THE EFFECT OF A PATENT FORAMEN OVALE ON THE HYPERCAPNIC VENTILATORY RESPONSE

by

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A patent foramen ovale (PFO) is an intracardiac shunt pathway present in approximately one-third of the general, healthy population that allows varying degrees of blood flow to pass directly from the right to the left heart, bypassing pulmonary circulation and gas exchange at the lungs. Subjects with a PFO (PFO+) exhibit higher alveolar to arterial oxygen difference ($AaDO_2$) values at rest than subjects without a PFO (PFO–), indicating that PFO+ subjects have a source of right-to-left shunt significant enough to decrease gas exchange efficiency at rest (Lovering *et al.,* 2011). Additionally, PFO+ subjects have blunted ventilatory acclimatization to hypoxia compared to PFO– subjects (Elliott *et al.,* 2015). Therefore, the aim of this research was to determine if the presence of a PFO affects an individual's response to acute hypercapnic conditions, as quantified by calculating each individual's hypercapnic ventilatory response (HCVR). It was hypothesized that, when compared to PFO- subjects, PFO+ subjects would exhibit a blunted HCVR during exposure to normoxic

hypercapnia and hyperoxic hypercapnia when compared to PFO- subjects. Accordingly, 31 healthy, non-smoking subjects – 16 PFO+ (9 female) and 15 PFO- (8 female) – participated in this study. Subjects completed both a normoxic hypercapnia (NH) and hyperoxic hypercapnia (HH) breathing trial, in a randomized and balanced order, separated by ≥ 40 minutes. End-tidal oxygen and carbon dioxide ($P_{ET}O_2$ and $P_{ET}CO_2$) were controlled utilizing a dynamic end-tidal forcing system (AirForce). During the HH trial, $P_{ET}O_2$ was clamped at 250 mmHg and $P_{ET}CO_2$ was increased in a stepwise fashion to target values of +3 mmHg, +6 mmHg and +9 mmHg of each subject's baseline $P_{ET}CO_2$. Each stage consisted of a 90 second steady-state data collection period after each increase. The procedure for the NH was identical to the HH trial except that $P_{ET}O_2$ was clamped at the resting baseline value for each subject and $P_{ET}CO_2$ increased as above. Hypercapnic ventilatory response (HCVR) was calculated as slope of the ventilation (V_E) versus end-tidal carbon dioxide ($P_{ET}CO_2$) regression line for each subject (L/min/mmHg CO_2). PFO+ subjects demonstrated a blunted HCVR in both the NH and HH trials compared to PFO- subjects ($p < .05$). Overall, these results indicate that PFO+ subject have a blunted response to acute hypercapnic challenges in both normoxic and hyperoxic conditions suggesting a difference in central but not peripheral chemosensitivity to increased CO_2. Therefore, PFO+ subjects may be more susceptible to developing sleep apnea and subsequent comorbidities as a result of differences in central CO_2 chemosensitivity.

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Abbreviations and Terms

$AaDO_2$: alveolar-arterial oxygen gradient; difference between the alveolar and arterial concentrations of oxygen

BMR: basal metabolic rate; rate of energy expenditure at rest

Central chemoreceptors: areas of highly sensitive tissue located in the medulla (brainstem); primary sensors of $PaCO_2$

CNS: central nervous system

CSF: cerebrospinal fluid

DEF: dynamic end-tidal forcing system; system utilized in this experiment to control and estimate arterial blood gases in place of an arterial line

ECF: extracellular fluid

$FiCO_2$: fraction of inspired carbon dioxide

FiO_2: fraction of inspired oxygen

HH: hyperoxic hypercapnia

Hypercapnia: condition of increased blood carbon dioxide

HCVR: hypercapnic ventilatory response; $\frac{\Delta V_E}{\Delta P_{ET}CO_2}$ measures change in ventilation compared to change in arterial partial pressure of carbon dioxide to quantify an individual's response to hypercapnic conditions. Calculated as the slope of the V_E vs. $P_{ET}CO_2$ regression line for each subject in each trial in this experiment.

Hyperoxia: condition of increased blood oxygen

Hyperthermia: condition of increased body temperature

Hypoxia: condition of decreased blood oxygen

HVR: hypoxic ventilatory response; $\frac{\Delta V_E}{\Delta SaO_2}$ measures change in ventilation compared to change in arterial oxygen saturation to quantify an individual's response to hypoxic conditions

Interatrial septum: heart wall separating the right and left atria; location of a patent foramen ovale (PFO)

IV: intravenous

NH: normoxic hypercapnia

Normoxia: condition of baseline level of oxygen, as determined by each individual's resting steady state

NTS: nucleus tractus solitarius

OSA: obstructive sleep apnea

P_ACO_2: alveolar partial pressure of carbon dioxide

$PaCO_2$: arterial partial pressure of carbon dioxide

PaO_2: arterial partial pressure of oxygen

PFO: patent foramen ovale; intracardiac shunt pathway between right and left atria of heart

Peripheral chemoreceptors: areas of highly sensitive tissue located in the carotid bodies and aortic arch; primary sensors of PaO_2

$P_{ET}CO_2$: end-tidal (expired) partial pressure of carbon dioxide

$P_{ET}O_2$: end-tidal (expired) partial pressure of oxygen

PFT: pulmonary function testing; technique utilized to assess lung function in all subjects and ensure that no subjects exhibit signs of lung disease

RR: respiratory rate (breaths/min)

RTLS: right-to-left shunt

Saline-contrast echocardiography: technique utilized for PFO screening. Microbubbles are injected into a peripheral vein and viewed in the heart using ultrasound imaging

SaO_2: arterial oxygen saturation

SpO_2: predicted arterial saturation of oxygen

Valsalva maneuver: technique utilized to increase right atrial pressure above left atrial pressure, causing shunting through a PFO if present

V_E: ventilation (L/min)

Ventilatory acclimatization: increase in ventilation (V_E) that occurs in response to extended exposure to high altitude

V_T: tidal volume (L)

Introduction:

For the past two years, I have had the privilege of working as an undergraduate research assistant in Dr. Andrew Lovering's Cardiopulmonary and Respiratory Physiology Lab within the Human Physiology Department at the University of Oregon. One of the chief research focuses of this lab is to investigate the physiological implications of a patent foramen ovale (PFO), an intracardiac shunt pathway present in ~25-40% of the general, healthy population (Woods *et al.,* 2010; Marriott *et al.,* 2013; Elliott *et al.,* 2013). Individuals with a PFO (PFO+) have varying degrees of blood flow traveling directly from the right to left atrium, and therefore bypassing gas exchange in the lungs. There are several established effects of PFO, including increased gas exchange inefficiency at rest due to shunting through the interatrial opening (Lovering *et al.,* 2011). PFO+ subjects have also been demonstrated to have ~0.4° C higher core temperature when compared to those without a PFO (PFO-) (Davis *et al.,* 2015). In addition, research suggests a difference in ventilatory acclimatization between PFO+ and PFO- populations (Elliott *et al.,* 2015). Despite its prevalence, relatively limited research investigating the potential impacts of a PFO on normal physiology exists.

