lam *et al.*, 2014), also evokes sensory long-term facilitation through activation of NADPH oxidase pathways and sensory long-term facilitation can be abolished through isolated blockade of NADPH oxidase with apocynin following exogenous application of angiotensin II (Peng *et al.*, 2011). Changes in chemoreflex sensitivity following chronic intermittent hypoxia can be also be eliminated with isolated blockade of NADPH oxidase or combined pharmacological blockade of xanthine oxidase (with allopurinol) and angiotensin II (with losartan) receptor (Morgan *et al.*, 2016). However, isolated blockade of xanthine oxidase attenuated but did not completely remove the change in chemosensitivity following chronic intermittent hypoxia (Morgan *et al.*, 2016). Importantly, evoking sensory long-term facilitation following chronic intermittent hypoxia appears to be dependent on initial release of 5-HT during carotid body depolarizations in response to reduced arterial PO_2 (Prabhakar, 2000). Lastly, Roy and colleagues (Roy *et al.*, 2018) recently demonstrated that following acute intermittent hypercapnic hypoxia induced sensory long-term facilitation occurs in isolated perfused carotid bodies of rats naïve to prior hypoxia exposure and report a novel mechanism involving vanilloid type 1 ($TRPV_1$) receptors acting within carotid body afferents and provide further insight on the role of 5-HT, angiotensin-II and ROS signaling.

1.3.5 Intermittent Hypoxia and Ventilatory Long-Term Facilitation

Ventilatory long-term facilitation (vLTF) is another form of neuroplasticity observed in the control of ventilation following intermittent hypoxia and can manifest as increased phrenic motor nerve output or increased activity of upper airway muscles culminating in augmented resting ventilation lasting over an hour following the removal of the stimulus (Mitchell & Johnson, 2003). Sustained elevations in phrenic motor neuron activity were first observed following repeated stimulations of the CSN in cats (Millhorn *et al.*, 1980), and was confirmed following acute intermittent hypoxia in rats which was absent following continuous hypoxia of a similar duration (Baker & Mitchell, 2000). Recently, long-term facilitation of the hypoglossal nerve, which innervates the upper airway muscles, was also demonstrated following acute intermittent hypoxia (Wilkerson *et al.*, 2018). A multitude of acute intermittent hypoxia models have consistently demonstrated sustained elevations in phrenic motor output in animals with exposures consisting of 3-10 hypoxic cycles, ranging in length from 2-5 minutes of hypoxia at arterial PO_2 levels of 28-60 mmHg, with 5-10 minutes of normoxic recovery interspersed (reviewed in Mateika & Sandhu, 2011). Chronic intermittent hypoxia exposure additionally demonstrates vLTF following removal of the stimulus (McGuire & Ling, 2005; McGuire *et al.*, 2015). The severity of the hypoxic stimulus has been argued as an important role in the overall magnitude of vLTF observed in rats (McGuire *et al.*, 2002). Additionally, the magnitude of long-term facilitation observed is attenuated following denervation of carotid bodies in rat models indicating a modulatory role of the carotid bodies in this response (Bavis & Mitchell, 2003).

Evidence suggests humans similarly demonstrate vLTF following intermittent hypoxia, although the response is less robust as that observed in animals with laboratories reporting contrasting results. The discrepant findings of developing vLTF in humans is likely due differences in study designs including severity of intermittent exposure, arousal state which influences 5-HT discharge (i.e. attenuated during sleep) and central drive for ventilation (i.e. reduced during sleep) and lastly whether precise control of CO_2 levels was made during the recovery phase. Mateika and colleagues (2004) first reported that vLTF does not manifest following intermittent hypoxia during recovery under hypocapnic conditions. With additional studies demonstrating a lack of vLTF following acute intermittent hypoxia without the maintenance of isocapnia or hypercapnia during recovery (McEvoy *et al.*, 1996; Xie *et al.*, 2000; Jordan *et al.*, 2002). vLTF is also absent during recovery with uncontrolled CO_2 following

intermittent hypercapnic hypoxia exposures (Diep *et al.*, 2007; Deacon *et al.*, 2017). However, one report under isocapnic conditions during intermittent hypoxia and suggest manifestation of vLTF is dependent on time of day and is enhanced with repeated daily exposures (Gerst *et al.*, 2010). Such that a smaller magnitude of vLTF was achieved in the morning compared to evening without manipulation of CO_2 status during the intermittent exposure. The contrast in findings is possibly due to varying intermittent hypoxia models, with differences in severity and overall duration of hypoxia. Harris and colleagues (Harris *et al.*, 2006) first demonstrated that a sustained hypercapnic background throughout the exposure and recovery phase could elicit vLTF and long-term facilitation of genioglossus muscle activity in humans following acute intermittent hypoxia with hypercapnia ($P_{ET}CO_2$ increased 4-5 mmHg above baseline levels) sustained throughout the exposure and recovery. Since the initial report, this laboratory has reported vLTF manifesting in various states utilizing a similar intermittent hypoxia exposure (8-12 repeated cycles of 2-4 minutes hypoxic exposure) with sustained hypercapnia in healthy men and women to a similar magnitude (Wadhwa *et al.*, 2008; Mateika & Syed, 2013), awake and sleep states (Syed *et al.*, 2013), OSA (Syed *et al.*, 2013), and spinal cord injury (Tester *et al.*, 2014). Similar to sensory long-term facilitation in rats, OSA patients treated with antioxidants demonstrate attenuated vLTF following intermittent hypoxia (Lee *et al.*, 2009). vLTF exhibited following intermittent hypoxia and sustained hypercapnia has shown to be independent of tonic peripheral chemoreceptor drive as inhibition of carotid body activity with hyperoxia did not alter the ventilatory depression observed before exposure or during recovery (Griffin *et al.*, 2012). A caveat to this sustained hypercapnia model is that a progressive increase in ventilation is evident in control experiments with isolated exposure to hypercapnia at matched exposure durations and this may be contributing to an overestimation of vLTF measured during the sustained hypercapnia with intermittent hypoxia (Griffin *et al.*, 2012). Additionally, the 2-4 minute bouts of hypoxia are not representative of the hypoxemic bouts observed in OSA. Two previous reports with intermittent hypercapnic hypoxia (both <15-minute total hypercapnic-hypoxic time) that closely mimic OSA did not report vLTF however both reports may not have employed an exposure with adequate severity or of long enough duration to elicit vLTF despite maintaining isocapnic conditions during recovery (Diep *et al.*, 2007; Deacon *et al.*, 2017). There is a need for additional studies utilizing intermittent hypercapnic hypoxia to better understand the exposure models required for development of vLTF in humans.

Two centrally acting redundant pathways (termed the "Q" and the "S" pathways) have been proposed in animal models as the mechanisms responsible for vLTF. Assessing direct measures of sensory long-term facilitation are not possible in humans, and thus utilizing hyperoxia in combination with attainable measures of long-term facilitation (i.e. vLTF) provide insight on sensory long-term facilitation in humans. Though it has been suggested in humans that tonic peripheral chemoreceptor drive is not responsible for vLTF during recovery, activity from the peripheral chemoreceptors is implicated in the manifestation of vLTF. The "Q"-pathway suggests that intermittent hypoxia leads to 5-HT release from the medullary Raphe neurons and consequently activates the 5-HT type 2 receptor on the phrenic motor neuron. Brain derived neurotrophic factor (BDNF) becomes upregulated through upstream Gq-coupled proteins activated by 5-HT type 2 receptors ultimately resulting in downstream secondary messengers increasing synaptic sensitivity to glutamate on the post-synaptic motor neuron (in this case the phrenic motor neuron) (Dale-Nagle *et al.*, 2010; Pamenter & Powell, 2013). 5-HT release is evident during intermittent hypoxia (Kinkead *et al.*, 2001), and further support of this pathway is demonstrated by the absence of long-term facilitation with 5-HT antagonists (Bach & Mitchell, 1996; Ling *et al.*, 2001). Independent of 5-HT activation, alpha-adrenergic receptor activation on the phrenic motor neuron has been shown to result in long-term facilitation of the phrenic motor neuron through this Gq-coupled protein pathway as well (Neverova *et al.*, 2007). The "S"-pathway is dependent on adenosine activating a Gs-coupled protein eliciting downstream molecular changes, summating in the increased sensitivity to pre-synaptic glutamate on the post-synaptic motor neuron similar to the "Q" pathway (Hoffman *et al.*, 2010; Nichols *et al.*, 2012; Pamenter & Powell, 2013). Evidence for the redundancy in these pathways is shown in mice with a genetic 5-HT depletion that still develop vLTF following 10-days of intermittent hypoxia (Hickner *et al.*, 2013). These pathways have not been identified in humans and whether integration from the carotid body and sensory long-term facilitation are required for the eliciting vLTF in humans is not fully understood.

1.3.6 Clinical Relevance of Intermittent Hypoxia Induced Ventilatory Long-Term Facilitation

Recently, there is a renewed interest in the therapeutic application of intermittent hypoxia induced vLTF and associated plasticity in respiratory motor function. In animal models of spinal cord

injuries, multiple studies indicate intermittent hypoxia can promote improvements in diaphragm and inspiratory muscle activity, phrenic output and breathing function with acute injuries that are dependent on 5-HT pathways (Golder, 2005; Navarrete-Opazo *et al.*, 2015, 2017; Lee *et al.*, 2017; Dougherty *et al.*, 2018). One report shows that humans with incomplete spinal cord injuries have improved respiratory muscle motor output and vLTF during recovery after acute intermittent hypoxia with sustained hypercapnia throughout the exposure and recovery (Tester *et al.*, 2014). The ability of intermittent hypoxia to promote improvement in respiratory function in spinal cord injuries is important, because these individuals present with diminished respiratory function (Ovechkin *et al.*, 2010). It has also been proposed that OSA populations may benefit from elevations in upper airway muscle activity, which would promote improved upper airway stability and less frequent recurring apneas during sleep (Mateika & Narwani, 2009). This does not appear to be the case however, as apnea severity is worsened in OSA participants following acute intermittent hypoxia (Yokhana *et al.*, 2011). The higher AHI observed in OSA participants following acute intermittent hypoxia may be explained by elevations in ventilatory loop-gain and consequent worsening of ventilatory stability (Alex *et al.*, 2019). Lastly, the concomitant decrements in cardiovascular function following intermittent hypoxia cannot be ignored (Mateika *et al.*, 2015; see also 1.2).

1.4 Overview of the Control of the Cerebral Vasculature

Cerebral blood flow is supplied from four vessels which originate from the subclavian arteries. Specifically, the left and right common carotid arteries, and the left and right vertebral arteries. The common carotid artery bifurcates into the external carotid artery, supplying superficial facial structures, and the internal carotid artery which is responsible for ~70% of overall cerebral blood flow (Zarrinkoob *et al.*, 2015). The vertebral artery branches to the basilar arteries and distal intracranial posterior cerebral artery and is responsible for ~30% of overall cerebral blood flow (Zarrinkoob *et al.*, 2015). The intracranial middle and anterior cerebral arteries originate from the terminus of the internal cerebral artery and communicate with the posterior cerebral artery through the Circle of Willis, a key structure providing redundancy in the cerebral circulation to maintain adequate perfusion in the event of ischemia or blockage. Ultrasonography is commonly used to non-invasively asses cerebral blood flow with two techniques demonstrated consistently in the literature. Transcranial Doppler (TCD) ultrasound allows interrogation of blood velocity of the

cerebral intracranial vessels albeit with the assumption of vessel diameter remaining stable across a range of stimuli, which has been a topic of debate in the literature (Poulin & Robbins, 1996; Willie *et al.*, 2011*a*; Ainslie & Hoiland, 2014; Coverdale *et al.*, 2014). Duplex ultrasound allows the simultaneous measurement of blood velocity and beat-by-beat diameter, although measures are commonly of the extracranial conduit arteries that perfuse the global cerebral vasculature (Thomas *et al.*, 2015). Both techniques are used to assess the functional regulation of the cerebral vasculature to physiological stress in humans.

1.4.1 Cerebral Blood Flow Regulation by Arterial Oxygen and Carbon Dioxide

The cerebral blood flow response to changes in arterial oxygen and carbon dioxide are well described. Roy and Sherrington (Roy & Sherrington, 1890) were one of the first to characterize the change in cerebral blood flow in response to alterations in arterial oxygen status. During sojourn to high altitude cerebral blood flow is increased in response to alterations in oxygen availability (Ainslie & Ogoh, 2010; Willie *et al.*, 2014), and laboratory studies demonstrate that the magnitude of the response is dependent on the severity of hypoxemia (Wilson *et al.*, 2011; Willie *et al.*, 2012). Elevations and/or reductions in arterial CO_2 result in respective increases and decreases in cerebral blood flow in both humans and animals (Atkinson *et al.*, 1990; Beaudin *et al.*, 2011; Hoiland *et al.*, 2016*b*; Parfenova *et al.*, 2017). Indeed, an interaction between hypercapnia and hypoxia has been suggested, such that cerebral blood flow reactivity to hypoxia measured with TCD is elevated under hypercapnic conditions compared to isocapnic or poikilocapnic conditions (Ainslie & Poulin, 2004). Detailed reviews present the redundant mechanisms relating to cerebral blood flow regulation to changes in arterial oxygen and carbon dioxide (Hoiland *et al.*, 2016*a*, 2019), which largely implicate vascular regulation through ATP (Bergfeld & Forrester, 1992), adenosine (Kalaria & Harik, 1988; Morii *et al.*, 2017), prostaglandins (Hoiland *et al.*, 2016*b*; Parfenova *et al.*, 2017) and NO (Van Mil *et al.*, 2002).

1.4.2 Intermittent Hypoxia and Cerebral Vascular Reactivity

Individuals with OSA have an increased risk of stroke (Yaggi *et al.*, 2005) and experience neurocognitive deficits (Lal *et al.*, 2012; Zhou *et al.*, 2016) which may be related to an impairment in cerebrovascular reactivity to altered arterial gases, such as those experienced during a nocturnal apnea. It has been demonstrated that cerebral blood flow velocity increases during an apnea and

is subsequently reduced below baseline following the initiation of breathing in OSA representing a potential for under perfusion of the cerebral vasculature and cerebral ischemia following nocturnal apnea (Bålfors & Franklin, 1994). Additionally, multiple investigations have reported reduced cerebral vascular reactivity to hypoxia in OSA populations and reactivity is improved following continuous positive airway pressure therapy (CPAP) (Diomedi *et al.*, 1998; Foster *et al.*, 2007; Reichmuth *et al.*, 2009; Morgan *et al.*, 2010; Coloma Navarro *et al.*, 2016). The cerebral reactivity to hypercapnia has been reported to be impaired in OSA versus controls (Reichmuth *et al.*, 2009; Morgan *et al.*, 2010; Ponsaing *et al.*, 2018) or not different (Foster *et al.*, 2009*b*) although differences in OSA cohorts between these studies may be responsible (i.e. varying severity of OSA or adherence to CPAP therapy). Changes in dynamic cerebral autoregulation has also been demonstrated in OSA populations, suggesting an impaired ability to maintain cerebral blood flow in the face of acute blood pressure fluctuations (Nasr *et al.*, 2009; Waltz *et al.*, 2016). Individuals with OSA have impaired executive function including working memory and problem solving (Incalzi *et al.*, 2004) which are further supported by impairments in perfusion to brain regions responsible for these functions measured through magnetic resonance imaging (Macey *et al.*, 2002). Changes in cerebral vascular and neurocognitive function may be a result of chronic intermittent hypoxia, or due to the robust cardiovascular comorbidities and general older age associated with OSA populations.

Intermittent hypoxia models in healthy individuals give further insight into the influence of intermittent hypoxia on cerebral vascular control and neurocognitive function in the absence of comorbid disease. Champod and colleagues (2013) demonstrated in healthy individuals that exposure to six hours of intermittent hypoxia over four consecutive days impairs working spatial memory. Further research is needed to determine if intermittent hypoxia effects additional metrics of neurocognitive function in healthy individuals, although exposure to 50-minutes of continuous hypoxia results in impaired cognitive function including impairments in visual and verbal memory, executive function, and reaction time in healthy individuals (Turner *et al.*, 2015). With respect to cerebral hemodynamics in healthy individuals following intermittent hypoxia resting cerebral blood flow velocity in healthy individuals is unchanged following intermittent hypoxia (Foster *et al.*, 2009*a*; Beaudin *et al.*, 2014). Further, mixed results are presented on the change in cerebral vascular response to hypoxia following intermittent hypoxia with some reports of impairments similar to OSA in cerebral blood flow velocity response (Querido *et al.*, 2008) and vascular

resistance responses to hypoxia (Foster *et al.*, 2009*a*), and others showing no change cerebrovascular reactivity to hypoxia (Ainslie *et al.*, 2007; Foster *et al.*, 2010). A similar trend of mixed results is present when cerebral vascular reactivity to hypercapnia is assessed, with some showing impairments in cerebral conductance response to hypercapnia (Zhang *et al.*, 2015), or no changes following intermittent hypoxia in healthy individuals (Foster *et al.*, 2009*b*; Querido *et al.*, 2015). These contrasting findings are likely linked to divergent methodologies with respect to the pattern, duration, and intensity of hypoxia during intermittent hypoxia paradigms. Additionally, many of these models do not fully mimic OSA as they do not include hypercapnia during repeated hypoxic bouts, which would typically be observed during apneas in OSA.