Ventilatory responses to hypercapnic and hypoxic ventilatory challenges have been studied in a variety of conditions and populations. Responses to hypercapnic environments can be quantified and compared by measuring the hypercapnic ventilatory response (HVCR). HCVR is calculated by comparing a change in ventilation to a change in arterial partial pressure of carbon dioxide ($PaCO_2$), which can be estimated using end-tidal CO_2 ($P_{ET}CO_2$). However, the influence of a PFO on these responses has yet to be elucidated. Therefore, the purpose of this study and thesis was to determine if

the presence of a PFO affects an individual's response to hypercapnic ventilatory challenges. This purpose was tested by measuring ventilatory responses to two hypercapnic conditions in male and female subjects with and without a PFO. It was hypothesized that, compared to PFO- subjects, PFO+ subjects would demonstrate a blunted HCVR to acute exposure to hyperoxic hypercapnia (HH) and normoxic hypercapnia (NH).

Background

Cardiopulmonary Physiology

The cardiopulmonary system functions primarily to transport oxygen (O_2) and additional nutrients to peripheral tissues while concomitantly removing carbon dioxide (CO_2) and other metabolic waste products. Oxygen poor, venous blood from the systemic circulation enters the right side of the heart before being pumped to the pulmonary circulation. Following gas exchange in the lungs, oxygenated blood travels to the left heart for distribution throughout the body (*Figure 1*). These are vital functions, as oxygen is necessary for cellular respiration and energy production, while carbon dioxide must be removed for pH buffering and maintenance of homeostatic conditions (Hall, 2011).

Patent Foramen Ovale

The foramen ovale is an opening in the interatrial septum of the fetal heart that allows blood to flow directly from the right to the left atrium. In utero, a human embryo receives oxygenated blood from its mother, as the developing lungs do not yet perform gas exchange. Therefore, the foramen ovale allows the majority of blood flow to bypass pulmonary circulation during gestation and flow directly into the left heart for distribution to the body (Rasanen, 1996).

While the foramen ovale is patent in all healthy humans during fetal development, this opening typically closes shortly after birth. When the lungs become

functional at the commencement of ventilation, pulmonary vascular resistance, and therefore pulmonary pressure, decrease causing left atrial pressure to exceed right atrial pressure. This pressure gradient alteration forces the septum primum, a flap of tissue in the left atrium, against the foramen ovale, resulting in functional closure. The tissue flap eventually fuses to the interatrial septum, permanently occluding the opening in the majority of humans. However, studies utilizing saline contrast echocardiography have shown that the hole fails to close completely in ~25-40% of the population (Woods et. al., 2010; Marriott et al., 2013; Elliott et al., 2013), and is termed a patent foramen ovale (PFO). In otherwise healthy subjects, this intracardiac shunt pathway can allow up to 5% of cardiac output to flow directly from the right heart to the left heart (*Figure 2)*, bypassing pulmonary circulation and therefore gas exchange (Devuyst, 2004).

Functions of the Lungs

While the lungs fulfill multiple roles in the human body, the primary function of the respiratory system is gas exchange at the level of the alveoli. Pulmonary alveoli, the saccular units of the lung, fill with air during respiration. Pulmonary capillaries deliver oxygen poor, carbon dioxide rich blood to the alveolus, where carbon dioxide diffuses out of the blood and into the alveolus for expiration. Oxygen concurrently diffuses from the alveolus into the capillary for transport back to the heart and eventual delivery to peripheral tissues (Hall, 2011).

Gas exchange efficiency can be quantified by determining the difference between the alveolar and arterial partial pressures of oxygen, known as the alveolar-arterial oxygen difference ($AaDO_2$). This value represents the ability of oxygen to

diffuse effectively from the alveoli to the blood. The smaller the $AaDO_2$ value and more minimal the difference in oxygen partial pressures, the greater the pulmonary gas exchange efficiency. However, conditions such as right-to-left shunt or ventilation-perfusion mismatch lead to increases in $AaDO_2$ values, denoting a greater degree of gas exchange inefficiency. Because blood traveling through a PFO bypasses pulmonary circulation, Lovering et al. sought to establish the effect of a PFO on pulmonary gas exchange efficiency in otherwise healthy humans. This research determined that PFO+ subjects exhibit a higher $AaDO_2$ at rest than PFO- subjects, indicating that PFO+ subjects have a higher gas exchange inefficiency in resting conditions, as would be predicted with an additional source of right-to-left shunt (Lovering *et al.*, 2011).

In addition to performing gas exchange, it has been suggested that the lungs facilitate some degree of heat dissipation through respiratory cooling. Cabanac and White established that when core temperature reaches a threshold of approximately 38.5°C in hyperthermic conditions, ventilation increases 2-3 fold. While the cause of this increase in ventilation observed in concert with increased core temperature is not yet fully understood, it is estimated that respiratory cooling as a secondary function of the lungs accounts for a 10% of heat loss. Therefore, ventilation may increase with increased core temperature in order to augment heat dissipation occurring at the lungs (Cabanac and White, 1995).

Chemical Control of Breathing

Respiratory rate (RR) is principally determined by oxygen, carbon dioxide and pH quantities in the blood. Arterial partial pressure of CO_2 ($PaCO_2$) is primarily sensed

by central chemoreceptors located in the ventrolateral portion of the medulla within the central nervous system (CNS). The central medullary chemoreceptors are composed of neural tissue that is highly sensitive to changes in the hydrogen ion (H^+) concentration, and therefore pH, of the brain extracellular fluid (ECF). Though CO_2 predominantly travels through the bloodstream as bicarbonate (HCO_3^-), the blood-brain barrier is relatively impermeable to bicarbonate as well as hydrogen ions, preventing chemoreceptors from sampling the CO_2 levels of the blood directly. However, carbon dioxide itself easily traverses the blood brain barrier, after which carbonic anhydrase catalyzes the conversion of CO_2 and water to carbonic acid (H_2CO_3), which then dissociates into bicarbonate and hydrogen ions within the ECF and cerebral spinal fluid (CSF). Central chemoreceptors sense hydrogen ion concentration and therefore indirectly monitor $PaCO_2$ through changes in ECF and CSF pH. The respiratory pattern generator within the medulla then integrates signals from central chemoreceptors and modulates a response based on the firing rate of the chemoreceptors. The signal is relayed to the diaphragm and accessory respiratory muscles to stimulate a change in the rate of contraction, which causes an increase in ventilation (West *et al.*, 2013).

While carbon dioxide is primarily sensed by central chemoreceptors, peripheral chemoreceptors located in the carotid and aortic bodies primarily maintain blood oxygen homeostasis. Peripheral chemoreceptors sense the arterial partial pressure of oxygen (PaO_2) and respond to conditions of low oxygen (hypoxia). Carotid bodies are composed of glomus cells, which sense oxygen and release transmitters in response to hypoxia (Prabhakar *et al.*, 2004). These chemoreceptors signal to the nucleus tractus solitarius (NTS), a specialized area of the medulla, which relays information to the

respiratory pattern generator, eventually leading to contraction of the muscles necessary for inspiration (Smith *et al.*, 2010). When PaO_2 decreases, peripheral chemoreceptor firing rate increases, resulting in increased ventilation in order to return oxygen to normal range levels (West *et al.*, 2013).

The central and peripheral chemoreceptors have traditionally been thought to operate independently. However, recent research suggests that the responses of these chemoreceptors are integrated and interdependent. Studies in canine models suggest a hyperadditive effect of chemoreception on ventilation, whereby the response of central chemoreceptors to increased $PaCO_2$ is influenced by and dependent upon a concomitant response by the peripheral chemoreceptors (Blain *et al.*, 2010). In the interdependent model, it is speculated that peripheral chemoreceptor outputs have the potential to affect central chemoreceptor outputs via signal transduction through the NTS. This model challenges traditional understanding of chemoreceptor influences on ventilation, whereby the central chemoreceptors and peripheral chemoreceptors signal to the central respiratory pattern generator independently (Smith *et al.*, 2010).

While both hypoxic and hypercapnic conditions provide powerful chemical drives to breathe, basal metabolic rate (BMR), and therefore rate of production of carbon dioxide, drives ventilation at sea level. At low elevation, the partial pressure of oxygen is high such that the need to maintain oxygen saturation of the blood only minimally drives ventilation, if at all. Carbon dioxide is produced as a byproduct of cellular metabolism and must be exhaled for elimination from the body. Therefore, the build-up of carbon dioxide from basal, or resting, metabolism determines respiratory rate in non-altitude conditions (Nattie *et al.*, 2005).

Hypercapnic and Hypoxic Ventilatory Responses

The hypercapnic ventilatory response (HCVR) measures the change in ventilation over the change in arterial partial pressure of carbon dioxide ($PaCO_2$) and provides a method to quantify an individual's response to hypercapnic, or high carbon dioxide, conditions. A greater HCVR denotes a larger response and chemosensitivity to a given change in arterial CO_2. Similarly, the hypoxic ventilatory response (HVR) compares change in ventilation to change in arterial oxygen saturation (SaO_2) and measures the response to hypoxic, or low oxygen, conditions. A larger HVR signifies a greater change in ventilation in response to hypoxia, and HVR has been shown to increase with ventilatory acclimatization to altitude (West *et al*., 2013).

Obstructive Sleep Apnea and PFO

Obstructive sleep apnea (OSA) is a sleep disorder characterized by partial or complete occlusions of the upper airway that result in repetitive hypopneas (reduced airflow) or apneas (cessation of airflow) and a periodic breathing pattern during sleep. The current prevalence of OSA in the United States is estimated to be 9% among 50 to 70-year-old women and 17% among 50 to 70-year-old men, with increases in prevalence as high as 55% in the past two decades (Peppard *et al.*, 2013). Sleep-disordered breathing, which leads to sleep fragmentation and nocturnal hypoxemia, can increase risk of severe health detriments, including systemic hypertension, coronary artery disease, insulin insensitivity, and stroke (Dempsey *et al.*, 2012). Thus, a large portion of the United States adult population is at risk of developing serious and life-

threatening health complications at some point in life secondary to obstructive sleep apnea.

There is a greater prevalence of PFO among patients with severe obstructive sleep apnea (43-72%) than in the general healthy population (Shanoudy *et al.*, 1998; Guchlerner *et al.*, 2012; Shaikh *et al.*, 2013). A recent study concluded that the presence of a PFO exacerbates the severity of obstructive sleep apnea and that PFO closure in patients with severe OSA can decrease apnea-hypopnea index (AHI) values, improve oxygen desaturation index (ODI), and decrease both nocturnal and daytime systolic blood pressure. Upper airway closure and subsequent breathing effort against the closed airway is believed to increase right atrial pressure above left atrial pressure, similar to the performance of a Valsalva maneuver, which increases flow across a PFO, worsening hypoxemia (Rimoldi *et al*., 2015). However, the exact physiological correlations between OSA and PFO have yet to be fully elucidated. One reason may be that PFO+ subjects may be prone to a relatively greater hypoventilation during sleep, which may subsequently predispose these subjects to central sleep apnea (Dempsey, 2010).

The Effect of Biological Sex on Ventilatory Responses

Sex hormones, namely testosterone, estrogen and progesterone, have significant effects on the central neural control of breathing (Behan *et al*., 2003). Progesterone, a steroidal sex hormone that is normally high during the luteal phase of the menstrual cycle, is a known ventilatory stimulant (White *et al*., 1983). Additional research has shown that sex hormones influence resting minute ventilation (V_E). In a recent comprehensive study, MacNutt *et al*. determined that there is a clear effect of menstrual

cycle phase on resting V_E. Body surface area (BSA) corrected V_E was demonstrated to be higher during the mid-luteal (ML) phase of the menstrual cycle, when estrogen and progesterone levels are high, compared to the early follicular (EF) and late follicular (LF) phases (MacNutt *et al*., 2012). However, the effects of biological sex and sex hormones on ventilatory responses to hypoxia and hypercapnia have been historically equivocal. Previous research has demonstrated hypoxic chemosensitivity in males to be greater (White *et al*., 1983), lesser (Aitken *et al.*, 1986), as well as no different (Jensen *et al.*, 2004) when compared to females in resting conditions. MacNutt *et al*. attribute the disparities among results to inconsistencies and inaccuracies in assessing menstrual cycle phase, as well as variability in methods used to measure hypoxic chemosensitivity. After correcting for these potential sources of discrepancy, it was ultimately determined that ventilatory responses to hypoxia and hypercapnia are unaffected by biological sex or menstrual cycle phase (MacNutt *et al.*, 2012). However, previous research into the effects of biological sex and sex hormones on ventilation has not taken into account the possible influence of the presence of a PFO on ventilatory responses.

The Effect of a Patent Foramen Ovale on Ventilatory Responses

While one may expect that PFO+ individuals, who have some amount of cardiac output bypassing gas exchange at the lungs, would exhibit augmented ventilatory responses to hypoxic and thermoregulatory challenges, recent research by Elliott *et al.* and Davis *et al.* suggests otherwise.