1.4.3 Cerebral Neurovascular Coupling

The limited ability of the brain to store glycogen for ATP generation requires a tight coupling between cerebral oxygen supply and metabolic demands during neural tasks for oxidative metabolism. This temporal matching of regional cerebral oxygen supply to demand is termed neurovascular coupling (NVC) (Phillips *et al.*, 2016). Pial vessels and penetrating arterioles downstream of larger conduit cerebral arteries with modulation through the neurovascular unit comprise the functional NVC response. The three main components of the neurovascular unit include the vascular smooth muscle, neuron and astrocyte glial cell (Attwell *et al.*, 2010; Phillips *et al.*, 2016). Feed-forward mechanisms of NVC involve glutamate signaling to both the neuron and astrocyte that stimulate Ca^{++} release and increased production of NO and prostaglandin which act to vasodilate cerebral vessels with increases in neuronal activity (Attwell *et al.*, 2010; Phillips *et al.*, 2016; Iadecola, 2017). Feed-back mechanisms involve local signalling of tissue PO_2 and PCO_2 from hypoxia and metabolite accumulation (Phillips *et al.*, 2016). Whether functional changes in NVC are responsible for the cognitive impairments observed in OSA, or how acute intermittent hypoxia influences NVC in healthy individuals remains unknown.

Much of our understanding of alterations in NVC to physiological stressors are limited to animal models, though recent investigations in humans are becoming more common with recent standardization of methods to assess NVC in humans (Phillips *et al.*, 2016). With relevance to OSA, Capone and colleagues (2012) demonstrated in rats that NVC was impaired following 35-days of chronic intermittent hypoxia, which was restored following NADPH oxidase inhibition, suggesting a link between NVC and oxidative stress developed during intermittent hypoxia.

Additionally, NVC is impaired in hypertensive rat models and angiotensin II receptor blockade prevented impairments in NVC (Takeda *et al.*, 2009). One study to date has assessed NVC in humans with OSA and showed no difference in NVC compared to otherwise healthy controls (Uzuner & Uzuner, 2017). However, the OSA cohort in this study had typical comorbidities of the general OSA population (hypertension, smokers, and obese BMI classifications) and no measures of cognitive performance were reported, therefore whether isolated intermittent hypoxia exposure influences NVC and the relationship to cognitive function in OSA remains unknown. Furthermore, NVC is shown to be reduced in healthy older males compared to younger cohorts (Nowak-Flück *et al.*, 2018) and is another confounding factor that may explain the previously shown similarity between OSA and healthy controls, which included older individuals in both groups (Uzuner & Uzuner, 2017). The influence of acute intermittent hypoxia on NVC in humans free from comorbid disease is poorly understood. Intermittent hypoxia can lead to reduced bioavailability of circulating NO (Pialoux *et al.*, 2009), oxidative stress (Pialoux *et al.*, 2011), elevated arterial pressure (Foster *et al.*, 2010) and reduced cerebrovascular reactivity (Foster *et al.*, 2009*a*) to hypoxia which may result in impaired NVC following exposure.

1.5 Summary of the Literature Review

OSA is a respiratory disorder characterized by chronic exposure to intermittent hypoxia and leads to robust comorbidities including increased risk of stroke, development of hypertension, and neurocognitive deficits. Models of intermittent hypoxia in healthy individuals show these changes are due to increases in sympathetic nerve activity, plasticity in the chemosensory response to changes in arterial oxygen and carbon dioxide that leads to lasting increases in ventilation (vLTF) although the requirement of sustained hypercapnia is suggested to fully express this response. Additionally, the cerebral blood flow response to arterial gases is altered with intermittent hypoxia and a change in the ability to match cerebral oxygen delivery to neuronal demand may be diminished and responsible for the neurocognitive deficits in OSA. Importantly, the influence of intermittent hypercapnic hypoxia on ventilatory plasticity and cerebral vascular control remains to be fully elucidated in humans.

Chapter 2: Specific Research Objectives and Hypotheses

2.1 Aim 1: The role of peripheral chemoreceptor drive on ventilatory long-term facilitation induced by acute intermittent hypercapnic hypoxia in men and women.

Brief Background:

Acute intermittent hypoxia elicits vLTF, a form of respiratory neuroplasticity that manifests in humans and animals as increased minute ventilation ($\dot{V}_I$) lasting up to 60-minutes following the removal of the stimulus (Mitchell & Johnson, 2003). vLTF is evident in both men and women during wakefulness and sleep (Syed *et al.*, 2013). However, previous studies that successfully elicit vLTF in humans have required a sustained hypercapnic stimulus ($P_{ET}CO_2$ +3-5 mmHg above baseline) throughout intermittent hypoxia exposure and the recovery phase (Harris *et al.*, 2006; Griffin *et al.*, 2012). It has been suggested that peripheral chemoreceptors are not responsible for vLTF elicited during this paradigm of intermittent hypoxia with sustained hypercapnia (Griffin *et al.*, 2012). Notably, vLTF is absent when CO_2 levels are uncontrolled during recovery despite pairing intermittent hypoxia with hypercapnia during exposure (Diep *et al.*, 2007; Deacon *et al.*, 2017). These disparate findings may be the result of differences between severity and duration of intermittent hypoxia protocols employed. Whether vLTF is evident during recovery without sustained hypercapnia following an intermittent hypercapnic hypoxia paradigm that more appropriately mimics severe OSA remains unknown. Furthermore, it is unclear whether peripheral chemoreceptor drive has a functional role in eliciting vLTF during isocapnic recovery. Investigating whether peripheral chemoreceptor drive modulates vLTF may provide insight into sensory long-term facilitation which is believed to be necessary for the plasticity of muscle sympathetic nerve activity and the rise in arterial blood pressure following intermittent hypoxia (Xie *et al.*, 2000; Foster *et al.*, 2010; Gilmartin *et al.*, 2010).

Research Objectives:

To determine (1) whether vLTF is evident in humans during isocapnic recovery following 40-minutes of intermittent hypercapnic hypoxia, and (2) whether tonic peripheral chemoreceptor drive modulates vLTF in men and women.

Research Hypotheses:

It is hypothesized that during isocapnic-normoxic recovery following an acute 40-minute intermittent hypercapnic hypoxia exposure, (1) $\dot{V}_I$ will be elevated compared to baseline for up to

50-minutes, and (2) during peripheral chemoreceptor inhibition with hyperoxia in changes in $\dot{V}_I$ will be augmented during recovery compared to baseline indicating a role for peripheral chemoreceptor drive in vLTF.

2.2 Aim 2: The effect of acute intermittent hypercapnic hypoxia on cerebral neurovascular coupling in healthy men and women.

Brief Background:

The physiological responses of healthy humans exposed to acute intermittent hypoxia are comparable to those of OSA patients exposed to chronic intermittent hypoxia during nocturnal apneas. These include increases in central sympathetic outflow, daytime blood pressure and a decrease in cognition (Xie *et al.*, 2000; Gilmartin *et al.*, 2010; Champod *et al.*, 2013). Such deterioration of neurocognitive function has been attributed to reduction in perfusion to brain regions responsible for cognitive function (Macey *et al.*, 2002) and an impairment of cerebral neurovascular coupling (NVC) (Guiney *et al.*, 2015). The matching of cerebral oxygen supply to metabolic demand, and its impairment may contribute to reductions in neurocognitive function following intermittent hypoxia. Furthermore, previous reports suggest reduced cerebrovascular reactivity to alterations in arterial O_2 and CO_2 in OSA patients (Foster *et al.*, 2007; Morgan *et al.*, 2010) and in healthy individuals following intermittent hypoxia (Foster *et al.*, 2009*a*). One study to date has investigated NVC in individuals with OSA and reported no difference compared to age-matched healthy controls (Uzuner & Uzuner, 2017). However, it is unknown if acute intermittent hypoxia either with or without paired hypercapnia alters cerebral NVC in healthy individuals free of comorbidities commonly expressed in OSA.

Research Objectives:

To characterize the change in cerebral neurovascular coupling responses following an acute 40-minute bout of intermittent hypercapnic hypoxia in men and women.

Research Hypothesis:

We hypothesize that cerebral neurovascular coupling responses will be attenuated following 40-minutes of acute intermittent hypercapnic hypoxia in men and women in line with previous reports of reduced cerebrovascular reactivity following intermittent hypoxia.

Chapter 3: Methods

3.1 Participants

The Clinical Research Ethics Board at the University of British Columbia provided ethical approval of all experimental procedures (CREB ID; H18-02513) and written informed consent was obtained from all participants. In total, 20 volunteers (11 male, 9 female) were recruited and participated in this study, however some analyses were completed with smaller sample sizes due to technical issues and is reported in detail in Chapter 4. Participants were excluded from the study if they were overweight (BMI > 30 kg/m^2), hypertensive based upon the American Heart Association guideline (SBP > 130, DBP > 80 mmHg; Whelton *et al.*, 2017), had abnormal pulmonary function (see below), had a reported history of cardiovascular or respiratory disease, had an overnight oxygen desaturation index (ODI) greater than 5 events/hour and were taking medications other than oral contraceptives. Previously, estrogen has been reported as a potent stimulus for ventilation and estrogen fluctuations throughout the menstrual cycle are shown to influence resting ventilation (Behan *et al.*, 2003; Macnutt *et al.*, 2012). Therefore, female participants were tested within 0-5 days of the early follicular phase of menstruation to ensure estrogen levels were at their lowest and had a minimal effect.

3.2 Screening Procedures

During a preliminary laboratory visit, participants completed a series of screening questionnaires and tests to assess inclusion/exclusion criteria. First, a health history questionnaire was completed to assess a history of hypertension, impaired renal or liver function or underlying cardiorespiratory disease (see appendix A). Next participants were tested for healthy pulmonary function including spirometry, diffusion capacity and anatomical lung volumes. Participants were seated in a commercially available whole-body plethysmography box (V62J, Sensormedics, Yorba Linda, CA) connected to a pulmonary function system (Vmax Encore 22, Sensormedics). In agreement with recommended guidelines, spirometry tests were conducted to assess forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1) and the ratio of FEV_1 to FVC (FEV_1/FVC) (Miller et al., 2006). Participants were excluded if they had a FEV_1/FVC of < 0.70 or < 80% of their predicted demographic specific values based on height and age for spirometry (Knudson *et al.*, 1983), lung volumes (Crapo *et al.*, 1982) and diffusion capacity (Crapo & Morris, 1981). Diffusion capacity of the lungs was quantified through a single breath carbon monoxide maneuver

(MacIntyre *et al.*, 2005). Lastly, total lung capacity (TLC), functional reserve capacity (FRC) and residual volume (RV) were assessed using full body plethysmography (Wanger *et al.*, 2005). Following pulmonary function testing participants received a take-home overnight pulse oximeter (WristOx2, Model 3150, Nonin, Plymouth, MN) to be worn for a single night. Nocturnal oximetry data was analyzed with nVision software (V6.4, Nonin) to determine the nocturnal ODI. A desaturation was classified as a reduction in $S_PO_2 \geq 4\%$ and participants were excluded if there was an ODI > 5 events/hour. Participants who met all inclusion criteria returned for completion of the experimental visit(s).

3.3 Experimental Procedures

All 20 participants visited the lab for one experimental day, and a subset of male subjects (n=10) returned for a second time-matched experimental room-air control visit a minimum of 72 hours following the first visit (Figure 1). Females were not included in the control visit due to technical difficulties in time aligning menstrual cycle phase to the first experimental visit. Participants were instructed to refrain from exercise, consumption of caffeine and/or alcohol a minimum of 12 hours prior, and fast for a minimum of 2 hours prior to their arrival to the lab for experimental testing. Upon arrival participants were instrumented for measurements of automated SBP, DBP, HR, left middle and right posterior cerebral artery blood velocity (MCA_V and PCA_V; respectively), S_PO_2, and a non-venting face mask to measure ventilation and end-tidal oxygen and carbon dioxide ($P_{ET}O_2$ and $P_{ET}CO_2$; respectively) (Figure 3).

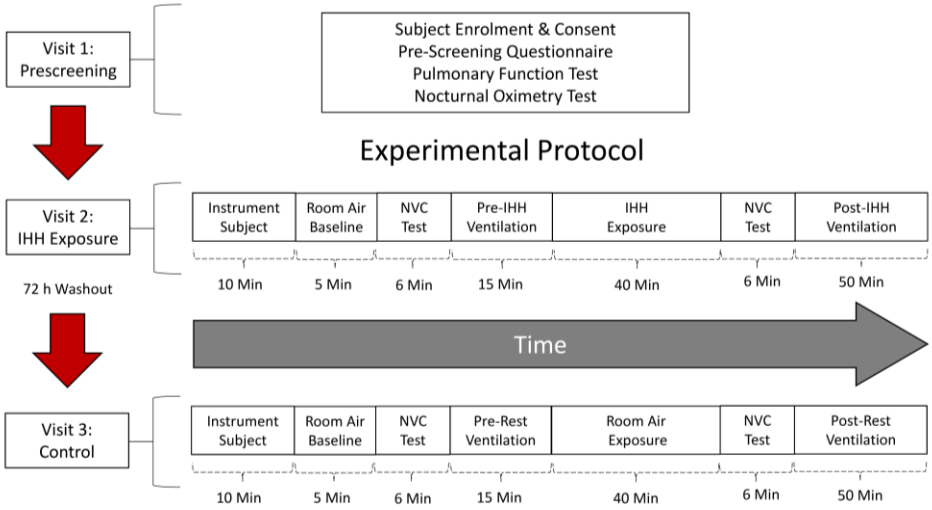

Figure 1. Timeline progression of experimental protocol.

Abbreviations: IHH, intermittent hypercapnic hypoxia, NVC, neurovascular coupling.

Figure 2 outlines the experimental protocol schematic with an individual participant's IHH exposure plotted. Following instrumentation, 5-min of baseline room air measurements were collected before initiating dynamic end-tidal forcing at the participants resting $P_{ET}O_2$ and $P_{ET}CO_2$. For the remainder of the experimental protocol participants remained breathing on the dynamic end-tidal forcing system. After a period of stable breathing a series of baseline tests were conducted before IHH (pre-IHH). First, a NVC test was conducted in a darkened and quiet room adhering to recommended guidelines (Phillips *et al.*, 2016). In short, following a 1-min baseline, NVC was assessed as five repeated cycles of alternating 30-sec eyes closed and 30-sec eyes opened. During the eyes open phase participants focused on a standardized alternating flashing checkerboard to stimulate the visual cortex while measuring the associated MCA_V and PCA_V response. Next, the effect of peripheral chemoreceptor inhibition on ventilation was determined. Participants were exposed to three, 5-min cycles comprised of 1-min of hyperoxia (target $P_{ET}O_2$ = 350 mmHg, $P_{ET}CO_2$ = isocapnic baseline) and 4-min of end-tidal O_2 and CO_2 clamped at baseline values. This duration and target of $P_{ET}O_2$ has previously been used to inhibit peripheral chemoreceptors in humans and has been shown to attenuate isolated carotid body afferent discharge (Lahiri *et al.*, 1983; Stickland *et al.*, 2008; Querido *et al.*, 2010; Griffin *et al.*, 2012).

After completion of pre-IHH measurements, participants were exposed to 40-min of IHH administered by dynamic end-tidal forcing at 1-min cycles comprised of 40-sec hypercapnic hypoxia (target nadir $P_{ET}O_2$ = 45 mmHg, peak $P_{ET}CO_2$ = +4 mmHg above baseline) and 20-sec of room air exposure. This protocol mimics the rate of oxyhemoglobin desaturations typically observed in severe OSA patients during nocturnal sleep (AHI $\geq$ 60 events/hour).

Following IHH, $P_{ET}O_2$ and $P_{ET}CO_2$ were restored and clamped at baseline levels determined prior to IHH. Following a 5-minute period after completing IHH to allow for stabilization of end-tidal gases, acquire manual blood pressure measurements and ensure optimal MCA and PCA signals, participants endured a second NVC test post-IHH. Next, to characterize the magnitude of vLTF, cardiovascular and respiratory variables were continuously measured for 50-minutes of recovery following the second NVC test. Lastly, during the 50-minutes of recovery, to measure the influence of peripheral chemoreceptor inhibition on vLTF, nine repeated cycles were conducted at 5-min intervals with $P_{ET}O_2$ and $P_{ET}CO_2$ clamped at baseline levels for 4-min and $P_{ET}O_2$ increased to ~350 mmHg for 1-min.