In 2012 the Cardiopulmonary and Respiratory Physiology Lab traveled to Mt. Chacaltaya, Bolivia to study the effect of PFO on ventilatory acclimatization. Twenty-one healthy, sea level residents (11 PFO+) were utilized for this study. Resting and exercise measurements were taken at sea level, following acute transport to 5260m (~17,000ft), and after 16 days at elevation. There were no significant differences in HVR after one day at altitude between PFO+ and PFO- groups. However, following 16 days at elevation, PFO- subjects demonstrated a significant increase in breathing when compared to day one measures. In addition, PFO- subjects had a significantly larger increase in breathing with acclimatization than PFO+ subjects at the day 16 time point. Conversely, PFO+ subjects exhibited no significant change in breathing from day 1 to day 16 at altitude, suggesting that individuals with a PFO have blunted ventilatory acclimatization to high altitude (Elliott *et al.*, 2015).

Similarly, unpublished data from a recent study conducted by Davis *et al.* shows that subjects with a PFO demonstrate a blunted ventilatory response to passive heating. For this experiment, PFO+ and PFO- subjects were immersed in a 40°C hot tub for 30 minutes while ventilation, among other factors, was measured. Although PFO+ subjects consistently maintain a ~0.4°C higher core temperature than PFO- subjects, PFO- subjects in this study showed a significantly greater increased ventilation in response to increased core temperature, compared to the PFO+ group.

While previous studies have explored the effect of a PFO on hypoxic and thermoregulatory challenges, the influence of a PFO on the hypercapnic ventilatory response (HCVR) remains unknown. Because PFO+ individuals have some degree of blood flow bypassing gas exchange at the lungs and therefore exhibit greater gas

exchange inefficiency and higher $PaCO_2$, it could be expected that PFO+ subjects would demonstrate a greater increase in ventilation and larger HCVR than PFO- subjects. However, Elliott *et al.* and Davis *et al*. have recently established that PFO+ subjects show a blunted ventilatory response to chronic hypoxia exposure, as well as acute exposure to heat. In combination, these results led to the purpose and hypothesis for this thesis.

Methods

This study was approved by the University of Oregon's Office for Protection of Human Subjects. Each subject was provided documentation outlining the experiment and gave written consent prior to participation. Experimental procedures were conducted in accordance with the *Declaration of Helsinki*.

Subjects

A total of 31 subjects (17 female) volunteered, and were included in this study. The experiment was explained in detail to all subjects both orally and in writing, and each individual provided written consent to participate. All subjects were non-smoking individuals with no history of cardiopulmonary disease. Of the 14 male subjects, there were 7 PFO+ subjects and 7 PFO- subjects and of the 17 female subjects, 9 were PFO+ and 8 were PFO-. There were no significant differences between PFO+ and PFO- groups in age, height, weight, or body surface area (*Table 1*).

PFO Detection: Saline-Contrast Echocardiography

The presence of a PFO was determined using saline-contrast echocardiography (Lovering and Goodman, 2012). The screening was performed with the subject breathing room air, seated, and positioned on their left side in an IV chair reclined at a 45° angle. The subject was instrumented with an intravenous catheter (20-22G) attached to a three-way stopcock and extension system in the antecubital fossa. Two syringes, one containing 3 ml of sterile saline and the other containing 1 ml of room air, were

attached to the stopcock. The contents of the two syringes were combined and agitated for 10-15 seconds and the resulting microbubbles were injected into a peripheral vein via the IV catheter. Ultrasound imaging (Philips ie33) was utilized to view all four chambers of the heart in an apical view (echocardiogram). Microbubbles were injected and viewed during normal tidal breathing, as well as immediately following the release of a Valsalva maneuver. The Valsalva maneuver is a technique utilized to increase right atrial pressure above left atrial pressure and allow right-to-left heart blood flow if a PFO is present. The Valsalva maneuver was determined effective if a leftward shift of the interatrial septum was observed.

Following each saline-contrast injection, the subsequent 20 cardiac cycles were recorded to determine the presence of a PFO. Within 3-5 cardiac cycles of injection, a cloud of microbubbles appeared within the right heart chambers. The presence of any microbubbles in the left heart within the next 20 heartbeats constituted evidence for the existence of either a PFO or intrapulmonary shunt. The subject was determined to have a PFO (PFO+) if contrast appeared within the left atrium or ventricle in ≤ 3 cardiac cycles (Marriott *et al.*, 2013; Fenster *et al.*, 2013). It has been previously established that the number of microbubbles viewed in the left heart is proportional to the amount of blood flow between the right and left atria (Fenster *et al.*, 2013). Therefore, microbubbles that appeared in the left heart were counted in order to classify the size of the PFO. After the release of a Valsalva maneuver, subjects that showed ≤ 12 microbubbles in the left heart within 3 cardiac cycles were classified as having a small PFO (PFO+), while subjects with ≥ 13 bubbles visualized in the left heart were determined to have a large PFO (PFO+), as before (Davis *et al.*, 2015, Norris *et al.*,

2015). If no microbubbles appeared in the left heart within 20 cardiac cycles of saline-contrast injection, the absence of both a PFO (PFO-), as well as blood flow through intrapulmonary shunts, was confirmed.

Pulmonary Function Testing

Pulmonary function testing (PFT) was performed to ensure that all subjects exhibited normal pulmonary function and did not demonstrate signs of lung disease. Baseline PFT measures were obtained using a computerized spirometry system (Ultima PFX, Medgraphics, St Paul, MN, USA) according to standards established by the American Thoracic Society/European Respiratory Society (ATS/ERS) (Macintyre *et al.*, 2005). Measures obtained for this experiment included forced vital capacity (FVC), volume of air forcefully expired in one second (FEV_1), mid-expiratory flow rate (FEV_{25-75}) and slow vital capacity (SVC). Whole-body plethysmography was utilized to determine lung volume measurements, including total lung capacity (TLC), residual volume (RV) and functional residual capacity (FRC) (Wanger *et al.*, 2005). Lung diffusion capacity for carbon monoxide (DLCO) was measured using a MedGraphics Elite Series Plethysmograph (MedGraphics Ultima PFX, Breeze v.6.3.006) and the single breath, breath-hold technique (Knudson *et al.*, 1987; Macintyre *et al.*, 2005). All subjects included in this study demonstrated FVC, FEV_1, and FVC/FEV_1 values $\geq$ 85% of predicted values determined by height and weight, indicating normal lung function.

End-Tidal Forcing System

A dynamic end-tidal forcing system (DEF) designed by Foster *et al.* (Querido *et al.*, 2013; Bain *et al.*, 2013; Foster *et al.*, 2014) was utilized to control and estimate arterial blood gases. The DEF method relies on real-time feedback information to regulate and maintain the end-tidal partial pressure of O_2 ($P_{ET}O_2$) and the end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) by analyzing expired air and adjusting fractions of inspired O_2 (FiO_2) and CO_2 ($FiCO_2$) on a breath-by-breath basis (Tymko *et al.*, 2015). Specific to this experiment, the end-tidal forcing system was employed to measure and quantify responses of the central and peripheral chemoreceptors to normoxic and hyperoxic hypercapnia conditions.

Throughout the study, all respiratory parameters were measured at 200 Hz using a personal computer and the DEF system interfaced with an analog-to-digital converter (Powerlab/16SP ML 880; ADInstruments, Colorado Springs, CO). Respiratory and cardiovascular data were analyzed using commercially available software (LabChart V7.1, ADInstruments, Colorado Springs, CO, USA). All trials required that subjects were instrumented with a nose clip and breathed through a mouthpiece with a biological filter and two-way nonrebreathing valve (7900 series, Hans Rudolph, Shawnee, KS). Prior to each trial, a pneumotachograph (HR 800L, Hans Rudolph) and differential pressure amplifier (ML 141, ADInstruments), utilized to measure respiratory flow, were calibrated with a 3-liter syringe.

End-tidal partial pressures of O_2 and CO_2 were maintained using the portable DEF system. Each trial required that varying proportions of N_2, CO_2 and O_2 were delivered to the subject for inhalation in order to regulate $P_{ET}O_2$ and $P_{ET}CO_2$ as

previously described (Foster *et al.,* 2014). The DEF system utilizes independent solenoid valves to deliver N_2, CO_2 and O_2, which are then combined and sent through a humidification chamber before delivery to the subject through the pneumotach's inspiratory port. $P_{ET}CO_2$, $P_{ET}O_2$, tidal volume (V_T), breathing frequency (F) and minute ventilation (V_E) are determined for each exhalation using specifically designed software (Labview 13.0, National Instruments, Austin, TX). The DEF system then utilizes this feedback information to determine the composition of inspired air needed to adjust $P_{ET}O_2$ and $P_{ET}CO_2$ to target values.

Study Protocol

Following PFO screening and pulmonary function testing, subjects returned to the Cardiopulmonary and Respiratory Physiology Lab in the Center for Medical Education and Research for one day to complete the study day protocol. After subject arrival, researchers obtained height and weight using a stadiometer and electronic scale (Ohaus Corporation, ES200L, Pinebrook, NJ, USA), respectively. In addition, female subjects were tested to rule out pregnancy as an exclusion criteria; no females were found to be pregnant. Each subject was then seated in an upright IV chair and instrumented with necessary devices for a wide range of data collection. A finger pulse oximeter (ADInstruments, Colorado Springs, CO, USA) was utilized to monitor oxygen saturation. The subject was instrumented with a 3-lead ECG on the right and left collarbone and right hip areas for continuous heart rate acquisition. In addition, the subject was outfitted with a standard blood pressure cuff for instantaneous blood pressure measures.

Each subject underwent two hypercapnic breathing trials in a randomized and balanced order. For each trial, the subject began breathing room air on a two-way nonrebreathing mouthpiece (model 2400, Hans Rudolph, Kansas City, MOS) and pneumotachograph (HR 800L, Hans Rudolph) with nose clip. The subject continued to breathe room air until $P_{ET}O_2$ and $P_{ET}CO_2$ values stabilized, at which "steady state" was achieved. Two breathing trials were then implemented with a 40-minute resting period between trials.

- *Hyperoxic Hypercapnia*: Subjects began breathing room air on the mouthpiece to establish baseline steady state values for $P_{ET}O_2$ and $P_{ET}CO_2$. Once baseline was established, end-tidal O_2 was clamped at 250 mmHg (hyperoxia) while resting $P_{ET}CO_2$ was maintained. Steady state was achieved when end-tidal values remained within 1 mmHg of desired values for three consecutive breaths and subjects then remained at steady state for five minutes. The fraction of inspired carbon dioxide ($FiCO_2$) was then adjusted to increase $P_{ET}CO_2$ to the baseline resting measure +3 mmHg. After reaching steady state, subjects remained at this stage for 90 seconds before moving to the next stage. This procedure was then repeated for $P_{ET}CO_2$ values of +6 and +9 mmHg above the established resting baseline value.
- *Normoxic Hypercapnia*: Subjects began breathing room air on the mouthpiece to establish baseline steady state values for $P_{ET}O_2$ and $P_{ET}CO_2$. Once baseline was established, end-tidal O_2 and CO_2 were clamped at resting values. Steady state was achieved when end-tidal values remained

within 1 mmHg of desired values for three consecutive breaths and subjects then remained at steady state for five minutes. $FiCO_2$ was then adjusted to increase $P_{ET}CO_2$ to the baseline resting measure +3 mmHg. After reaching steady state, subjects remained at this stage for 90 seconds before moving to the next stage. This procedure was then repeated for $P_{ET}CO_2$ values of +6 and +9 mmHg above resting value.

Statistical Analysis

GraphPad Prism software (v 7.0b) was used for data analysis. Anthropometric data was analyzed using unpaired t-tests. Overall and group descriptive statistics (mean and standard deviation) were calculated for all test variables. To determine differences in ventilatory responses between groups, HCVR was calculated as the slope of the V_E versus $P_{ET}CO_2$ regression line for each subject in each trial. Group HCVR values were calculated as the average of the slopes of each individual subject. Group averages were compared and analyzed using unpaired t-tests with an α-level of .05. All other cardiovascular variables were compared using a two-way repeated measures ANOVA. One PFO+ subject was excluded from the hyperoxic hypercapnia trial after data analysis determined that the subject's HCVR was greater than two standard deviations above the group mean, fulfilling qualifications for a statistical outlier.

Results

Anthropometrics and Lung Function

There were no significant differences between groups in anthropometric, pulmonary function, or DLCO measures ($p > .05$) (*Table 1*).

Cardiorespiratory Measures

There was no effect of PFO on V_E, V_T, RR, $P_{ET}O_2$ or $P_{ET}CO_2$ during the normoxic hypercapnia (NH) or hyperoxic hypercapnia (HH) trials ($p > .05$) (*Table 2*).

Hypercapnic Ventilatory Response

All subjects demonstrated a hypercapnic ventilatory response and significantly increased ventilation in response to acute normoxic (*Figure 3A*) and hyperoxic (*Figure 3B*) hypercapnia, as expected. There was a main effect of PFO on HCVR during acute exposure to normoxic hypercapnia and hyperoxic hypercapnia. During the NH trial PFO+ subjects exhibited a significantly lower HCVR when compared to PFO- subjects ($p < .05$) (*Figure 4A*). Additionally, PFO+ subjects demonstrated a blunted HCVR in the HH trial compared to PFO- subjects ($p > .05$) (*Figure 5A*).