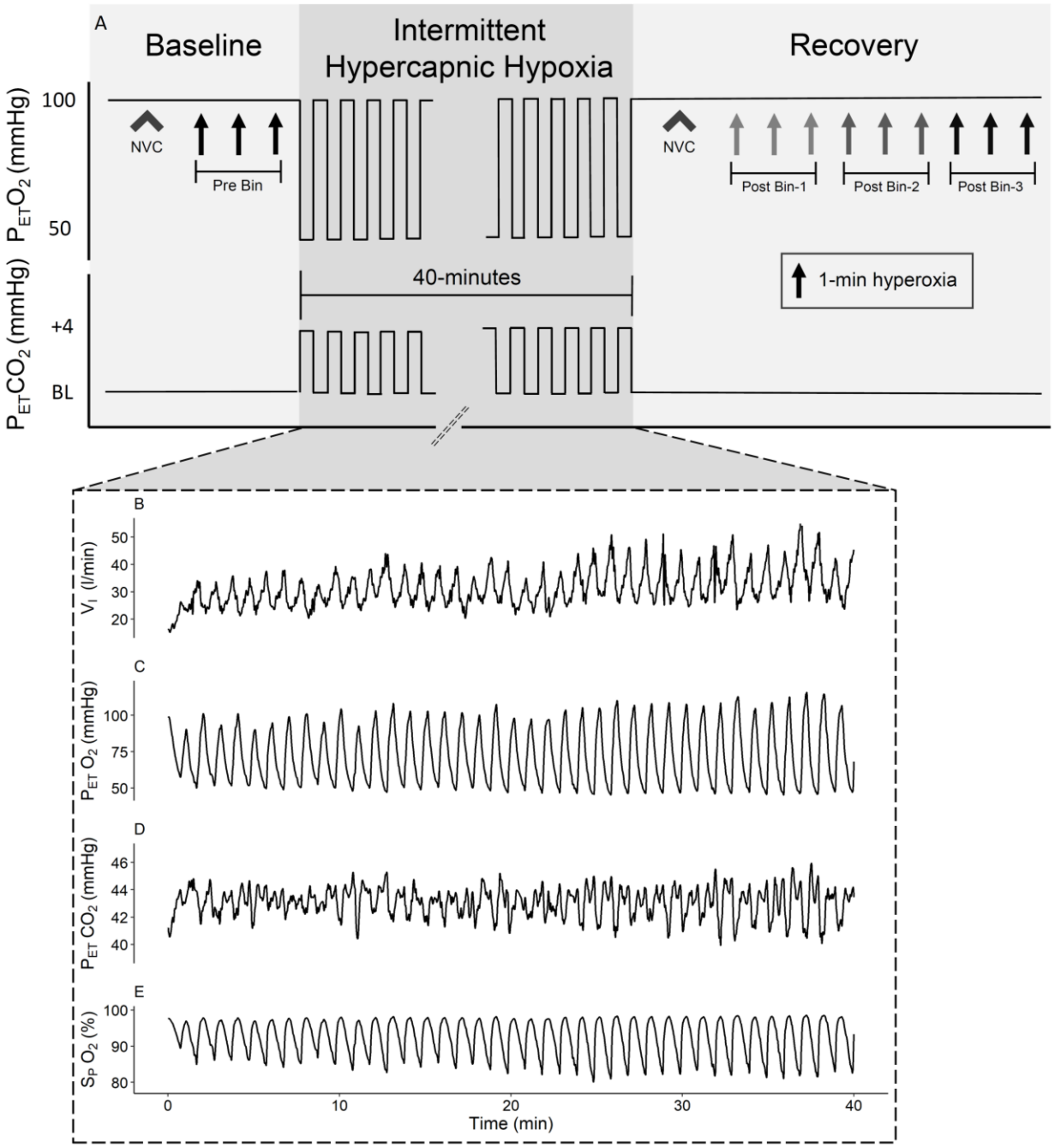

Figure 2. Schematic of experimental protocol and intermittent hypercapnic hypoxia. A: Schematic of Pre- and Post-exposure measures recorded throughout experiment and schematic for target end-tidal values during the 40-minutes of IHH exposures. Arrows denote when 1-minute bouts of hyperoxia were administered, and bins denote the three consecutive hyperoxic bouts cycle-averaged together. B, C, D & E denote raw minute ventilation ($\dot{V}_I$), partial pressure of end-tidal O_2 ($P_{ET}O_2$), partial pressure of end-tidal CO_2 ($P_{ET}CO_2$), and peripheral oxyhemoglobin saturation (S_PO_2) traces respectively for an individual subject throughout 40-minutes of IHH.

Male participants who returned for a second experimental control visit adhered to identical restrictions prior to the arrival to the lab. Participant instrumentation was identical, notably, care was taken to ensure the MCA and PCA insonation sites and transcranial Doppler ultrasound settings were the same as the first experimental visit including vessel depth, ultrasound power, gate size, B-mode and Doppler gain. The pre-IHH and post-IHH experimental protocol was identical as described previously, however instead of 40-min IHH exposure, participants breathed room air without dynamic end-tidal forcing. Continuous measurements were made throughout the time-matched room air exposure.

3.4 Specific Methodology

3.4.1 Cardiorespiratory Measurements

All cardiorespiratory measurements were collected at 200 Hz using commercially available software (Labchart V8.0, ADInstruments, Colorado Springs, CO) and an analog-to-digital converter (Powerlab 16/35SP ML 880, ADInstruments) interfaced with a personal computer. A lead-II configuration electrocardiogram (FE132, ADInstruments) was used to measure HR, a finger probe and pulse oximeter (7500FO, Nonin Medical) measured oxyhemoglobin saturation, and finger pulse photoplethysmography (Finometer PRO, Finapress Medical Systems, Amsterdam, The Netherlands) measured beat-by-beat blood pressure. The blood pressure signal was calibrated to a reconstructed brachial artery waveform via return-to-flow calibration prior to starting room-air baseline, and a second return-to-flow calibration was performed during stable end-tidal O_2 and CO_2 control following IHH exposure (Guelen *et al.*, 2003). Additionally, an automated brachial blood pressure monitor (Carescape V1000 Vital Signs Monitor, GE, Fairfield, CT) was placed on the left arm and used to verify the beat-by-beat blood pressure measurements during room-air baseline and following IHH.

Throughout the experiment, participants breathed through a non-venting full-face mask (Ultra Mirage NV, ResMed, San Diego, CA) attached in series to a pneumotachograph (HR800L, Hans Rudolph, Shawnee, KS) with differential pressure transducer (PA-1, Hans Rudolph) to measure respiratory flow and frequency, and lastly, a custom-built sequential gas delivery circuit modified from the design outlined by Farra and colleagues (Farra *et al.*, 2016) (Figure 3). The pneumotachograph was calibrated prior to and confirmed for accuracy immediately following the end of experiments with a 3-liter syringe. The custom sequential gas delivery circuit was

connected to a dynamic end-tidal forcing system (Tymko *et al.*, 2015, 2016). Respired gases were sample at the mouth and analyzed by a gas analyzer (ML206, ADInstruments) to measure fraction of oxygen (FO_2) and carbon dioxide (FCO_2) and used to calculate the partial pressure of oxygen and carbon dioxide (PO_2 and PCO_2 respectively). These values were time aligned to the end of expiration to measure $P_{ET}O_2$ and $P_{ET}CO_2$. Prior to experiments the gas analyzer was calibrated with gases of known concentration and confirmed for accuracy immediately following experiment completion.

3.4.2 Transcranial Doppler

Cerebral blood flow velocity of the MCA and PCA was non-invasively measured using transcranial Doppler ultrasound (PMD150B, Spencer Technologies, Seattle, WA) throughout baseline, IHH and removed following completion of the post-IHH NVC test. Two low-frequency (2 MHz) ultrasound probes were placed bi-laterally at the temporal windows and held into position using a custom fit headpiece (M600 Bilateral Head Frame, Spencer Technologies). Previously described methods for the identification and location of the left side MCA and right side PCA were observed (Willie *et al.*, 2011*a*). In short, the PCA was confirmed by measuring the PCA_V response to a visual finger tracking task following a brief eyes-closed period. The MCA was confirmed by measuring the MCA_V response following a brief compression of the common carotid artery.

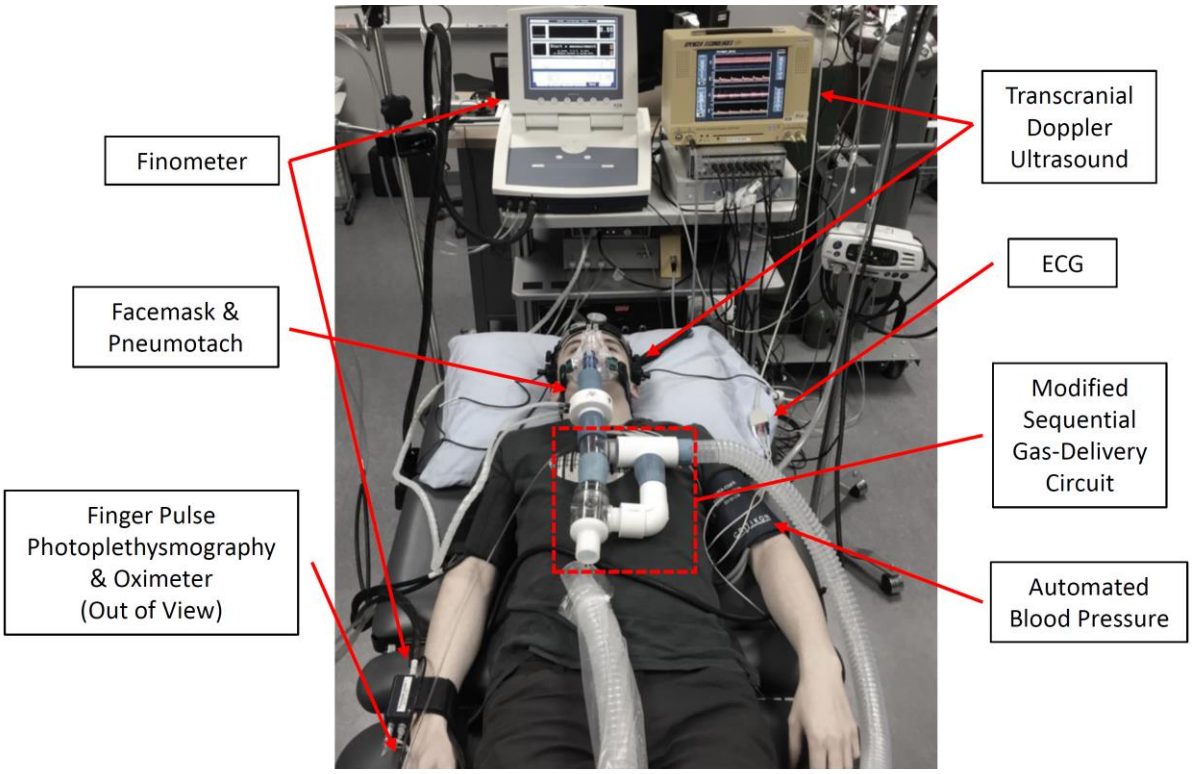

Figure 3. Experimental setup and instrumentation.

3.4.3 Dynamic End-tidal Forcing

Throughout the protocol $P_{ET}O_2$ and $P_{ET}CO_2$ were controlled at desired levels using a dynamic end-tidal forcing system. This custom-built fast gas delivery system allows for the quick manipulation of inspired gases through solenoid valves individually controlling delivery of O_2, CO_2, and N_2 gas into a mixing and humidification chamber in-line with an inspiratory circuit (Tymko *et al.*, 2015, 2016). Purpose designed software was used (Labview 13.0, National Instruments, Austin, TX) where feed-back control is accomplished through proportional and integral error. Using information regarding $P_{ET}O_2$, $P_{ET}CO_2$, inspired V_T, expired V_T the system delivers the adequate gas mixture to maintain the desired $P_{ET}O_2$ and $P_{ET}CO_2$ values. Feed-forward control of the inspirate is based on estimates of metabolic O_2 consumption and CO_2 production and the required F_IO_2 and F_ICO_2 to maintain desired $P_{ET}O_2$ and $P_{ET}CO_2$ are determined using the alveolar gas equation. This system has been previously used to effectively deliver a similar IHH protocol used in the current investigation (Stuckless *et al.*, 2018), and control end-tidal gases during various physiological stressors (Bain *et al.*, 2013; Smith *et al.*, 2016; Vermeulen *et al.*, 2018).

3.5 Data Analysis

3.5.1 Cardiorespiratory Parameters

Flow measurements through a pneumotachograph are sensitive to changes in the total composition of O_2, CO_2 and N_2 gases which influence gas viscosity and resistance relative to atmospheric room air (Johns *et al.*, 1982; Yeh *et al.*, 1984). We therefore employed a continuously adapting correction factor to the respiratory flow signal to account for changes in gas composition and pneumotachograph temperature according to equation 1 and as outlined by Yeh et. al., (1984).

Equation 1:

$$\dot{V} = \frac{\Big(\big(FO_2 \times \big(200 + (0.2985 \cdot T)\big)\big) + \big(\big(FCO_2 \times 150.7 + (0.4560 \cdot T)\big)\big) + \big((1 - FO_2 - FCO_2) \times 167.7 + (0.4530 \cdot T)\big)\Big)}{\big(174.4 + (0.4207 \cdot T)\big)}$$

Where $\dot{V}$ = flow, T = ambient temperature of inspired air in Celsius; FO_2 = fraction of oxygen in respired air and FCO_2 = fraction of carbon dioxide in respired air.

The viscosity corrected inspired flow signal was used for calculation of respiratory variables including inspired V_T and f_B which were multiplied together to calculate $\dot{V}_I$ which was expressed as body temperature, pressure saturated (BTPS). Ventilatory data was extracted on a breath-by-breath basis for further offline analysis.

HR was measured by calculating the rate of R-wave intervals determined by the lead-II ECG, and S_PO_2 was determined by the continuous finger probe signal. Mean MCA_V and PCA_V was measured from their respective waveforms. The peak and nadir values from the beat-by-beat blood pressure waveform were extracted to measure SBP and DBP respectively and used to calculate MAP as the sum of (1/3*SBP) + (2/3*DBP). All cardiovascular variables were extracted and analyzed offline on a beat-by-beat basis.

All ventilatory and cardiovascular variables were time-aligned and averaged at specific time intervals using custom built software available online (Silo v2.7.6, https://github.com/lindseyboulet/silo), built using the Shiny web application and R programming language (Chang *et al.*, 2015; R Core Team, 2018). Within each 5-minute cycle (three times before and nine times following IHH) variables were averaged over 30-sec during normoxic breathing immediately prior and, the last 30-sec of the following hyperoxia exposure for all pre- and post-IHH cycles. The three pre-IHH cycles were averaged for all individual subjects and a single pre-

IHH isocapnic normoxic value is reported. The normoxic 30-sec bins are reported in successive order across 50-minutes of recovery. To determine the influence of peripheral chemoreceptor inhibition, the absolute delta change in cardiorespiratory variables was calculated by subtracting the normoxic value from the corresponding hyperoxic value within each respective time bout. To account for variability in the hyperoxic response, delta values from the three pre-IHH cycles, and over 3 consecutive post-IHH cycles were averaged together (outlined in Figure 2), such that four delta time-points are reported: Pre, Post-Bin 1 (Post-5, 10 & 15 mins), Post-Bin 2 (Post-20, 25 & 30 mins) and Post-Bin 3 (Post-35, 40 & 45 mins). Absolute delta variables were averaged across 3-bins to strengthen the contention that differences observed in the hyperoxic response between time points were not due to variability in the response. These data were analyzed statistically to determine resting ventilatory and cardiovascular changes from baseline during isocapnic recovery following IHH, and to determine influence of peripheral chemoreceptor inhibition (see 3.6 Statistical Analysis).

Data during IHH exposure was interpolated on a 1-sec time-scale and analyzed for mean, nadir and maximum S_PO_2 and $P_{ET}CO_2$ within each 1-min normoxic-hypercapnic hypoxic cycle. The total duration spent below 95%, 90% and 85% S_PO_2 was calculated to characterize the severity and total hypoxic time of the exposure. The Severinghaus transformation was applied to PO_2 data throughout IHH exposure to determine S_PO_2 without circulatory delay from the peripheral finger transducer (Severinghaus, 1979).

3.5.2 Cerebral Neurovascular Coupling

Hemodynamic and cardiovascular variables were extracted on a beat-by-beat basis for the 6-min NVC test pre- and post-IHH and analyzed with custom software developed in MATLAB (Phillips *et al.*, 2016). The peak response to five repeated eyes-closed, eyes-opened cycles was used to characterize NVC. The software measures resting hemodynamic variables during 1-min baseline and automatically detects the absolute peak MCA_V and PCA_V response within the eyes-open neuronal activation phase (i.e. eyes-open) and calculates the absolute and percent relative change from baseline. Cerebrovascular conductance was calculated as the quotient from beat-by-beat MAP and PCA_V or MCA_V (PCA_{CVC} and MCA_{CVC} respectively). NVC was quantified as the peak absolute and percent increase (relative to eyes-closed) during visual stimulation cycle-averaged over the 5 repeated cycles.

3.6 Statistical Analysis

All statistical analyses were conducted within R-Studio (version 3.5.1) using the R-programming language (R Core Team, 2018) and statistical packages (lme4; (Bates *et al.*, 2014), lmerTest (Kuznetsova *et al.*, 2017)). Significance was set at an alpha value of $P < 0.05$ for all comparisons. Paired within subjects' comparisons were made between all pre- and post- exposure (within the IHH or control cohort) analyses, and unpaired samples comparisons were conducted to analyze differences between IHH or control conditions within specified time points. Participant anthropometric parameters, nocturnal oximetry and pulmonary function data were analyzed with an independent sample Student's t-test to determine differences between men and women.

3.6.1 Ventilatory Long-Term Facilitation Statistical Analysis

A linear mixed effect model was conducted on each variable to determine how cardiorespiratory parameters changed from baseline during 50-minutes of isocapnic-normoxic recovery following IHH or time-matched control. Within the experimental IHH cohort, the fixed effects were sex (two levels; men and women) and time (11 levels; pre-IHH and post-5 through post-50 minutes at 5-minute intervals) while subjects were included as a random effect. For the time matched control cohort, the fixed effect was time (11 levels; pre-CON and post-5 through post-50 minutes at 5-minute intervals) with subjects included as a random effect. When significant main effects were determined a Tukey's post-hoc analysis was conducted to determine where specific differences exist. When a significant time-by-sex interaction effect was present, an independent samples Student's t-test was run within each time level to determine where differences exist between men and women. To determine whether differences exist between the experimental IHH cohort and time-matched control cohort inclusive of all participants an independent samples Student's t-test was run within each level of time during pre- exposure and isocapnic-normoxic recovery.

Absolute delta change from normoxia to hyperoxia within each time level was calculated and cycle averaged together across three consecutive 5-minute bins prior to and following IHH or time matched control. Separate linear mixed effect models were run on each experimental cohort to determine how the delta responses changed from baseline throughout recovery. Time (4 levels; Pre-Bin, Post-Bin 1, Post-Bin 2 and Post-Bin 3; Figure 2) was included as a fixed factor, and subjects were included as a random effect. When a significant main effect of time was determined a Tukey's post-hoc analysis was conducted to determine where specific differences lay. To

compare the absolute delta responses between the experimental IHH and time-matched control cohorts an independent samples Student's t-test was conducted within each cycle averaged time bin.

To compare the difference in magnitude of observed changes during recovery between the experimental IHH and time-matched control cohorts a linear mixed effect model was run on recovery data including only individuals with complete datasets from both IHH and control conditions (n = 9 all males). The pre exposure time point was compared with a paired samples Student's t-test and was not included in the mixed effect model. The absolute change from pre exposure at each time point of recovery were calculated and used in the model. Time (10 levels; post-1 through post-10) and condition (2 levels; IHH and control) were included as fixed factors and subjects was included as a random effect. When significant F-ratios were present Tukey's post-hoc analysis was conducted to determine where specific differences lay.