Effect of Biological Sex

There was no effect of biological sex on HCVR in the HH and NH trials. When examining individual sexes, there were no significant differences between PFO+ subjects and same sex PFO- subjects, likely driven by relatively low subject numbers. In the NH trial, the female PFO+ subjects trended toward a blunted HCVR when compared to female PFO- subjects (*Figure 4B, p* = .06). In addition, there were no significant differences between the male PFO+ and PFO- subjects, though the data trended in the direction of differences seen in all subjects (*Figure 4C, p* = .17). Similarly, in the HH trial female PFO+ subjects trended toward a blunted HCVR when compared to female PFO- subjects (*Figure 5B, p* = .06). Similar to the NH trial, there were no significant differences between the male PFO+ and PFO- subjects in the HH trial, though the data trended in the direction of differences seen in all subjects (*Figure 5C, p* = .15). Furthermore, there was no effect of biological sex on HCVR within the PFO+ and PFO- groups ($p > .05$, data not shown).

Discussion

The purpose of this study was to determine the effect of a patent foramen ovale (PFO) on the hypercapnic ventilatory response (HCVR). It was hypothesized that subjects with a PFO (PFO+) would exhibit a blunted HCVR in response to acute hyperoxic hypercapnia (HH) as well as acute normoxic hypercapnia (NH) when compared to subjects without a PFO (PFO-).

The Hypercapnic Ventilatory Response

The human hypercapnic ventilatory response (HCVR) is a well-established and extensively researched phenomenon. Unsurprisingly, all subjects included in this study demonstrated an increase in resting minute ventilation (V_E) in response to normoxic hypercapnic (*Figure 3A*) and hyperoxic hypercapnic (*Figure 3B*) experimental conditions. The results of this experiment support previous findings, which demonstrate changes in ventilation in response to corresponding changes in $PaCO_2$ in both normoxic and hyperoxic hypercapnic conditions in healthy, non-smoking subjects (Duffin, 2007). Thus, we were able to successfully increase $P_{ET}CO_2$ in a step-wise fashion and elicit an increase in V_E, as expected. Although all subjects when grouped together exhibited an anticipated HCVR, separating subjects based on the presence or absence of a PFO revealed differing responses between groups.

Effect of a PFO on the Hypercapnic Ventilatory Response

Individuals with a PFO have previously demonstrated blunted ventilatory responses to chronic hypoxia (Elliott *et al.*, 2015) as well as acute thermoregulatory challenges (Davis *et al.*, in review). Similarly, the results of this experiment indicate a difference in ventilatory responses between PFO+ and PFO- subjects. PFO+ subjects demonstrated a blunted, significantly lower HCVR to normoxic hypercapnic (*Figure 4A*) as well as hyperoxic hypercapnic (*Figure 5A*) conditions compared to PFO- subjects. These data suggest that the presence of a PFO is correlated with decreased chemoreceptor sensitivity to increased CO_2. PFO+ subjects exhibited a diminished ventilatory response to increased $P_{ET}CO_2$ compared to PFO- subjects in the NH and HH trials, despite the fact that $P_{ET}CO_2$ values were similar between groups (*Table 3*). As speculated by Elliott *et al*. regarding differences in HVR between PFO+ and PFO- subjects after 16 days at altitude, it is possible that the blunted HCVR in PFO+ subjects may hint at an appropriate, advantageous response in individuals with right-to-left intracardiac shunting (2015). Increasing ventilation in response to hypoxic or hypercapnic challenges increases metabolic demand due to increased recruitment, and therefore energy demand and waste production, of muscles required for respiration. Right-to-left shunting in PFO+ subjects impairs their ability to increase PaO_2 and decrease $PaCO_2$. Therefore, it is possible that the increased waste production and metabolic demand associated with increasing ventilation would negate the benefits of increasing ventilation to decrease $PaCO_2$ in PFO+ subjects, leading to decreased HCVR compared to PFO- subjects.

The results of both the NH and HH trials, when evaluated together, suggest that there is a central chemoreceptor component to the differences in ventilatory responses between PFO+ and PFO- subjects to acute hypercapnia, but a negligible peripheral chemoreceptor component. PFO+ subjects had a ~0.5 L/min/mmHg CO_2 lower average HCVR in both the NH and HH trial compared to PFO- subjects, indicating that dampening the output of the peripheral chemoreceptors in the HH trial had no impact on the difference between PFO+ and PFO- HCVR and that the difference in responses between groups are likely centrally driven (*Figure 6*). These results support the findings of an accompanying study conducted by the Cardiopulmonary and Respiratory Physiology Lab, which demonstrates no differences in ventilation between PFO+ and PFO- subjects in response to acute exposure to isocapnic and poikilocapnic hypoxia (unpublished data). In addition, these data are supported by previous research that indicates that there are no significant differences between PFO+ and PFO- subjects in the hypoxic ventilatory response (HVR) after one day of exposure to hypoxia at high altitude (Elliott *et al.*, 2015). The results of all three studies, taken in combination, suggest that there is no effect of PFO on peripheral chemoreceptor sensitivity, and therefore no differences in ventilatory responses as a result of peripheral chemoreceptor activity, during acute exposure to hypoxia or hypercapnia.

Effect of Biological Sex and PFO on the Hypercapnic Ventilatory Response

Female PFO+ subjects trended toward a lower HCVR than female PFO- subjects in both the HH (*Figure 4B*) and NH (*Figure 5B*) trials. Similarly, male PFO+ subjects trended toward a lower HCVR than male PFO- subjects in both the HH (*Figure*

4C) and NH (*Figure 5C*) trials. Thus, there was no influence of biological sex on the effect of PFO on HCVR. These results denote that without controlling for menstrual cycle phase, PFO+ subjects exhibit a lower HCVR than PFO- subjects, suggesting that the differences observed between groups were driven by PFO, rather than biological sex. In addition, there was no effect of biological sex on HCVR within the PFO- and PFO+ groups ($p > .05$, data not shown). The results of the present study are consistent with and confirm the findings of MacNutt *et al.,* validating that even without controlling for the presence of a PFO, there was no effect of biological sex on HCVR (2012).

Clinical Relevance and Significance

Ventilatory control and chemoresponsiveness have previously been identified as important factors in obstructive sleep apnea (OSA) pathogenesis, as hypoventilation and obstructive events occur during stages of low respiratory drive (Dempsey *et al.,* 2005; Eckert *et al.,* 2008). Therefore, a relatively greater hypoventilation during sleep in individuals with PFO may represent an important potential contributing factor to OSA susceptibility and severity. As described before, multiple studies have established an increased prevalence of PFO among individuals with OSA (Shanoudy *et al.,* 1998; Guchlerner *et al.,* 2012; Shaikh *et al.,* 2013). However, the physiological mechanisms underlying this correlation were previously undetermined. The results of this study represent novel findings that may provide meaningful insight into the connection between OSA and PFO and contribute to clinical understanding of this life-threatening sleep pathology.

The present study determined that PFO+ subjects have a significantly lower HCVR than PFO- subjects. Thus, PFO+ subjects are prone to hypoventilate at increased $PaCO_2$ levels. A comprehensive review into the pathophysiology of sleep apnea determined that both hyperventilation and hypoventilation in conditions of fixed, resting CO_2 production destabilize the respiratory control system and increase susceptibility for apnea. By this model, hypoventilation, which results in higher resting P_ACO_2, places an individual at a greater risk of apnea, as a smaller increase in ventilation is required to overshoot the apneic threshold and result in a cessation of breathing (*Figure 2A*, Dempsey, 2005). A decreased HCVR, and therefore slope of the ΔV_E - ΔP_ACO_2 relationship, appears to decrease susceptibility to apnea (*Figure 2B*, Dempsey, 2005). However, overlaying these two figures and combining decreased HCVR with hypoventilation, which may be representative of some PFO+ individuals, leads to a similar increase in susceptibility of crossing the apneic threshold and developing a periodic breathing pattern during sleep.

As previously described, the decreased HCVR observed in PFO+ subjects in this study signifies decreased chemoreceptor sensitivity to increased $PaCO_2$. An important aspect of obstructive sleep apnea pathophysiology is a loss of muscle tone to the genioglossus, or tongue, muscle that occurs during NREM and REM sleep, resulting in partial or full blockage of the upper airway. Horner *et al.* utilized a rodent model to demonstrate decreased chemoreceptor stimulation to the genioglossus muscle despite significant increases CO_2 during NREM and REM sleep compared to a wakeful state. This study concluded that chemoreceptor stimulation to the genioglossus muscle is blunted during sleep and greater increases in $PaCO_2$ are required to elicit a response

from the genioglossus muscle during NREM sleep compared to wakefulness. Further, no degree of increased $PaCO_2$ was sufficient to increase genioglossus tone during REM sleep. Decreased genioglossus muscle tone during sleep is an important contributing factor to the loss of upper airway patency observed in OSA. Therefore, the decreased chemoresponsiveness to increased $PaCO_2$ observed in PFO+ subjects in the present study may indicate that these subjects also have decreased chemoreceptor stimulation to the genioglossus muscle, increasing the probability of obstruction and upper airway closure. However, this remains speculative.

In addition to potentially increasing the probability of obstruction, the presence of a PFO may influence the severity of the effects of obstruction when it does occur. A study conducted by Johansson *et al*. determined that oxygen desaturation occurs more often in proportion to the number of blockages in PFO+ subjects than in subjects without a PFO. The study concluded that there was a significantly higher prevalence (60%) of large PFO among subjects with a high proportional desaturation (PD) value, as measured by the oxygen desaturation index (ODI)/ apnea-hypopnea index (AHI) ratio, than among subjects with a low PD value (13%). Thus, subjects with a large PFO experienced a greater degree of hypoxemia per apnea (Johansson *et al.*, 2007). In addition, Beelke *et al.* found that right-to-left shunting occurred in 9 out of 10 PFO+ subjects during an obstructive apnea lasting longer than 17 seconds (2003). Taken in combination with the results of the present study, these conclusions may provide insight into the decreased severity of sleep apnea observed after PFO closure (Rimoldi *et al.*, 2015). The present study determined that PFO+ subjects have a decreased HCVR compared to PFO- subjects. Therefore, it is possible that a greater increase in $PaCO_2$ as

a result of apnea is necessary to elicit a response significant enough to arouse an individual with a PFO, potentially leading to longer apneas in PFO+ subjects. If this were true, the conclusions of Beelke *et al.* would suggest that longer apneas in PFO+ subjects would also lead to right-to-left shunting (RTLS), increasing the degree of hypoxemia as a result of the apnea. This proposed pathway aligns with the findings of Johansson *et al.;* longer apneas as a result of decreased HCVR, leading to increased RTLS and a greater degree of hypoxemia, could explain the greater proportional oxygen desaturation observed in subjects with large PFO. Thus, PFO and a corresponding decreased HCVR may lead to a greater severity of the effects of obstruction, worsening OSA severity. However, additional research is required to confirm the proposed mechanisms outlined whereby a PFO may contribute to central and obstructive sleep apnea (*Figure 7*).

Conclusion

While previous research has determined several effects of a PFO on normal physiology, it was undetermined if the presence of a PFO influences ventilatory responses to acute hypercapnic challenges. The high prevalence of PFO in the general, healthy population (25-40%) and even higher prevalence in some disease populations (43-72%) validates the importance of research into how this right-to-left shunt pathway may affect various physiological processes. The results of this study indicate that PFO+ individuals have a blunted ventilatory response to both normoxic and hyperoxic hypercapnia compared to PFO- individuals, suggesting that the presence of a PFO influences central chemosensitivity to increased $P_{ET}CO_2$. These findings may provide insight into the increased prevalence of PFO in sleep apnea patients and contribute to understanding of the pathogenesis of both central and obstructive sleep apnea.

Limitations

This experiment did not measure and control for menstrual cycle phase of female subjects. Consequently, it was assumed that the sample of females included in the study represents a range of sex hormone levels and varied stages of the menstrual cycle. Thus, although unlikely, it is possible that a disproportionate number of subjects participated in the experiment during any specific phase of the menstrual cycle, potentially skewing data and contributing to, or retracting from, the results observed. However, previous research demonstrates no effect of menstrual cycle phase on HCVR or HVR (MacNutt *et al.*, 2012). Therefore, potential effects of menstrual cycle on the observed results are improbable and likely negligible.

In addition, this study utilized end-tidal gases using a dynamic end-tidal forcing system (DEF) as an estimation of arterial blood gases. DEF induced hypercapnia is known to increase $P_{ET}CO_2$ relative to $PaCO_2$ as a proportion of inspired CO_2 occupies dead space and mixes with expired air, leading to inflated $P_{ET}CO_2$ values. However, the end-tidal-to-arterial PCO_2 gradient is typically insignificant at rest (Tymko *et al.*, 2016). All breathing trials were performed in resting conditions, indicating that end-tidal gases likely served as an accurate estimation of arterial blood gases and only minimally contributed to experimental error.