3.6.2 Cerebral Neurovascular Coupling Statistical Analysis

Resting cerebral hemodynamics, cardiovascular parameters and neurovascular coupling responses within the experimental IHH cohort were statistically analyzed with a linear mixed effect model with time (2 levels; Pre-IHH and Post-IHH) and sex (2 levels; men and women) included as fixed factors and subjects included as random effects. When significant time-by-sex interactions were determined Tukey's post-hoc analysis was conducted to determine specific differences. Control data was compared with a paired samples Student's t-test to determine changes between pre- and post- measures. Lastly, to determine differences in the change from pre- to post- between the IHH and control cohorts, an independent samples Student's t-test was run on the calculated delta values.

Chapter 4: Results

4.1 Participants

A total of 20 participants met the inclusion and exclusion criteria for these experiments. Anthropometric characteristics, nocturnal oximetry and pulmonary function screening parameters are listed in Table 1. Compared with women, men were taller and had greater body mass but both cohorts were otherwise healthy presenting with similar BMI. All participants had normal lung function, and no evidence of undiagnosed sleep apnea.

Table 1. Participant anthropometrics, nocturnal oximetry, and pulmonary function.

	Men (n=11)	Women (n=9)	P =	All (n=20)
Age (years)	22 ± 1	23 ± 1	NS	22 ± 1
Height (cm)	180 ± 2	165 ± 3	<0.01	173 ± 2
Body Mass (kg)	78.5 ± 3.1	62.7 ± 2.8	<0.01	71.4 ± 2.8
BMI (kg/m^2)	24 ± 1	23 ± 1	NS	24 ± 1
ODI (/hr)	1.3 ± 0.4	1.0 ± 0.3	NS	1.2 ± 0.2
FVC (l)	6.0 ± 0.3	4.1 ± 0.2	<0.01	5.2 ± 0.3
FEV_1 (l)	4.7 ± 0.2	3.4 ± 0.1	NS	4.1 ± 0.2
FEV_1/FVC (% Measured)	78 ± 2	81 ± 2	NS	80 ± 1
FEV_1/FVC (% of Predicted)	94 ± 2	93 ± 2	NS	93 ± 1
TLC (l)	7.2 ± 0.3	5.2 ± 0.2	<0.01	6.3 ± 0.3
D_LCO (ml/min/mmHg)	40 ± 2	26 ± 1	<0.01	34 ± 2
D_LCO/V_A (ml/min/mmHg/l)	6.1 ± 0.3	5.6 ± 0.2	NS	5.9 ± 0.2

Abbreviations: BMI, body mass index; ODI, oxygen desaturation index; FVC, forced vital capacity; FEV_1, forced expired volume in one second; TLC, total lung capacity; D_LCO, diffusion capacity of the lung for carbon monoxide transfer; D_LCO/V_A, D_LCO corrected for alveolar volume; NS, not significant. Data represent mean ± SEM.

4.2 Intermittent Hypercapnic Hypoxia

Figure 2 panels B through E represent the cyclical respiratory characteristics of 40-minutes of IHH at 1-minute cycles in an individual subject. Within each 1-minute cycle across the total exposure the mean nadir S_PO_2 was 83.3 ± 1.0 %, mean peak S_PO_2 was 97.4 ± 0.2 %, mean delta $P_{ET}CO_2$ was 3.2 ± 0.3 mmHg calculated as the difference between the recorded peak and nadir $P_{ET}CO_2$ within

each 1-minute cycle. During IHH the total duration spent with S_PO_2 below 95, 90, and 85% was 24.3 ± 1.2, 12.6 ± 0.9, and 5.8 ± 0.8 minutes respectively.

4.3 Ventilatory Long-Term Facilitation

Statistical analysis was conducted on 19 individuals (9 female) included in the IHH cohort, and 9 males who returned for a time-matched control. Of the females originally recruited, one was excluded from analysis due resting heart rate ~20 /min above the group mean that persisted throughout the experiment did not normalize throughout a prolonged period of baseline. One male in the experimental group was unable to return for a time-matched control visit due to a time-constraint.

4.3.1 Effect of Intermittent Hypercapnic Hypoxia on Ventilation

Figure 4 outlines the changes in $\dot{V}_I$ prior to and following 40-minutes of IHH in the experimental and control cohorts. Following IHH $\dot{V}_I$ was elevated from baseline at all time points throughout the 50-minute duration of isocapnic nomoxic recovery suggesting the presence of vLTF. The magnitude of vLTF was similar between sexes throughout recovery (Figure 4, Panel B). vLTF occurred predominantly as a function of increases in f_B, but elevations in V_T were also present (Table 2). During recovery $P_{ET}O_2$ and $P_{ET}CO_2$ were not different from baseline levels indicating good end-tidal control throughout the recovery period (Table 2).

In the time-matched control group, $\dot{V}_I$ was not increased during the first 20-minutes nor at 30- and 35 minutes of recovery, however became elevated at 25-minutes and the during the last 15-minutes of recovery (Figure 4, Panel C). This modest increase in $\dot{V}_I$ occurred through nonsignificant changes in both V_T and f_B in the control cohort (Table 2). Similar to the experimental group, $P_{ET}O_2$ and $P_{ET}CO_2$ were tightly controlled at the respective baseline values throughout 50-minutes of recovery (Table 2). Notably, during the first 20-minutes of recovery the increase in $\dot{V}_I$ was significantly larger in the experimental IHH cohort compared to the time-matched control (Figure 4, Panel A), but $\dot{V}_I$ was not different between groups during the final 30-minutes of recovery.

Comparing the magnitude of change from baseline in participants with complete data sets in both IHH and control conditions, the delta for each time point during recovery was calculated from the pre exposure to determine the magnitude of change throughout recovery in both cohorts.

Pre exposure f_B, V_T and $\dot{V}_I$ were not significantly different between the IHH and control conditions. A main condition effect was evident in f_B, such that at all recovery time points f_B was higher in the IHH condition compared to control (+1.6 ± 0.5 vs. +0.1 ± 0.5 /min respectively; $P < 0.01$), although no interaction effect was present. Similarly, a main condition effect was evident in $\dot{V}_I$, such that at all recovery time points $\dot{V}_I$ was higher in the IHH condition compared to control (+5.2 ± 0.7 vs. +1.8 ± 0.7 l/min respectively; $P < 0.01$) although no interaction effect was present. Between the IHH and control conditions no difference in V_T was evident (condition effect, $P = 0.2$).

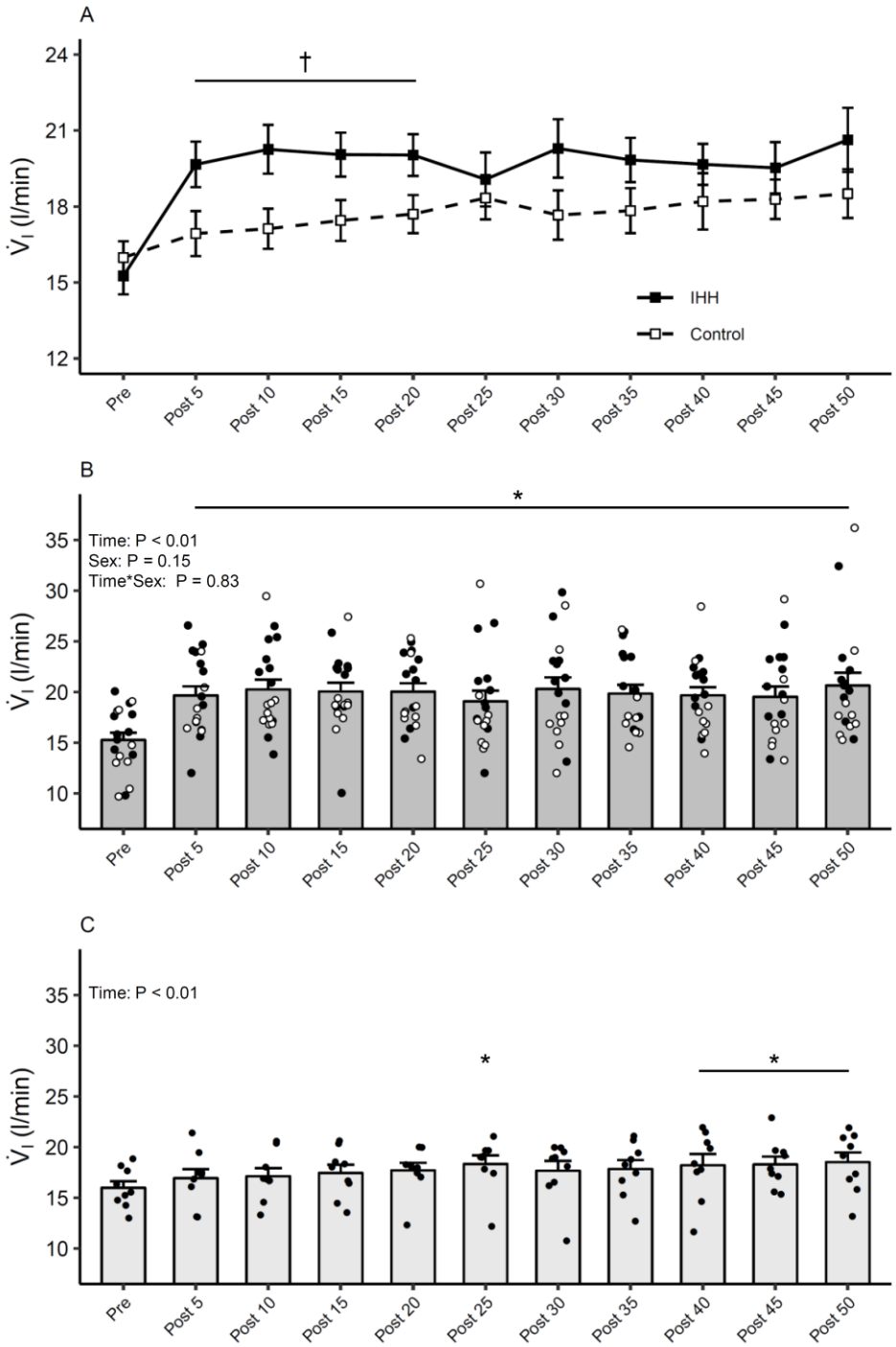

Figure 4. Minute ventilation prior to and following 40-minutes of IHH or room-air control.

A: Values represent mean ± SEM in both IHH and control group. B: Individual data points in the IHH cohort, bars represent mean ± SEM. White filled circles represent women, black filled circles represent men. C: Individual data points in the control cohort, bars represent mean ± SEM. * denotes $P < 0.05$ from respective "Pre" condition, † denotes $P < 0.05$ between IHH and control at the respective time point. Abbreviations: $\dot{V}_I$, minute ventilation; IHH, intermittent hypercapnic hypoxia.

Table 2. Respiratory parameters prior to and following 40-minutes of experimental exposure.

	V_T (litres)		f_B (/min)		$P_{ET}O_2$ (mmHg)		$P_{ET}CO_2$ (mmHg)	
	IHH	**CON**	**IHH**	**CON**	**IHH**	**CON**	**IHH**	**CON**
Pre	1.1 ± 0.1	1.1 ± 0.1	14 ± 1	13 ± 1	94 ± 1	93 ± 1	40 ± 1	40 ± 1
Post-5	1.1 ± 0.1	1.3 ± 0.2	17 ± 1 *	13 ± 1 †	94 ± 1	95 ± 1	40 ± 1	40 ± 1
Post-10	1.2 ± 0.1	1.2 ± 0.1	17 ± 1 *	14 ± 1	94 ± 1	94 ± 1	40 ± 1	40 ± 1
Post-15	1.2 ± 0.1	1.3 ± 0.1	16 ± 1 *	13 ± 1 †	93 ± 1	93 ± 1	40 ± 1	40 ± 1
Post-20	1.2 ± 0.1	1.3 ± 0.1	16 ± 1	13 ± 1 †	94 ± 1	94 ± 1	40 ± 1	40 ± 1
Post-25	1.2 ± 0.1	1.3 ± 0.1	16 ± 1	14 ± 1	95 ± 1	94 ± 1	40 ± 1	40 ± 1
Post-30	1.3 ± 0.1 *	1.3 ± 0.2	15 ± 1	14 ± 1	94 ± 1	95 ± 1	40 ± 1	40 ± 1
Post-35	1.2 ± 0.1	1.2 ± 0.1	16 ± 1	14 ± 1	94 ± 1	95 ± 1	40 ± 1	40 ± 1
Post-40	1.2 ± 0.1	1.3 ± 0.1	16 ± 1	13 ± 1	93 ± 1	93 ± 1	40 ± 1	41 ± 1
Post-45	1.2 ± 0.1	1.3 ± 0.1	16 ± 1	13 ± 1 †	94 ± 1	94 ± 1	40 ± 1	40 ± 1
Post-50	1.3 ± 0.1 *	1.3 ± 0.1	16 ± 1	15 ± 1	94 ± 1	94 ± 1	40 ± 1	40 ± 1

IHH cohort N=19; CON cohort N=9, * denotes $P < 0.05$ from respective "Pre" time point, † denotes $P < 0.05$ compared to IHH condition at the respective time point. Abbreviations: IHH, intermittent hypercapnic hypoxia; CON, control; $\dot{V}_I$, minute ventilation; V_T, tidal volume; f_B, breathing frequency; $P_{ET}O_2$, partial pressure of end-tidal O_2; $P_{ET}CO_2$, partial pressure of end-tidal CO_2. Data represent mean ± SEM.

4.3.2 Respiratory Response to Hyperoxia and Peripheral Chemoreceptor Inhibition

During 1-minute hyperoxic bouts, the average $P_{ET}O_2$ achieved during Pre, Post-Bin 1, Post-Bin 2, and Post-Bin 3 was 348 ± 6, 349 ± 6, 351 ± 6, and 363 ± 5 mmHg respectively in the IHH cohort and 341 ± 10, 333 ± 10, 333 ± 9, and 340 ± 9 mmHg respectively in the control cohort. The corresponding absolute delta respiratory parameters are listed in Table 3. The absolute delta $\dot{V}_I$ during hyperoxia are further represented in Figure 5 for both IHH and control cohorts. In the IHH cohort, a main effect of time was evident comparing the absolute delta $\dot{V}_I$ across the four-time bins ($P < 0.01$). Post-hoc analysis determined the magnitude of depression in $\dot{V}_I$ was augmented compared to baseline following IHH exposure during Post-Bin 2 (corresponding to 20, 25 and 30-minutes of recovery) however was not different during Post-Bin 1 (corresponding to 5, 10 and 15-minutes of recovery; $P = 0.24$) and Post-Bin 3 (corresponding to 35, 40 and 45-minutes of recovery; $P = 0.47$) compared to baseline. There was no difference in the response across the three post-IH time points. In the time-matched control group, the change in $\dot{V}_I$ during hyperoxia was similar compared to baseline at all time points during recovery (Figure 5 and Table 3, $P = 0.97$). Additionally, the depression in $\dot{V}_I$ was significantly larger in the IHH cohort compared with control within Post-Bin 2 (Table 3). The observed change in $\dot{V}_I$ was not due to significant changes evident in V_T or f_B. Importantly, $P_{ET}CO_2$ did not significantly differ during hyperoxia exposure in either the IH or control cohorts.

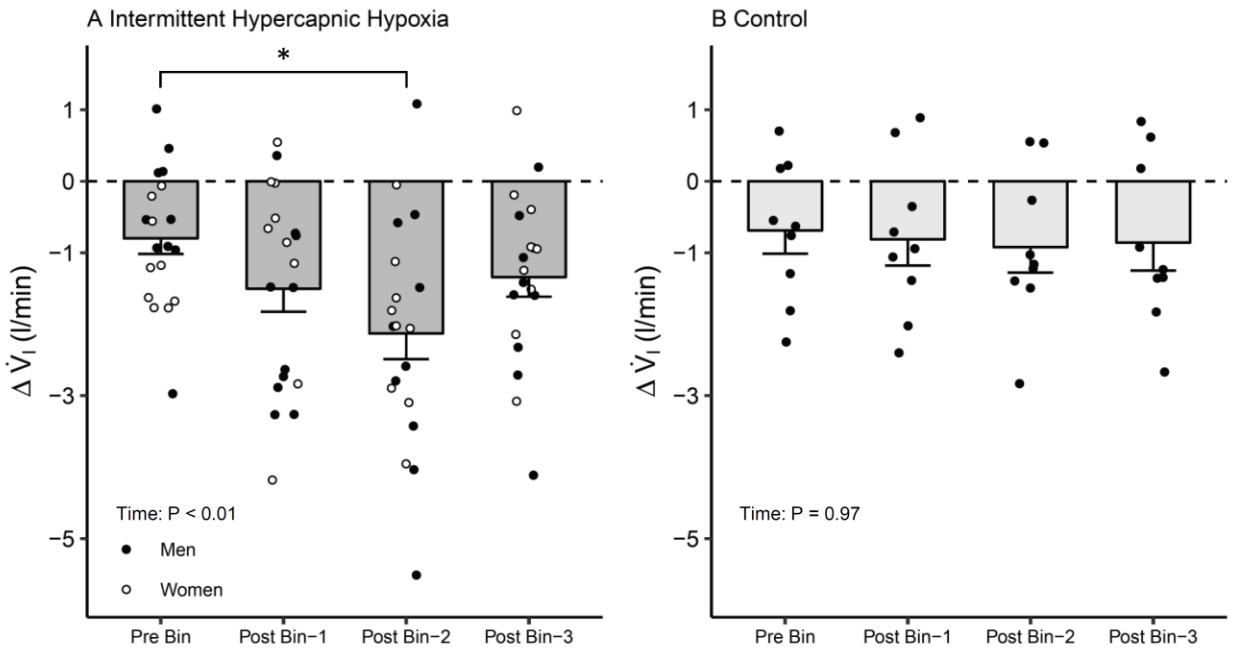

Figure 5. Group mean and individual delta changes in minute ventilation during hyperoxia exposure. Filled bars represent mean ± SEM. * denotes P < 0.05 from "Pre-Bin". Abbreviations: $\dot{V}_I$, minute ventilation.

Table 3. Delta response in respiratory parameters during hyperoxia exposure before and following IHH.

	Pre		Post Bin-1		Post Bin-2		Post Bin-3	
	IHH	**CON**	**IHH**	**CON**	**IHH**	**CON**	**IHH**	**CON**
Δ $\dot{V}_I$ (l/min)	-0.8 ± 0.2	-0.7 ± 0.3	-1.5 ± 0.4	-0.8 ± 0.4	-2.1 ± 0.4 *	-0.9 ± 0.4 †	-1.3 ± 0.3	-0.9 ± 0.4
Δ V_T (ml)	-1 ± 28	-23 ± 19	-2 ± 24	-92 ± 20 †	-57 ± 31	-48 ± 34	-44 ± 28	-90 ± 68
Δ f_B (/min)	-0.4 ± 0.2	-0.3 ± 0.4	-1.0 ± 0.4	0.1 ± 0.4	-0.6 ± 0.4	-0.2 ± 0.4	-0.6 ± 0.3	0.4 ± 0.6
Δ $P_{ET}O_2$ (mmHg)	254 ± 6	247 ± 10	256 ± 5	239 ± 10	257 ± 7	239 ± 10	270 ± 5 *	246 ± 9 †
Δ $P_{ET}CO_2$ (mmHg)	-0.6 ± 0.1	-0.7 ± 0.1	-0.4 ± 0.2	-0.9 ± 0.3	-0.3 ± 0.1	-0.5 ± 0.2	-0.5 ± 0.1	-0.8 ± 0.1

IHH Cohort N=19; CON Cohort N=9, * denotes $P < 0.05$ from respective Pre time point, † denotes $P < 0.05$ compared to IHH condition at the respective time point. Abbreviations: IHH, intermittent hypercapnic hypoxia; CON, control; $\dot{V}_I$, minute ventilation; V_T, tidal volume; ml, milliliters; f_B, breathing frequency; $P_{ET}O_2$, partial pressure of end-tidal O_2; $P_{ET}CO_2$, partial pressure of end-tidal CO_2. Data represent mean ± SEM.

4.3.3 Sex Differences in Respiratory Parameters and Patterns Eliciting vLTF

Sex specific changes in V_T and f_B are represented in Figure 6. No difference between men and women was evident at baseline in either V_T or f_B. A main effect of time was observed within f_B such that f_B was elevated from baseline during normoxic recovery in both men and women at 5 through 20 minutes of recovery and was not elevated during the remainder of recovery. Women trended towards having an augmented elevation in f_B throughout recovery following IHH, but no main effect of sex or time-by-sex interaction was present. A significant time-by-sex interaction was evident in V_T values throughout the protocol. Post-hoc analysis determined that men had larger tidal volumes compared to women at 5 through 15-minutes and 30 through 50-minutes of normoxic recovery (Figure 6). Additionally, men increased V_T at 30, 40, and 50 minutes of recovery, whereas women did not change V_T from baseline throughout any timepoints during recovery. In summary, vLTF appears to be elicited through changes in V_T in men, and changes in f_B in women. As previously reported, $P_{ET}O_2$ and $P_{ET}CO_2$ were held consistently at baseline levels throughout recovery (Table 2), however main effects of sex determined differences were evident between in $P_{ET}O_2$ ($P < 0.01$) and $P_{ET}CO_2$ ($P < 0.01$) values in men and women. Across all time points women had higher $P_{ET}O_2$ (95.0 ± 0.3 vs. 92.0 ± 0.2 mmHg) and lower $P_{ET}CO_2$ (38.4 ± 0.0 vs. 41.1 ± 0.1 mmHg) compared to men despite similar $\dot{V}_I$ (Figure 4).

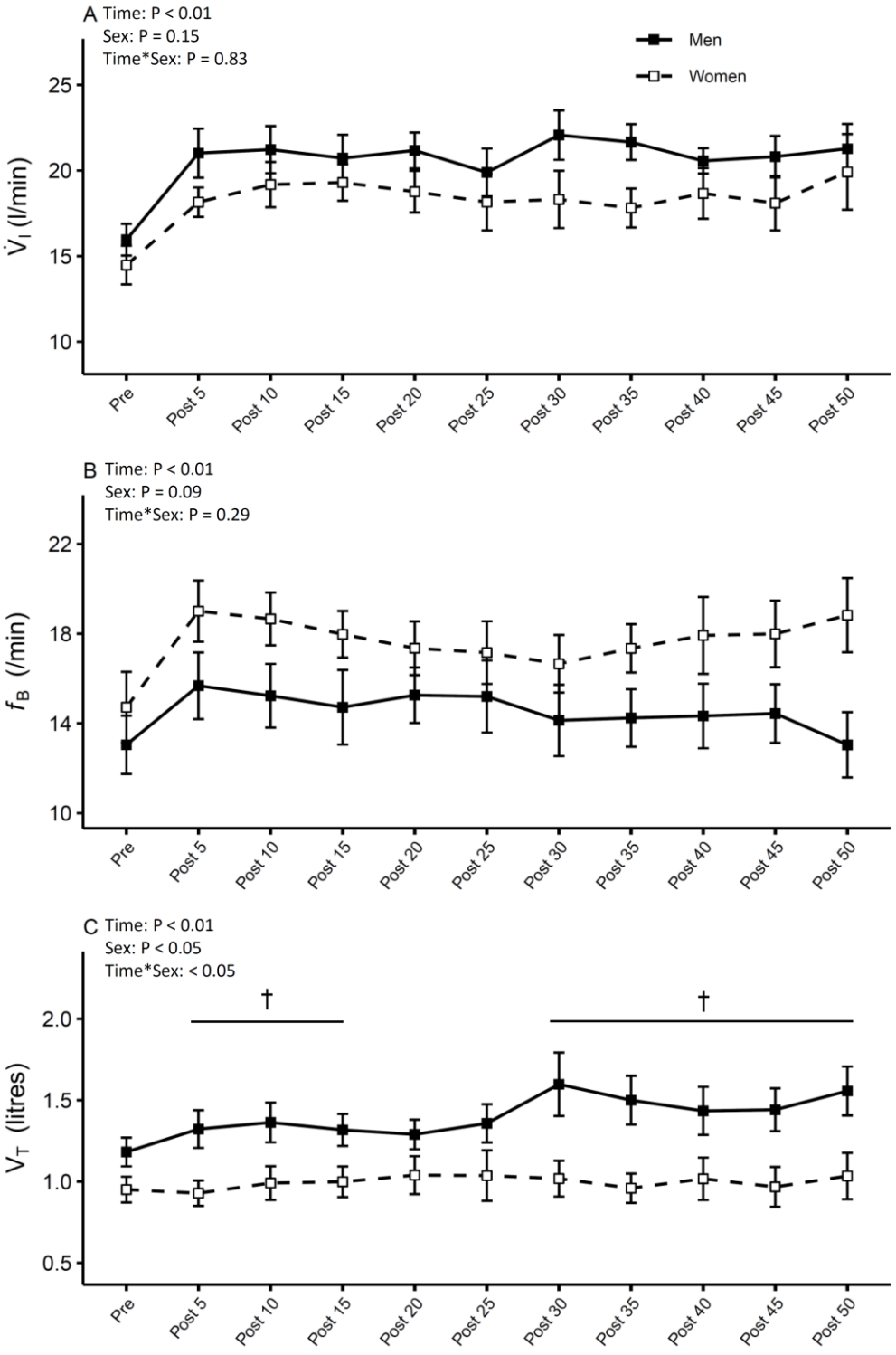

Figure 6. Sex comparisons in respiratory patterns throughout normoxic recovery. A: Sex differences in breathing frequency (f_B) throughout recovery. B: Sex differences in tidal volume (V_T) throughout recovery. Values represent mean ± SEM. † denotes $P < 0.05$ between men vs. women at the specified time point.

4.3.4 Cardiovascular Responses During Normoxic Recovery

Table 4 outlines the cardiovascular parameters at baseline and throughout 50-minutes of isocapnic-normoxic recovery in both experimental and control groups. No difference was present in SBP throughout recovery when compared to baseline ($P = 0.41$) and was not different between sexes ($P = 0.53$). In the control group, SBP increased during recovery compared to baseline ($P < 0.01$) and post-hoc analysis determined the only significant elevation in SBP occurred at 50-minutes recovery. Both men and women had similar SBP across all experimental time points ($P = 0.53$). No difference in SBP was evident between the IHH and control groups at any experimental stage.

When compared to baseline DBP increased in both the IHH and control groups during recovery (main effects of time, both $P < 0.01$). Post-hoc analysis determined that at all time points throughout recovery DBP was significantly elevated in the IHH cohort however DBP was only elevated during the final 20-minutes of recovery in the control cohort (Post-35 through to Post-50 minutes; Table 4). Additionally, DBP in the IHH cohort were significantly higher than control during Post-5 through to Post-15 minutes but similar through the remainder of recovery (Table 4). Additionally, in the IHH cohort a main effect of sex was observed ($P < 0.01$) such that females had higher DBP compared to males at all experimental time points (71.9 ± 1.2 vs. 61.9 ± 1.2 mmHg respectively), although there was not evidence of a time-by-sex interaction ($P = 0.98$).

Changes in MAP were primarily due to changes in DBP. A main effect of time was found in both the IHH and control cohorts such that MAP increased during recovery compared to baseline in both groups (both $P < 0.01$). Post hoc analyses determined in the IHH cohort that MAP was significantly elevated compared to baseline during recovery at Post-15, Post-20, Post-45 and Post-50 (Table 4). In the control cohort, similar to DBP, MAP was increased compared to baseline in the final 20 minutes (Post-35 through to Post-50; Table 4). However, no significant differences in MAP were present at any time points when comparing between IH and control. A main effect of sex was also observed for MAP ($P < 0.01$), such that women had higher MAP compared to males at all experimental time points (87.0 ± 1.5 vs. 79.9 ± 1.4 mmHg respectively), although like DBP a time-by-sex interaction was not presented ($P = 0.94$).

No significant changes in HR were shown in either the IHH or control group at any experimental time points (Table 4, $P = 0.07$ and $P = 0.06$ respectively). Additionally, in the IHH cohort, men and women had similar HR across all time points ($P = 0.07$).

Table 4. Cardiovascular parameters at baseline and during recovery following 40-minutes of IHH or time-matched control.

	SBP (mmHg)		DBP (mmHg)		MAP (mmHg)		HR (/min)	
	IHH	**CON**	**IHH**	**CON**	**IHH**	**CON**	**IHH**	**CON**
Pre	114 ± 2	115 ± 4	62 ± 2	59 ± 2	79 ± 2	77 ± 2	66 ± 2	57 ± 2 †
Post-5	115 ± 2	115 ± 3	66 ± 2 *	60 ± 1 †	82 ± 2	78 ± 2	64 ± 2	59 ± 3
Post-10	114 ± 2	115 ± 3	66 ± 2 *	61 ± 1 †	83 ± 1	79 ± 2	65 ± 2	59 ± 2
Post-15	116 ± 2	115 ± 3	68 ± 1 *	62 ± 2 †	84 ± 1 *	80 ± 2	65 ± 1	58 ± 2 †
Post-20	116 ± 2	116 ± 4	68 ± 1 *	63 ± 2	84 ± 1 *	81 ± 2	64 ± 2	61 ± 3
Post-25	115 ± 2	117 ± 3	67 ± 2 *	62 ± 1	83 ± 1	81 ± 2	66 ± 2	59 ± 2 †
Post-30	114 ± 2	119 ± 3	66 ± 2 *	64 ± 2	83 ± 2	82 ± 3	66 ± 2	56 ± 2 †
Post-35	114 ± 2	122 ± 3	67 ± 2 *	66 ± 2 *	83 ± 2	85 ± 2 *	67 ± 2	59 ± 2 †
Post-40	114 ± 3	122 ± 3	67 ± 3 *	66 ± 2 *	83 ± 2	85 ± 2 *	68 ± 3	61 ± 2
Post-45	117 ± 2	125 ± 4	68 ± 2 *	68 ± 3 *	85 ± 2 *	87 ± 3 *	68 ± 2	60 ± 3 †
Post-50	117 ± 2	126 ± 4 *	69 ± 1 *	69 ± 2 *	85 ± 1 *	88 ± 2 *	67 ± 2	61 ± 2

IHH Cohort N=19; CON Cohort N=9, * denotes $P < 0.05$ from respective Pre time point, † denotes $P < 0.05$ compared to IHH condition at the respective time point. Abbreviations: IH, intermittent hypercapnic hypoxia; CON, control; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate. Data represent mean ± SEM.

4.4 Cerebral Neurovascular Coupling

Statistical analysis was completed on 18 individuals (8 females) and 8 males returned for a time-matched control. Of the 10 females initially recruited for participation in this study, one was excluded due to an outlier in resting HR from the group norm suggesting this person was not in a rested state (see 4.3). A second was excluded due to unresponsive PCA_V during visual stimulation, suggesting unreliable insonation of the PCA. In one male, NVC measures were forgone due to unreliable TCD measurements. One male did not return for a control visit. An additional male was dropped from the control group due to an unexplained 6 mmHg shift in resting $P_{ET}CO_2$ during the 40-minutes of room air exposure which did not allow for adequate controlling of $P_{ET}CO_2$ to baseline levels. Lastly, due to unreliable MCA measurements in one male the MCA responses in the IHH cohort includes 17 individuals, and the control cohort includes 7 individuals.

4.4.1 Changes in Cerebral Hemodynamics and Neurovascular Coupling Following Intermittent Hypercapnic Hypoxia

Within the IHH cohort, linear mixed effect modeling determined there were no sex differences across cerebral hemodynamic or neurovascular coupling measurements, therefore grouped data across men and women are presented. Resting cerebral hemodynamics are presented in Table 5. Following both 40-minutes of IHH and time matched room air exposure, resting PCA_V was reduced, however there was no change in MCA_V following experimental exposure in both cohorts. Resting PCA_{CVC} and MCA_{CVC} were both reduced in the IHH cohort, and PCA_{CVC} was similarly reduced in the control cohort, however MCA_{CVC} did not change.

Figure 7 shows the group cycle averaged absolute and percent relative change in PCA_V and PCA_{CVC} for the eyes-closed to eyes-opened transition in the IHH cohort. Figure 7 also outlines the peak PCA_V and PCA_{CVC} as a percent relative to baseline for the IHH cohort. Additional PCA and MCA neurovascular coupling metrics in the IHH and control cohort are presented in Table 5. Despite reduced resting PCA_V in both IHH and control groups, the absolute and percent relative peak PCA_V during neurovascular coupling was unchanged following IHH or time matched control. Absolute and percent relative peak MCA_V were also unchanged in both IHH and control groups following experimental exposures. Absolute peak PCA_{CVC} was reduced in both IHH and control cohorts following IHH exposure although an increased percent relative peak PCA_{CVC} was evident

in the IHH cohort suggestive of improved NVC following IHH exposure that was not evident following room-air exposure in the control cohort. Similarly, percent relative MCA_{CVC} was improved following IHH and was not changed in the time-matched control group. The MAP response during visual stimuli was not different from pre- to post-IHH when presented as either an absolute delta (3.4 ± 0.8 vs. 2.2 ± 0.7 mmHg respectively, P = 0.12) or percent relative (104.5 ± 1.2 vs. 102.8 ± 1.0 % respectively, P = 0.11) response. The absolute delta PCA_V during visual stimulation was not different between pre- and post-IHH conditions (8.2 ± 0.8 vs. 8.5 ± 0.8 cm/s respectively, P = 0.49), and compared to baseline the absolute delta PCA_{CVC} trended to be higher following IHH (0.110 ± 0.011 vs. 0.120 ± 0.010 cm/s/mmHg, P = 0.07) suggesting a trend toward improved NVC in absolute terms. Similarly, during visual stimulation absolute delta MCA_V was not different between pre- and post-IHH (4.7 ± 0.9 vs. 5.4 ± 0.7 cm/s respectively, P = 0.32), however absolute delta MCA_{CVC} was higher compared to baseline following IHH (0.066 ± 0.014 vs. 0.091 ± 0.001 cm/s/mmHg respectively, P = 0.02). When comparing the delta from pre- to post- between IHH and control cohorts, there were no differences across any cerebrovascular parameters reported.

Table 5. Cerebral hemodynamics and neurovascular coupling parameters.

	Intermittent Hypercapnic Hypoxia			Control		
	Pre	**Post**	**P =**	**Pre**	**Post**	**P =**
Resting Cerebral Hemodynamics						
PCA_V (cm/s)	39.8 ± 3.0	38.2 ± 3.0 *	0.045	41.8 ± 2.0	38.7 ± 2.1 *	<0.01
MCA_V (cm/s)	65.1 ± 3.2	64.2 ± 3.9	NS	56.6 ± 5.5	55.5 ± 5.9	NS
PCA_{CVC} (cm/s/mmHg)	0.51 ± 0.04	0.47 ± 0.04 *	<0.01	0.55 ± 0.03	0.50 ± 0.03 *	<0.01
MCA_{CVC} (cm/s/mmHg)	0.84 ± 0.04	0.78 ± 0.05 *	<0.01	0.76 ± 0.07	0.73 ± 0.08	NS
Cerebral Neurovascular Coupling Responses						
Peak PCA_V (cm/s)	48.1 ± 3.4	46.8 ± 3.4	NS	49.2 ± 2.3	47.4 ± 2.5	NS
Peak MCA_V (cm/s)	69.9 ± 3.5	69.9 ± 4.0	NS	61.0 ± 5.8	60.8 ± 5.9	NS
Peak PCA_{CVC} (cm/s/mmHg)	0.62 ± 0.04	0.59 ± 0.04 *	<0.01	0.67 ± 0.03	0.62 ± 0.03 *	<0.01
Peak MCA_{CVC} (cm/s/mmHg)	0.91 ± 0.05	0.87 ± 0.05	0.08	0.84 ± 0.08	0.81 ± 0.08	NS
Rel Peak PCA_V (%)	121.3 ± 1.9	123.3 ± 1.8	NS	117.9 ± 1.6	122.0 ± 2.6	NS
Rel Peak MCA_V (%)	107.5 ± 1.4	108.8 ± 1.3	NS	108.1 ± 1.6	111.0 ± 2.6	NS
Rel Peak PCA_{CVC} (%)	122.5 ± 2.0	127.0 ± 2.0 *	<0.01	121.9 ± 2.5	123.5 ± 1.9	NS
Rel Peak MCA_{CVC} (%)	108.1 ± 1.7	112.2 ± 1.5 *	<0.01	111.6 ± 1.7	112.5 ± 2.6	NS

PCA parameters, IHH cohort N=18, CON cohort N=8; IHH MCA parameters, IHH cohort N=17, CON cohort N=7; * denotes P<0.05 from respective Pre and Post condition. Abbreviations: MCA_V, middle cerebral artery blood velocity; PCA_V, posterior cerebral artery blood velocity; MCA_{CVC}, middle cerebral artery vascular conductance; PCA_{CVC}, posterior cerebral artery vascular conductance; NS, not significant. Data represent mean ± SEM.

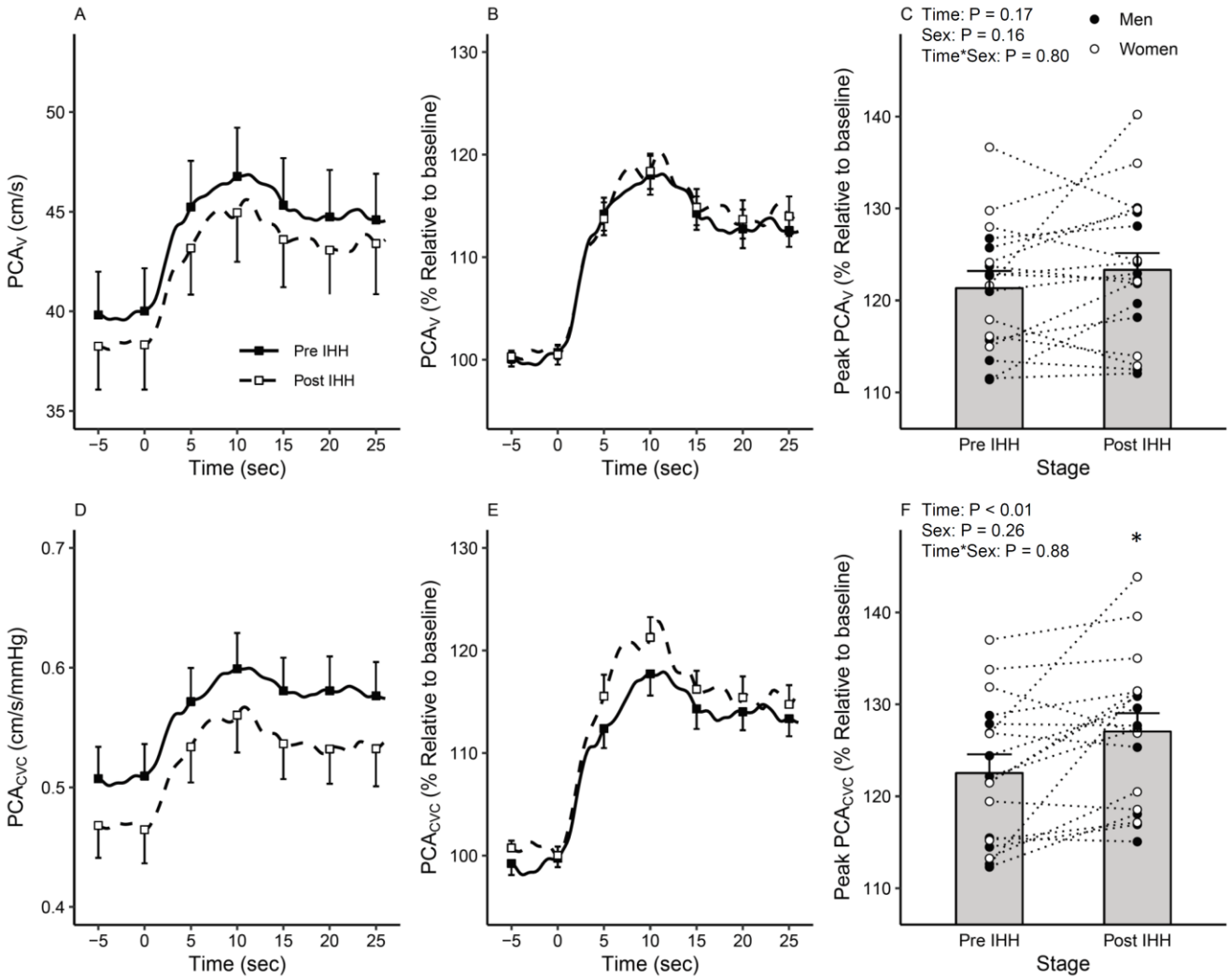

Figure 7. PCA responses during neurovascular coupling prior to and following IHH exposure. N=18. A: Absolute PCA_V response during NVC. B: Percent relative PCA_V response. C: Individual and group mean ± SEM peak relative PCA_V response. D: Absolute PCA_{CVC} response during NVC. E: Percent relative PCA_{CVC} response during NVC. F: Individual and group mean ± SEM peak relative PCA_{CVC} response. * denotes $P < 0.05$ between Pre-IHH and Post-IHH conditions.

4.4.2 Cardiorespiratory Changes Following Intermittent Hypercapnic Hypoxia

Table 6 outlines cardiorespiratory parameters prior to and following IHH or time-matched control in participants where NVC measurements were taken (N=18). In both groups, SBP was unchanged following IHH or time-matched control. DBP was increase in both groups immediately following exposure, however only the IHH cohort demonstrated elevated MAP compared to baseline. Resting HR was unchanged from baseline in both cohorts. Like the vLTF results reported elsewhere, $\dot{V}_I$ was increased in both groups (not different between sexes; $P = 0.2$) immediately following IH and time-matched control, and $P_{ET}O_2$ and $P_{ET}CO_2$ were unchanged as expected with dynamic end-tidal forcing maintaining baseline parameters. Within the IHH cohort, a main effect of sex was evident in both DBP and MAP (both $P < 0.01$) such that women had elevated DBP (69 ± 2 vs. 59 ± 2 mmHg) and MAP (84 ± 2 vs. 77 ± 2 mmHg) compared to men prior to and following IHH exposure, however significant time-by-sex differences were not present. When comparing the deltas from pre- to post- between the IHH and control cohorts the change in $\dot{V}_I$ was greater in the IHH cohort compared to the control cohort ($P < 0.05$), however no other significant differences were present between the IHH and control cohorts for the remaining cardiorespiratory parameters reported.

Table 6. Cardiorespiratory parameters in IHH and control groups where NVC measures were obtained.

	Intermittent Hypercapnic Hypoxia			Control		
	Pre	**Post**	**P =**	**Pre**	**Post**	**P =**
Cardiovascular Parameters						
SBP (mmHg)	113 ± 2	114 ± 2	NS	113 ± 3	116 ± 3	NS
DBP (mmHg)	61 ± 2	66 ± 2 *	<0.01	58 ± 2	60 ± 1 *	<0.05
MAP (mmHg)	78 ± 2	83 ± 2 *	<0.01	76 ± 1	77 ± 1	NS
HR (/min)	66 ± 2	64 ± 2	NS	60 ± 2	59 ± 2	NS
Respiratory Parameters						
$\dot{V}_I$ (l/min)	15.1 ± 0.8	19.3 ± 1.0 *	<0.01	16.2 ± 0.7	17.4 ± 0.9 *	<0.05
$P_{ET}O_2$ (mmHg)	93 ± 1	94 ± 1	NS	94 ± 1	95 ± 1	NS
$P_{ET}CO_2$ (mmHg)	40 ± 1	40 ± 1	NS	40 ± 1	40 ± 1	NS

IHH cohort, N=18; Control cohort, N=8, * denotes P<0.05 from respective Pre and Post condition. Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; $\dot{V}_I$, minute ventilation; $P_{ET}O_2$, partial pressure of end-tidal O_2; $P_{ET}CO_2$, partial pressure of end tidal CO_2; NS, not significant. Data represent mean ± SEM.

Chapter 5: Discussion

5.1 Aim 1: The role of peripheral chemoreceptor drive on ventilatory long-term facilitation induced by acute intermittent hypercapnic hypoxia.

5.1.1 Main Findings

The purpose of this aim was to determine whether acute IHH (1) induces vLTF during 50-minutes of isocapnic-normoxic recovery, and (2) determine whether tonic peripheral chemoreceptor drive modulates vLTF during recovery. Our data show that vLTF is evident during 50-minutes of isocapnic-normoxic recovery in both men and women In contrast to previous reports that the peripheral chemoreceptors do not have a functional role in vLTF (Griffin *et al.*, 2012), our data show that inhibition of peripheral chemoreceptor drive attenuates $\dot{V}_I$ 20-30 minutes of recovery following the end of IHH but does not abolish vLTF. This effect may reflect sensory long-term facilitation of carotid body afferents which is known to develop over a similar time course in isolated rat carotid body preparations (Prabhakar *et al.*, 2007). Lastly, secondary analysis showed that women develop vLTF primarily through changes in f_B, whereas males rely on changes in V_T to elicit a similar magnitude of vLTF.

5.1.2 Intermittent Hypoxia and Ventilatory Long-Term Facilitation

We observed an increase in resting $\dot{V}_I$ in the IHH cohort throughout 50-minutes of isocapnic-normoxic recovery compared to baseline that was greater than the time matched control cohort during the initial 20-minutes of recovery (Figure 4). The observed magnitude of vLTF in the current study is similar to previous reports, although continuous hypercapnia throughout exposure and recovery was not a requirement. Sustained hypercapnia was shown to be necessary in prior investigations to successfully induce vLTF in humans (Harris *et al.*, 2006; Griffin *et al.*, 2012; Syed *et al.*, 2013). Moreover, our findings are in contrast with two prior investigations which administered an acute IHH stimulus similar to that used in the current study and failed to elicit vLTF (Diep *et al.*, 2007; Deacon *et al.*, 2017). These discrepant findings with IHH exposures may be the result of variations in the severity of IHH paradigms used, with Diep *et al.*, (2007) and Deacon *et al.*, (2017) using less than 15-minutes of total hypercapnic hypoxia at 30-second intervals versus the 27-minute total hypercapnic hypoxia exposure at 40-second intervals used currently. Additionally, discrepancies may be due to the absent of precise control of CO_2 during

recovery in prior reports (Diep *et al.*, 2007; Deacon *et al.*, 2017). The influence of CO_2 status during recovery is important for inducing vLTF, and our data show for the first time vLTF during recovery can be induced under isocapnic conditions in awake men and women with paired intermittent hypercapnic hypoxia. Increased resting $\dot{V}_I$ was evident at 25-minutes and from 40- to 50-minutes of recovery compared to baseline in the control cohort. Griffin and colleagues (2012) similarly observed increased $\dot{V}_I$ during a time-matched control with exposure to isolated hypercapnia similar to the control group in the current study. The increase in $\dot{V}_I$ observed in the control cohort does not represent vLTF and may reflect sympathoexcitation or discomfort from remaining sedentary for a prolonged period. Thus, in the IHH cohort measurements made in the initial 30-minutes of recovery likely reflects vLTF and increases in $\dot{V}_I$ shown after 30-minutes of recovery may not fully represent the vLTF manifested following IHH.

Animal models provide insight on the mechanisms responsible for vLTF and propose an increased sensitivity to glutamate on the post-synaptic phrenic motor neuron that results in higher discharge rate and strength following intermittent hypoxia (Pamenter & Powell, 2013). Increased glutamate sensitivity is due to activation of spinal 5-HT, adenosine or alpha-adrenergic receptor activation on the post-synaptic neuron and through secondary downstream Gq-protein or Gs-protein coupled signaling mechanisms (McGuire *et al.*, 2004; Nichols *et al.*, 2012; Hickner *et al.*, 2013; Pamenter & Powell, 2013). Tonic peripheral chemoreceptor inhibition did not fully abolish vLTF, and it is likely that central signaling mechanisms including 5-HT, adenosine and alpha-adrenergic pathways have a functional role in vLTF observed in the integrative human model. The specific central pathways and mechanisms responsible for vLTF are difficult to discern and remain incompletely understood in healthy humans.

5.1.3 Hyperoxic Ventilatory Depression: A Link to Sensory Long-Term Facilitation?

Carotid body sensory long-term facilitation is characterized by prolonged increases in carotid body afferent activity, is related to prolonged elevations in sympathetic vasomotor outflow (sympathetic long-term facilitation) elicited by intermittent hypoxia and shown to be dependent on 5-HT signaling (Peng *et al.*, 2003, 2006; Roy *et al.*, 2018). Additionally, vLTF is evident in animals following repeated stimulation of the CSN, indicative of a relationship between peripheral carotid bodies and vLTF. Whether sensory long-term facilitation is implicated in the vLTF response observed in humans is unknown. Griffin and colleagues (2012) reported that peripheral

chemoreceptor inhibition with hyperoxia did not modulate the observed vLTF following intermittent hypoxia, as reductions in $\dot{V}_I$ were similar prior to and following intermittent hypoxia. Although, prior conclusions were drawn with a sustained hypercapnic background during hyperoxia and limit comparison to the current data. The current results contrast previous reports as our data show that tonic peripheral chemoreceptor drive has a modulatory role in vLTF. Compared to baseline, hyperoxia exposure attenuated $\dot{V}_I$ to a greater magnitude throughout recovery with the largest reductions occurring between 20 to 30-minutes of isocapnic-normoxic recovery. This time-dependent response was not evident in the time-matched control group in which similar reductions in $\dot{V}_I$ were observed at baseline and during recovery (Figure 5). The augmented reduction in $\dot{V}_I$ observed following IHH with hyperoxic inhibition of peripheral carotid bodies may, for the first time in humans, indicate sensory long-term facilitation in humans as it has a modulatory role in $\dot{V}_I$. However, direct measurements of carotid body afferents are not possible in humans and limits the conclusion that sensory long-term facilitation occurred following IHH.

5.1.4 Sex Differences in Eliciting Ventilatory Long-Term Facilitation

Secondary analysis of the current results demonstrate that men and women both developed vLTF to a similar magnitude during isocapnic-normoxic recovery (Figure 5). This finding is consistent with prior investigations which also report that healthy men and women both develop vLTF during hypercapnic recovery following intermittent hypoxia (Wadhwa *et al.*, 2008; Syed *et al.*, 2013). Additionally, we demonstrate for the first time that during isocapnic-normoxic recovery, vLTF manifests in men primarily through changes in V_T whereas women develop vLTF primarily through changes in f_B (Figure 6). Syed and colleagues (2013) previously demonstrated that at 30-minutes of hypercapnic recovery following intermittent hypoxia, men had elevated V_T compared to women regardless of arousal state. However, men also had higher V_T at baseline which likely contributed to the higher V_T consistently reported throughout recovery compared to women (Syed *et al.*, 2013). Importantly, in the current data no significant differences were observed between men and women for V_T or f_B at baseline (Figure 6). Furthermore, both Syed et al., (2013) and Wadhwa et al., (2008) previously reported no difference in f_B during hypercapnic recovery between men and women regardless of arousal state. In support of a difference in respiratory recruitment patterns between men and women, a similar disparity is evident during incremental exercise, where

women increased f_B to a greater extent and males increased V_T greater than women at matched submaximal workloads (Kilbride *et al.*, 2003). Women tend to have smaller airways compared to men, and during exercise can experience expiratory flow limitations more commonly compared to men (Guenette *et al.*, 2007; Sheel *et al.*, 2009; Dominelli *et al.*, 2015*a*, 2018). Additionally, women have an increased work of breathing compared to men at matched ventilatory workloads (Dominelli *et al.*, 2015*b*). As a result, it is plausible that vLTF is achieved in women by increasing breathing frequency rather than tidal volume to minimize added respiratory work.

Women were tested between 0-5 days of the early follicular phase as sex hormones are reported to be a potent stimulus for ventilation (Behan *et al.*, 2003; Behan & Wenninger, 2008). Controlling menstrual cycle to 0-5 days ensured estrogen had a minimal influence on the observed results. Controlling menstrual cycle in the current investigation may demonstrate that the differing respiratory patterns is a result of anatomical and ventilatory energetic sex differences rather than a hormonal influence on the carotid body. As the menstrual cycle progresses women tend to increase resting $\dot{V}_I$ compared to the follicular phase (White *et al.*, 1983; Macnutt *et al.*, 2012) and may be a result of increased estrogen levels upregulating 5-HT receptor activity throughout the menstrual cycle (Biegon *et al.*, 1980, 1983). Data from anesthetized rats show young males develop greater magnitudes of phrenic long-term facilitation compared to aged matched females regardless of circulating hormone status, which is reversed by middle-age where females develop greater phrenic long-term facilitation (Zabka *et al.*, 2001). To date no data are available characterizing how vLTF is altered throughout the menstrual cycle in healthy women.

5.1.5 Effect of Intermittent Hypoxia on Arterial Blood Pressure

Following IHH exposure DBP was significantly elevated throughout the 50-minute duration of isocapnic-normoxic recovery, DBP was also elevated compared to the time matched control group during the first 15-minutes of recovery. This indicates that IHH exposure resulted in increases in blood pressure for a minimum of 30-minutes following the removal of the stimulus. However, increased blood pressure was evident in the time-matched control group during the final 20-minutes of recovery and elevated blood pressure past 30-minutes of recovery in the IHH group may therefore not represent an effect of IHH exposure. Changes in blood pressure are supported by previous investigations which report elevated arterial blood pressure following both acute and chronic intermittent hypoxia in healthy individuals (Foster *et al.*, 2009*a*; Gilmartin *et al.*, 2010;

Tremblay *et al.*, 2016). Elevated blood pressure following IHH likely occurred through upregulation of the renin-angiotensin system from increases in sympathetic vasomotor outflow towards the kidney during intermittent hypoxia and likely contributes to the sustained DBP response shown throughout recovery (Foster *et al.*, 2010; Gilmartin *et al.*, 2010). Implication of a renin-angiotensin dependent blood pressure response is supported by pharmacological blockade of angiotensin-II type 1 receptor which was able to abolish the increase in arterial blood pressure following 6-hours of intermittent hypoxia (Foster *et al.*, 2010) and reduce the magnitude of augmented sympathetic vasomotor outflow following intermittent hypoxia (Jouett *et al.*, 2017). Sympathetic vasomotor outflow remains increased for a minimum of 20-minutes following removal of a continuous or intermittent hypoxic stimulus (Xie *et al.*, 2000, 2001) that likely remains elevated during peripheral chemoreceptor inhibition with hyperoxia (Querido *et al.*, 2010). Additionally, we have reported augmented sympathetic neurovascular transduction following IHH such that forearm vascular conductance and DBP are more sensitive to changes in sympathetic outflow following IHH (Stuckless *et al.*, 2018). This relationship provides a potential link between elevated sympathetic vasomotor outflow to arterial blood pressure following intermittent hypoxia.

Women had higher DBP and MAP at baseline and throughout recovery, but there were no time-by-sex interactions suggesting a similar arterial blood pressure response to acute IHH exposure between sexes. To our knowledge we are the first to characterize the arterial blood pressure response in healthy men and women following acute intermittent hypoxia (with or without concomitant hypercapnia). In women it is suggested that β-adrenergic receptor activation offsets the vasoconstrictive effect of elevations in sympathetic vasomotor activity (Hart *et al.*, 2011). Moreover, Samora *et. al.*, (2019) recently demonstrated that women have a blunted arterial blood pressure response to exercise compared to men that was unchanged with β-adrenergic receptor inhibition despite reducing the arterial blood pressure response to exercise in men. The lack of sex differences in arterial blood pressure responses during recovery from IHH may indicate that women develop elevated arterial blood pressure independent of β-adrenergic receptor tone. Although the current volunteers were all normotensive at rest and had similar HR ($P = 0.07$), the DBP and MAP difference between men and women add to the mixed body of literature that show young healthy women having lower (Williams *et al.*, 2018), or similar (Hart *et al.*, 2011; Briant *et al.*, 2016; Williams *et al.*, 2016, 2017; Samora *et al.*, 2019) resting arterial blood pressure

parameters compared to young men. Importantly, the resting MAP values of men and women in the current investigation are lower than previously reported normative values for healthy adults at an average age of 25 (Joyner *et al.*, 2015), and likely indicates an influence of fitness level contributing to the observed sex differences.

5.1.6 Methodological Considerations

The paradigm of intermittent hypoxia included paired hypercapnic-hypoxia at a high frequency of 1-cycle/minute with 40-seconds of hypercapnic-hypoxia and 20-seconds of normoxic recovery for a total duration of 40-minutes. This duty cycle and paired hypercapnic-hypoxia exposure mimics severe OSA (> 30 events/hour) and the accompanied arterial blood O_2 and CO_2 changes typical during an apneic event. Previous investigations eliciting vLTF administered intermittent hypoxia at durations of 2-4 minutes a bout, with similar durations of normoxic recovery for 8-12 repeated bouts while having a sustained hypercapnic background throughout the exposure (Harris *et al.*, 2006; Griffin *et al.*, 2012; Syed *et al.*, 2013). Prior reports utilizing IHH exposures although unable to elicit vLTF had shorter duration bouts lasting 30-seconds of hypercapnic-hypoxia and administered 15 (Diep *et al.*, 2007) or 24 (Deacon *et al.*, 2017) repeated bouts with 90-seconds of recovery interspersed. The more severe exposure paradigm used currently which lengthened hypercapnic-hypoxic bouts at a higher-frequency for longer duration compared to prior investigations may have been necessary to elicit vLTF. We further show that while shortening the intermittent hypoxia and recovery cycle durations, vLTF is present during isocapnic-normoxic recovery while achieving similar magnitude of desaturations with full resaturations during normoxic recovery, and total hypoxic time as reported previously (See 4.2) (Harris *et al.*, 2006; Griffin *et al.*, 2012).

The control cohort included only males as time-matched control testing occurred 1-week following the IHH experimental protocol and women would no longer be within 0-5 days of the follicular period of menses. Time-matched control testing for women on the subsequent follicular period was not feasible.

Hyperoxia was used to diminish tonic peripheral chemoreceptor drive and the hyperoxic level used was similar to previous reports. In humans this magnitude of hyperoxia has been shown to reduce $\dot{V}_I$ during sustained hypercapnia, sympathetic vasomotor activity during exercise, and sustained elevations of sympathetic vasomotor activity following continuous hypoxia (Stickland

et al., 2008; Querido *et al.*, 2010; Griffin *et al.*, 2012). Hyperoxia exposure of > 450 mmHg is not able to completely abolish firing of the CSN in isolated felines, though CSN firing is significantly reduced at the level used in the current investigation (i.e. ~350 mmHg) (Biscoe *et al.*, 1970; Lahiri *et al.*, 1983). Low-dose dopamine infusions have previously been used to inhibit the peripheral chemoreceptor drive and shown to improve apnea time in breath-hold divers (van de Borne *et al.*, 1998; Bain *et al.*, 2015). The non-invasive nature of the current experiment precluded the use of intravenous dopamine infusions. Further, application of low-dose dopamine requires a continuous infusion and would not allow for the temporal administrations required to transiently inhibit the peripheral chemoreceptors at 5-minute intervals.

5.1.7 Physiological Relevance of Ventilatory Long-Term Facilitation

We demonstrate that vLTF can be elicited in healthy awake individuals for 30-minutes of isocapnic-normoxic recovery following acute IHH and add to the growing number of intermittent hypoxia paradigms that are able to elicit respiratory plasticity. The proposed benefits of inducing vLTF include improvements in upper away muscle activity that is evident in both humans and animals (Harris *et al.*, 2006; Wilkerson *et al.*, 2018) that could improve respiratory airway stability and potentially limit recurrent apneas (Mateika & Narwani, 2009). However, mild intermittent hypoxia has recently been shown to increase loop gain and the number of nocturnal apneic events in individuals with OSA (Alex *et al.*, 2019). Elevated loop gain is an indication for worsened ventilatory stability which limits the application of intermittent hypoxia for therapeutic benefits (Deacon-Diaz & Malhotra, 2018). Additionally, the robust cardiovascular co-morbidities including hypertension and reduced vascular function expressed similarly in OSA and healthy individuals exposed to chronic intermittent hypoxia limit the therapeutic relevance of daily intermittent hypoxia exposure (Grote *et al.*, 2000; Yim-Yeh *et al.*, 2010; Kraiczi *et al.*, 2017). The therapeutic application of intermittent hypoxia remains incompletely understood, however our results provide a further understanding of the mechanisms responsible for promoting vLTF in humans.

5.2 Aim 2: The effect of acute intermittent hypercapnic hypoxia on cerebral neurovascular coupling in healthy men and women.

5.2.1 Main Findings

The purpose of this aim was to characterize the changes in cerebral NVC that occur following acute IHH exposure in healthy men and women. Contrary to the initial hypothesis and evidence of intermittent hypoxia related impairments of cerebrovascular reactivity to arterial blood gas challenges, our results demonstrate that metrics of cerebral NVC (Peak relative PCA_{CVC} and MCA_{CVC}) are improved following acute IHH exposure to a similar extent in both men and women. This novel finding in humans demonstrates an improved hyperemic response in matching cerebral oxygen delivery to neuronal demands following IHH. This change occurred despite observed reductions in resting cerebral hemodynamics, and robust increases in resting MAP and DBP following IHH.

5.2.2 Intermittent Hypoxia and Cerebral Neurovascular Coupling

We demonstrate that cerebral NVC when measured as the peak relative PCA_{CVC} response to visual stimulus is improved following IHH but not following the time-matched room-air control. When presented as an absolute delta PCA_{CVC} response to visual stimulus, a trend for an improved response was observed. Importantly, the MAP response during visual stimulus was not changed following IHH suggesting the increase in PCA_{CVC} response following IHH was not the result of an altered MAP response evoked during visual stimulus, despite increases in resting DBP and MAP following IHH (Table 6). To our knowledge, this is the first investigation to characterize the changes in NVC that occur following acute IHH in healthy humans. In addition, we observed similar NVC responses in men and women before and after IHH despite women having greater resting MAP. A lack of sex difference in NVC is in support of Willie *et. al.,* (2011*b*) who similarly report no sex differences in NVC at baseline or during exercise. There is evidence to support that improvements in NVC can occur by administering oxygen therapy to individuals with respiratory disease (Hoiland *et al.*, 2018*b*). The current results are in contrast to our hypothesis that cerebral NVC would be attenuated following IHH similar to an impaired cerebrovascular reactivity to hypoxia in healthy individuals following intermittent hypoxia (Querido *et al.*, 2008; Foster *et al.*, 2009*a*), and in patients with OSA (Foster *et al.*, 2007; Morgan *et al.*, 2010), and impaired cognitive

function (Champod *et al.*, 2013). Measurements of cerebrovascular reactivity were not acquired in the current investigation which limit direct comparisons to reports characterizing cerebral blood flow changes following intermittent hypoxia administered over multiple days. The reduction in cognitive function following intermittent hypoxia previously reported by Champod *et al.*, (2013) were observed following four repeated days of 6-hour exposures to intermittent hypoxia. Whether acute IHH impacts cognitive function is unknown, considering the improvement in NVC observed in our study, it is unlikely that cognitive function was impaired by 40 min of IHH.

Cerebral NVC demonstrates the close temporal matching between oxygen delivery for a given neural demand in the cerebral vasculature. The NVC response is dependent on an intact neurovascular unit, which is comprised of the vascular smooth muscle, astrocyte, and neuron. Feed-forward control of NVC occurs through receptors sensitive to glutamate on (1) the neuron, resulting in NO production through activation of neuronal NO synthase or (2) the astrocyte, resulting in arachidonic acid production, and vasodilation through relaxation of vascular smooth muscle (Phillips *et al.*, 2016; Iadecola, 2017; Hosford & Gourine, 2019). A potential explanation of the improved NVC observed following IHH is an increased sensitivity to glutamate through N-methyl-D-aspartate receptors (NMDAR) occurring on the neuron, upregulating the release of NO and resulting in a larger propagated vasodilatory response. Secondly, increased sensitivity on the astrocyte causing a similar upregulated vasodilatory response to a given neural demand (in this case a standardized visual stimulus). The importance of NO in the NVC response has been elegantly shown in prior investigations for which neuronal NO synthase inhibition substantially reduces the NVC response in animal preparations (Cholet *et al.*, 1997; Burke & Bührle, 2006; Kitaura *et al.*, 2007; Stefanovic *et al.*, 2007). The current results may represent potential for plasticity within the neurovascular unit that is similar to long-term facilitation of the phrenic motor neuron which lasts for up to 60-minutes following intermittent hypoxia exposure (Pamenter & Powell, 2013). Increased glutamate sensitivity through NMDAR on the phrenic motor neuron has been suggested as the functional mechanism relating to vLTF (Pamenter & Powell, 2013). We did not make serial measurements of NVC throughout the recovery period, although whether plasticity is present in the cerebral hemodynamic response to a neural demand warrants investigation. Furthermore, whether the proposed increased glutamate sensitivity and the subsequent NO production at the neurovascular unit is responsible for the functional increase in NVC is difficult

to discern in humans. Additional investigations in animal preparations could delineate these specific responses to intermittent hypoxia.

5.2.3 Intermittent Hypoxia on Resting Cardiovascular Parameters and Cerebral Hemodynamics

Resting PCA_V was reduced by 1.6 cm/s while MCA_V was unchanged following IHH. Previous experiments with intermittent hypoxia also report no change in the MCA_V but there is no data available with respect to PCA_V following IHH (Foster *et al.*, 2009*a*; Beaudin *et al.*, 2014). This may represent alterations in regional cerebral blood flow at rest, as the PCA is a branch from the vertebral artery, and MCA branches at the terminus of the internal carotid artery. Resting PCA_{CVC} and MCA_{CVC} were both reduced following IHH mediated through increases in MAP. Following 40-minutes of time-matched rest, PCA_V and PCA_{CVC} were reduced despite no change in MCA_V or MCA_{CVC} which may represent regional differences in reduced cerebrovascular function during prolonged inactivity. In support of this, peripheral vascular function is reduced following just 30-minutes of supine inactivity (Lewis *et al.*, 2017), and 4-hours of inactivity reduces resting MCA_V (Carter *et al.*, 2018).

Following IHH, DBP and MAP were increased compared to baseline and is consistent with the blood pressure changes observed in prior acute intermittent hypoxia exposures of much longer duration (Foster *et al.*, 2009*a*; Gilmartin *et al.*, 2010; Tremblay *et al.*, 2016). Although women had higher DBP and MAP compared to males prior to and following IHH, there was no time-by-sex interaction suggesting both men and women have similar arterial blood pressure responses to IHH. The observed sex difference in arterial blood pressure may be the result of training status, since men tended to have a lower resting HR compared with women prior to and following IHH ($P = 0.06$).

5.2.4 Methodological Considerations

In the current investigation the intermittent hypoxia paradigm used mimicked severe OSA for 40-minutes at 1-minute cycles comprised of 40-seconds of paired hypercapnic hypoxia and 20-seconds of normoxic recovery. Prior reports assessing the change in cerebrovascular reactivity to hypoxia and/or hypercapnia utilized acute or chronic intermittent hypoxia exposures without paired hypercapnia. We did not assess cerebrovascular reactivity prior to or following IHH

exposure which limits comparisons of changes in cerebrovascular control. Arterial blood pressure was augmented to a similar extent as demonstrated in prior investigations with intermittent hypoxia (Foster *et al.*, 2009*a*; Beaudin *et al.*, 2014).

Serial measurements of cerebral blood flow velocity with TCD has been reported frequently (Querido *et al.*, 2008, 2015; Foster *et al.*, 2009*a*; Caldwell *et al.*, 2018). Insonation parameters such as vessel depth, ultrasound power, maximum, minimum, and mean velocities were recorded during the IHH experimental visit, and matched insonation parameters with TCD were acquired in male subjects who returned for time-matched control testing. The utility of TCD ultrasound has been heavily debated with concerns surrounding the ability to accurately measure cerebral blood flow velocities over a range of CO_2 known to cause changes in the diameter of cerebral conduit artery (Poulin & Robbins, 1996; Willie *et al.*, 2011*a*; Coverdale *et al.*, 2014). To limit the influence of changes in $P_{ET}CO_2$ following IHH, dynamic end-tidal forcing clamped $P_{ET}CO_2$ values at the level recorded during the pre- exposure in everyone. Further, a standardized visual stimulus (flashing alternating checkerboard) was administered identically in both the pre- and post- NVC assessments.

Currently no data exist on whether NVC is altered throughout the menstrual cycle in women. Women were not included in the time-matched control, because the experimental control was conducted 1-week following IHH protocol. If women were included in the time-matched control, differences in menstrual cycle phase could have been a confounder. Time-match control testing during the early follicular phase of the subsequent cycle was not feasible due to difficulties in time aligning the time-matched control visit to that of the IHH visit. Although no data exist on NVC throughout menses, cerebral blood flow regulation during hypoxia and hypercapnia has been shown to change in women throughout menses (Debert *et al.*, 2012; Peltonen *et al.*, 2016). It is unclear whether intermittent hypoxia influences cerebral blood flow differently throughout the menstrual cycle.

5.2.5 Physiological Relevance of Improved Neurovascular Coupling

The importance of matching cerebral oxygen delivery to a neural demand is demonstrated by functional MRI studies that show reduced cerebral perfusion in regional areas of the brain required for a demanding task that can contribute to impaired cognitive function (Lal *et al.*, 2012). Furthermore, acute or chronic continuous reductions in oxygen availability (either normobaric or

hypobaric hypoxia) result in impaired cognitive function across a wide variety of assessments (Turner *et al.*, 2015) and is suggested to be the result of regional changes in cerebral blood flow to task specific regions (Lawley *et al.*, 2017; Hoiland *et al.*, 2018*a*). However, severe acute hypoxia (30-minutes at $P_{ET}O_2$ of 45 mmHg) led to impairments in reaction time without affecting NVC in healthy individuals (Caldwell *et al.*, 2018). Despite the growing evidence of OSA related impairments in cognitive function (Lal *et al.*, 2012) and the previously reported relationship between NVC and cognitive function (Sorond *et al.*, 2013), NVC appears to be preserved in OSA populations compared to age-matched healthy controls (Uzuner & Uzuner, 2017). The underlying role for changes in NVC on cognitive impairments is incompletely understood, and whether the improvements in NVC shown in the current investigation relate to improvements in cognitive function, or if the observed improvements persist with other intermittent hypoxia paradigms (i.e. longer exposures and/or repeated over multiple days) is unknown.

Chapter 6: Conclusion

We demonstrate for the first time that vLTF is evident during isocapnic-normoxic recovery in healthy men and women following acute IHH. We show men and women develop similar magnitudes of vLTF, although sex differences exist within the respiratory pattern required in the development of vLTF. Men and women had similar arterial blood pressure responses to IHH that persisted throughout recovery. Immediately following IHH, cerebral NVC responses to visual stimuli were increased compared to baseline in both men and women. These results add to the growing literature in humans demonstrating the integrative changes in cardiovascular and cerebrovascular control following acute intermittent hypoxia. Understanding of these functional changes aid in the development of potential therapeutic application of intermittent hypoxia to promote neuroplastic changes, and the understanding of early cardiovascular and cerebrovascular changes that can occur in OSA.

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Appendices

Appendix A: Forms

A.1 Inclusion/Exclusion Criteria Assessment

THIS INDIVIDUAL MAY BE A CANDIDATE FOR THE INTERMITTENT HYPOXIA AND VENTILATORY FACILITATION STUDY

PLEASE DISCUSS WITH VOLUNTEER

INCLUSION CRITERIA (all must be yes):	**YES**	**NO**
AGE 18-45 years	☐	☐
Body mass index < 30 kg/m^2	☐	☐
Normotensive (<140/90, >90/60)	☐	☐

EXCLUSION CRITERIA (all must be no)	**YES**	**NO**
≥5 hypopnea/hour during sleep	☐	☐
Known hypertension	☐	☐
Known impaired renal function	☐	☐
Known liver disease	☐	☐
Known heart failure	☐	☐
Known myocardial infarct	☐	☐
Known coronary artery disease	☐	☐
Known history of stroke	☐	☐
Known COPD or Asthma	☐	☐
Known central sleep apnea	☐	☐
Known obstructive sleep apnea	☐	☐
Taking any medications (over the counter or prescribed)	☐	☐
Smoker (within last year)	☐	☐

NOT INTERESTED ☐ **INTERESTED IN PARTICIPATION** ☐

For more information contact Tyler Vermeulen:
tyler.vermeulen@alumni.ubc.ca
250.807.8083

A.2 Pre-Screening Questionnaire

OKANAGAN

Subject Demographics and Prescreening Questionnaire

The effect of acute intermittent hypoxia exposure on neurovascular coupling and ventilatory long term facilitation

Subject Identification Code: _____

Weight (Kg): _____ Height (cm):_____ BMI: _____

Gender: _____ Age: _____ Time of last meal: _____

Please answer **Yes/No** for each question (if yes, please explain):

Have you refrained from caffeine, alcohol and vigorous exercise 12 hours prior to the experimental day?
YES NO

Do you have a history of fainting or have ever experienced a syncopal episode (i.e. fainting)?
YES NO

Do you have a previous history of or a current respiratory disease or abnormality (e.g., asthma, chronic bronchitis, cystic fibrosis)?
YES NO

Do you have a previous history of or a current cardiovascular disease or abnormality (e.g., cardiac arrhythmia, hypertension, myocardial infarction)?
YES NO

Do you have a previous history of or a current neurological disease or abnormality (e.g., epilepsy, chronic migraines, stroke)?
YES NO

Are you currently on any kind of medication, particularly ones that may alter blood pressure or cerebral blood flow other than oral contraceptives (e.g., opiates, diuretics, vasodilators)?
YES NO

Do you have type I or II diabetes?
YES NO

Do you suffer from any gastro-intestinal or liver diseases and/or other abnormality (e.g., ulcers, inflammatory bowel disease, or gastro-intestinal bleeding)?
YES NO

Do you partake in regular physical activity (e.g., moderate physical activity 3-5 days/ week)?
YES NO

Do you smoke?
YES NO

Do you have any drug allergies?
YES NO

Do you have a known sulfa allergy, or do you know if you have a G6P dehydrogenase deficiency?
YES NO

Have you ever been to high altitude (above 3,000m) and if so did you become sick?
YES, and became sick. Yes, but did not become sick. NO

Have you had all of your questions or concerns addressed?
YES NO

Subjects will only be identified by their unique identification code

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A.3 Participant Information and Informed Consent

THE UNIVERSITY OF BRITISH COLUMBIA

School of Health & Exercise Science
ART 151 - 3333 University Way
Kelowna, B.C., Canada V1V 1V17
Tel: (250) 807-8224 Fax: (250) 807-9865

PARTICIPANT INFORMATION AND CONSENT FORM

TITLE OF PROJECT:	The effect of acute intermittent hypoxia exposure on neurovascular coupling and ventilatory long term facilitation
SHORT TITLE:	Intermittent hypoxia and cardiorespiratory control
PRINCIPAL INVESTIGATOR:	Glen. E. Foster, Ph.D, Associate Professor School of Health and Exercise Sciences University of British Columbia 250.807.8224
CO-INVESTIGATORS:	Tyler D. Vermeulen, M.Sc. Student Lindsey M. Boulet, M.Sc., Ph.D. Student Brooke M. Shafer, Ph.D. Student
SPONSORS:	This research is sponsored by (1) the Natural Sciences and Engineering Research Council of Canada, (2) the Canadian Foundation for Innovation, (3) the Michael Smith Foundation for Health Research and (4) the Heart and Stroke Foundation of Canada. Participants may request details of the funding if they so wish.
EMERGENCY CONTACT:	Glen Foster (778) 214-9402. Available 24 hrs/day, 7 days/wk

INVITATION: Thank you for showing an interest in this project. You are being invited to take part in this research study because you are a healthy male or female volunteer between the ages of 18 and 45. You are not obese, do not smoke, non-diabetic, and have no known heart, lung, kidney, or brain diseases.

YOUR PARTICIPATION IS VOLUNTARY: Your participation is voluntary. You have the right to refuse to participate in this study. If you decide to participate, you may still choose to withdraw from the study at any time without any negative consequences to the medical care, education, or other services to which you are entitled or are presently receiving.

This consent form describes the inclusion criteria and experimental procedures that are being carried out for research purposes. Please review the consent document carefully when deciding whether or not you wish to be part of the research and sign this consent only if you accept being a research participant.

If you wish to participate in this study, you will be asked to sign this form. You will be able to read the results after the study is published. Upon request we can provide you with a reprint of the published manuscript.

Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

WHO IS CONDUCTING THE STUDY? This study is being conducted/sponsored by the Cardiopulmonary Laboratory for Experimental and Applied Physiology, The Natural Sciences and Engineering Research Council of Canada (NSERC), the Canadian Foundation for Innovation (CFI), the Michael Smith Foundation for Health Research and the Heart and Stroke Foundation of Canada. Otherwise there are no conflicts of interest to disclose.

BACKGROUND: Intermittent hypoxia is characterized by periodic exposure to low oxygen levels and can be experience by sleep apnea patients or athletes such as elite swimmers, or water polo players. Intermittent hypoxia leads to lasting increases in sympathetic activity from the control centers in the brain stem. These control centers are also responsible for the control of breathing rate and depth which may also be increased for extended periods following exposure to intermittent hypoxia. Additionally, intermittent hypoxia can lead to impairments in spatial working memory and may be related to a change in regulating the ratio of brain oxygen delivery and brain oxygen demand termed neurovascular coupling.

WHAT IS THE PURPOSE OF THE STUDY? The purpose of this study is to (1) determine the effect of intermittent hypoxia on neurovascular coupling and (2) characterize any lasting effects of intermittent hypoxia on ventilation.

WHO CAN PARTICIPATE IN THIS STUDY? You are eligible for this study if you are male or female, between the ages of 18-45 years, have normal blood pressure, free from diseases that affect your heart, blood vessels, or lungs, and are not currently taking any prescribed or over-the-

counter medications other than oral contraceptives. Normal blood pressure according to the American Heart Association 2017 Guideline is considered less than 130/80 mmHg & greater than 90/60 mmHg (systolic blood pressure/diastolic blood pressure). We will be recruiting 30 participants in total to complete this research study.

WHO SHOULD NOT PARTICIPATE IN THIS STUDY? You should not participate in the study if you have a history of high blood pressure, impaired kidney function, liver disease, heart failure, heart attack, coronary artery disease, stroke, chronic obstructive pulmonary disease, asthma, obstructive or central sleep apnea, or smoked within the past year. You will be screened for sleep apnea with a take-home sleep test, and will be excluded if you display 5 or more episodes of oxygen desaturation per hour. You are also ineligible if you are currently taking any medications, prescribed or over-the-counter other than oral contraceptives, or if you are obese (BMI>30 Kg/m2).

WHAT DOES THE STUDY INVOLVE? You will be required to visit the laboratory on three occasions. The first for a screening visit followed by two experimental visits separated by a minimum of 72 hours. Following the initial screening visit you will be sent home with a sleep monitoring system that will measure your oxygen saturation periodically while you sleep. The following morning you will return to the lab and be randomly assigned to receive intermittent hypoxia or a sham treatment for the first experimental visit. The study pre-screening and experimental protocol and its specific measurements are described in detail in this document. In total, your involvement in this study will involve approximately 6 hours of your time; a 1-hour screening visit and two 2.5-hour visits to perform the experimental protocol.

Visit 1

Sleep Monitoring:

You will be instructed how to use the overnight finger pulse blood oxygen monitor. You will take the system home and wear it for the night following your prescreening visit.

***Prescreening*:**

You will perform basic breathing exercises and lung gas exchange testing within a body plethysmography box (pictured) to ensure normal lung function. This process is called spirometry. A body plethysmography box is a common tool used clinically and in research to determine lung volumes and function. Your entire body will be inside this box, where they will be instructed to perform a variety of breathing exercises. In total, you will be in the box for 30 minutes although the

door will only be closed for a few minutes at a time. Following the lung function protocol, participants will be screened for hypertension with an automated blood pressure cuff. Following these pre-screening assessments, you will be scheduled for your second and third experimental visits. This will be done by coordinating an available time slot in the lab with a time that fits your commitments. If any incidental findings are found during the pre-screening visit you will be referred to a primary care physician (e.g. undiagnosed sleep apnea or existing hypertension). In the event of incidental findings your family physician will be notified of your participation in this study so that your family doctor can provide proper medical care.

Visit 2 & 3

Experimental:

Both experimental days will involve a 40-minute intermittent hypoxia test with a neurovascular coupling test before and after this exposure. The specific methods are detailed below. You will be randomized (i.e. by flipping a coin) to receive either intermittent hypoxia or a sham protocol prior to the experimental day. Following intermittent hypoxia, we will measure your breathing for 60-minutes following the exposure. The experimental setup will be identical for both visits 2 & 3.

***Breathing Protocol*:**

You will breathe through a mouthpiece while wearing a nose clip so that we can measure your breathing depth and frequency, and we will place devices on your fingers to measure the level of oxygen saturation in your blood, and your blood pressure. Additionally, ultrasound probes will be placed around the top of your ears (temporal zone) of your head to measure blood flow to brain regions. You will then rest quietly for 10-15 minutes to ensure stable baseline recordings of physiological variables.

Following a 10-minute baseline period, you will undergo a neurovascular coupling test followed by a 40-minute breathing protocol where we will periodically change the gas concentrations to simulate intermittent hypoxia (periodic low oxygen, and high carbon dioxide exposure) using a device that is capable of controlling the concentration of inspired gases (dynamic end-tidal forcing). On the sham visit the gas concentrations will remain at room air values for the 40-minute duration. Following the intermittent hypoxia or sham exposure we will repeat the neurovascular coupling test followed by measurement of breathing for 60 minutes.

Neurovascular coupling test. During this test you will be asked to initially close your eyes for 1 minute followed by 5 cycles of eyes open and closed for 30 seconds each. During the eyes open portion you will be instructed to stare at a checkerboard screen. This test lasts 6 minutes in duration.

All tests and data collection will take place in the Cardiopulmonary Laboratory for Experimental and Applied Physiology located in Room 185 of the Arts building at the University of British Columbia – Okanagan.

WHAT ARE MY RESPONSIBILITIES? As a participant in this study you are responsible for attending all sessions in the laboratory. We ask that you do not consume caffeinated beverages, alcohol, or exercise heavily within 24 hours of an experimental session and that you eat your last meal at least 6 hours before a laboratory session.

WHAT ARE THE POSSIBLE HARMS AND DISCOMFORTS? You will feel strong urges to breathe during hypoxia and may feel light-headed and nauseated. However, all symptoms will pass once you return to breathing room air.

It must be noted that individual responses to the experimental procedures exist and you are encouraged to report any unusual sensations or symptoms to the investigator. You are permitted to end testing at any time for any reason. All procedures used to collect physiological data will pose no risk to your continued health and well-being. Below are some of the possible harms for the specific methods we will be using.

***Intermittent Hypoxia*:** Intermittent hypoxia will be facilitated through a computer-controlled gas-mixing system under direct supervision of a trained operator. The mixing system has been used in previous studies with no adverse events. In addition, the same operator will operate the forcing system, and this individual has years of experience with this system. You will be under constant supervision during experimental protocol, and all recording devices, especially respiratory gas and oxygen levels within the blood, will be under constant and strict supervision to ensure participant safety. You could experience symptoms such as tingling sensations throughout your body during hypoxia bouts. These sensations will go away very quickly when you breathe room air. Your responses to the exposures to low levels of oxygen will be monitored during the test, and the test will be terminated if abnormal responses are observed (not anticipated). There is no risk of developing altitude illness. You may feel discomfort from lying in the same position for several hours. These discomforts will be alleviated once the testing is terminated and you are permitted to move around. The experiment will be terminated if your oxygen saturation drops below 70%, if the amount that you inhale and exhale in a minute exceeds the abilities of the gas control system, if the concentration of expired gases cannot be controlled adequately by the gas control system or if you wish for experimentation to be ceased.

***Ultrasound*:** Ultrasound is a non-invasive technique used for measuring blood flow velocity in this study. Though relatively harmless, prolonged insonation (exposure to ultrasound probe) can lead to redness of the skin or some minor bruising.

WHAT ARE THE POTENTIAL BENEFITS OF PARTICIPATING? You will not directly benefit from this study. However, you will gain information regarding your ventilatory responses to hypoxia, and pulmonary funtion. Your participation will lead to a better understanding of the mechanisms related to the control of breathing and physiological adaptations to intermittent hypoxia

WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?
You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, you have the right to request the withdrawal of

your information [and/or samples] collected during the study. This request will be respected to the extent possible. Please note however that there may be exceptions where the data [and/or samples] will not be able to be withdrawn for example where the data [and/or sample] is no longer identifiable (meaning it cannot be linked in any way back to your identity) or where the data has been merged with other data. If you would like to request the withdrawal of your data [and/or samples], please let your study doctor know. If your participation in this study includes enrolling in any optional studies, or long-term follow-up, you will be asked whether you wish to withdraw from these as well.

CAN I BE ASKED TO LEAVE THE STUDY? If you are not able to follow the requirements of the study or for any other reason, the study doctor may withdraw you from the study and will arrange for your care to continue. On receiving new information about the study, your research doctor might consider it to be in your best interests to withdraw you from the study without your consent if they consider that it would be better for your health. If you are asked to leave the study, the reasons for this will be explained to you and you will have the opportunity to ask questions about this decision.

HOW WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL? Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the investigator or his or her designate by representatives of the Natural Sciences and Engineering Research Council of Canada, and the UBCO Faculty of Health and Social Development, or the clinical research ethics board at the University of British Columbia for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number, SIN, or your initials, etc.). Only this number will be used on any research-related information collected about you during the course of this study, so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your rights to privacy are legally protected by federal and provincial law that require safeguards to ensure that your privacy is respected you also have the legal right to access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information.

WHAT HAPPENS IF SOMETHING GOES WRONG? By signing this form, you do not give up any of your legal rights and you do not release the Primary investigator, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided at no additional cost to you. The costs of your medical treatment will be paid by your provincial medical plan.

In the case of a serious medical event, please report to an emergency room and inform them that you are participating in a clinical study and that the following person can then be contacted for further information: Glen Foster at telephone number: (778) 214-9402.

A trained research assistant will be available on every occasion to explain the procedure and answer any questions. The individual is specifically trained in cardiovascular and respiratory physiology. The research team has undertaken these measures (previously without complications) in many individuals of your age. In the event of an emergency, staff will immediately dial 911 to activate emergency services. The co-investigators involved in this study, as well as university security staff, are trained in first aid and CPR and an automated external defibrillator is available on site.

WHAT WILL THE STUDY COST ME? All research-related care and treatment and any related tests that you will receive during your participation in this study will be provided at no cost to you.

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION? If you have any questions or desire further information about this study before or during participation, or if you experience any adverse effects, you can contact Glen Foster at (778) 214-9402

WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A PARTICIPANT? If you have any concerns or complaints about your rights as a research participant and/or your experiences while participaing in this study, contact the Research Participant Complaint Line in the University of British Columbia Office of Research Ethics by e-mail at RSIL@ors.ubc.ca or by phone at 604-822-8598 (toll free: 1-877-822-8598). Please reference the study number [H18-01740] when calling so the Complaint Line staff can better assist you.

OPEN ACCESS AND FUTURE USE OF RESEARCH DATA. Your de-identified research data may be published or deposited into a publicly accessible location at the time of publication. This enhances the transparency of the research, but also allows others to access the data. This should not increase risks to you, but it does mean that other researchers may analyze the data for different reasons other than those described in this consent form. Once data is made publicly available, you will not be able to withdraw your data. The extent of the risk of you being identified through public data is unknown, but currently appears to be low.

The effect of acute intermittent hypoxia exposure on neurovascular coupling and ventilatory long term facilitation

PARTICIPANT CONSENT

My signature on this consent form means:

- I have read and understand the information in this consent form.
- I have had enough time to think about the information provided.
- I have been able to ask for advice if needed.
- I have been able to ask questions and have had satisfactory responses to my questions.
- I understand that my participation in this study is voluntary.
- I understand that I am completely free at any time to refuse to participate or to withdraw from this study at any time, and that this will not change the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me.

I will receive a signed copy of this consent form for my own records.

I consent to participate in this study.

_____	_____	_____
Participant's Signature	Printed name	Date

_____	_____	_____	_____
Person obtaining consent	Printed name	Study Role	Date

If this consent process has been done in a language other than that on this written form, with the assistance of an interpreter/translator, indicate: Language: _____

Was the participant assisted during the consent process in one of the ways listed below?

☐ YES ☐NO [For typical situations where the person conducting the consent discussion simply reads the consent with the participant to ensure that informed consent is properly obtained, check "no"]

If yes, please check the relevant box and complete the signature space below:

☐ The consent form was read to the participant, and the person signing below attests that the study was accurately explained to, and apparently understood by, the participant (Please check if participant is unable to read).

☐ The person signing below acted as an interpreter/translator for the participant, during the consent process (please check if an interpreter/translator assisted during the consent process).

_____	_____	_____
Signature of Person Assisting	Printed name	Date