Figures

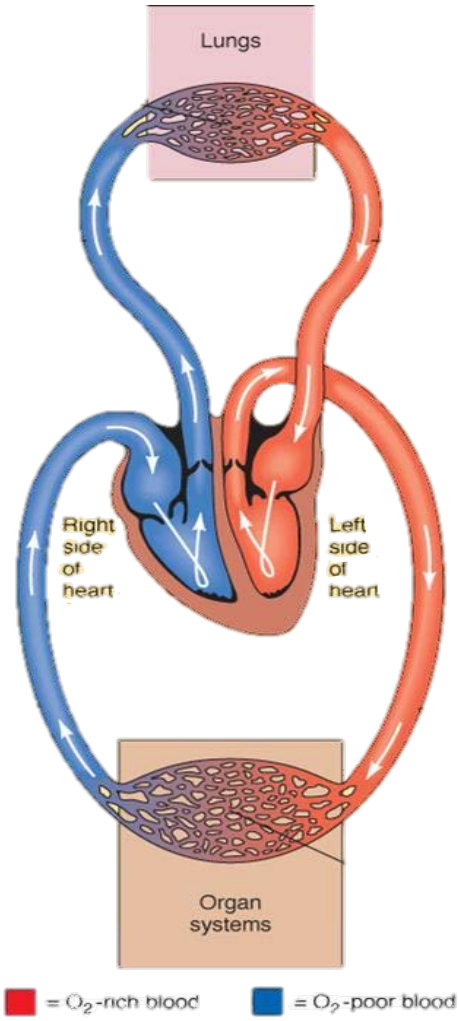

Figure 1: Cardiopulmonary circulation.

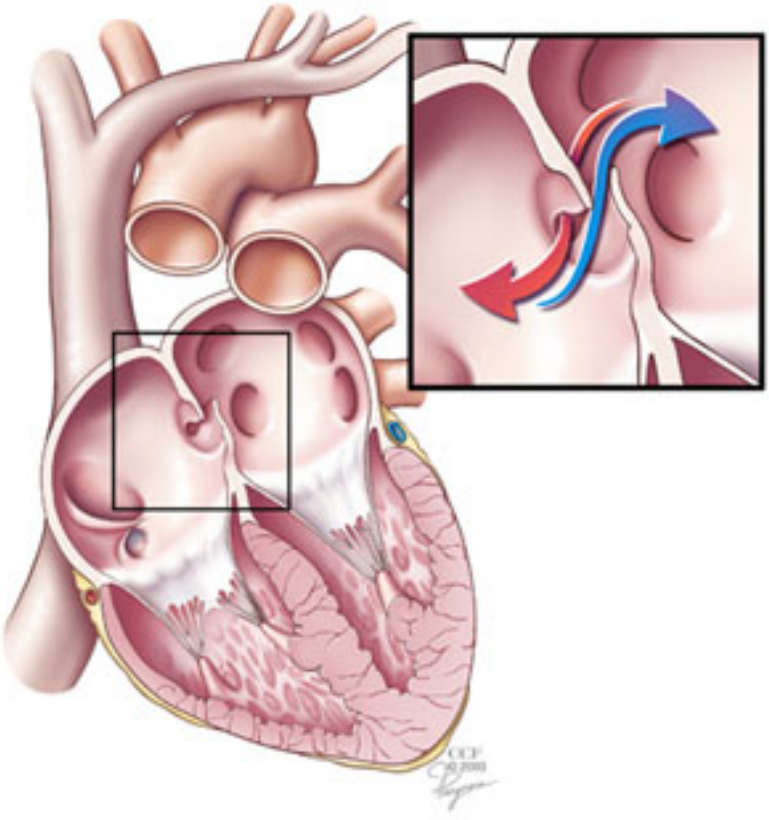

Figure 2: The pathway of blood flow through a patent foramen ovale (PFO).

Tables

	PFO+			PFO-			All		
	Male *n* = 7	Female *n* = 8	Overall *n* = 15	Male *n* = 7	Female *n* = 9	Overall *n* = 16	Male *n* = 14	Female *n* = 17	Overall *n* = 31
Age (y)	28 ± 6	21 ± 1	24 ± 5	28 ± 9	25 ± 10	27 ± 9	28 ± 7	23 ± 7	25 ± 7
Height (cm)	180 ± 9	163 ± 8	171 ± 12	180 ± 6	165 ± 6	172 ± 10	180 ± 7	164 ± 7	171 ± 11
Weight (kg)	82 ± 10	59 ± 8	69 ± 14	82 ± 15	59 ± 5	71 ± 16	82 ± 12	59 ± 6	70 ± 15
BSA (m^2)	2.0 ± 0.2	1.6 ± 0.1	1.8 ± 0.2	2.0 ± 0.2	1.6 ± 0.1	1.8 ± 0.2	2.0 ± 0.2	1.6 ± 0.1	1.8 ± 0.2
FVC (L)	5.6 ± 1.1	3.7 ± 0.4	4.5 ± 1.2	5.4 ± 0.5	3.9 ± 0.6	4.6 ± 0.9	5.5 ± 0.8	3.8 ± 0.5	4.6 ± 1.1
FEV1 (L)	4.5 ± 0.9	3.1 ± 0.4	3.9 ± 1.0	4.3 ± 0.5	3.2 ± 0.5	4.0 ± 0.7	4.4 ± 0.7	3.1 ± 0.5	3.7 ± 0.9
DLCO (ml/ min/Torr)	45 ± 8	28 ± 6	38 ± 10	40 ± 6	32 ± 4	36 ± 7	42 ± 7	30 ± 5	37 ± 8

Table 1: Anthropometric and pulmonary function testing data. All values are means ± standard deviations. No significant differences between groups ($p > .05$).

		Rest		+3		+6		+9	
		PFO–	PFO+	PFO–	PFO+	PFO–	PFO+	PFO–	PFO+
HH	V_E ($L \cdot min^{-1}$)	15.6 ± 3.7	17.3 ± 3.7	20.5 ± 4.3	19.7 ± 3.7	26.5 ± 6.1	23.6 ± 4.8	32.2 ± 8.2	29.5 ± 5.4
	V_T (L)	1.4 ± 0.6	1.4 ± 0.5	1.5 ± 0.6	1.5 ± 0.5	1.8 ± 0.6	1.7 ± 0.6	2 ± 0.7	2 ± 0.6
	RR ($breaths \cdot min^{-1}$)	13.1 ± 5	13.7 ± 3.7	14.9 ± 5.2	14 ± 4.2	16.1 ± 5.6	14.6 ± 4.1	17.4 ± 6	15.5 ± 3.3
	$P_{ET}O_2$ (mmHg)	237 ± 50.6	269.9 ± 32.4	250.5 ± 10	251.4 ± 9.6	249.7 ± 4.5	251.6 ± 4.6	249.7 ± 2.9	250.1 ± 4.1
	$P_{ET}CO_2$ (mmHg)	40.1 ± 3.4	41.2 ± 3.4	43.1 ± 3.3	44.1 ± 3.4	46.4 ± 3.4	47.3 ± 3.4	49.5 ± 3.5	50.3 ± 3.5
	HR (bpm)	66.2 ± 13.7	65.1 ± 7.3	68 ± 14.5	65.5 ± 7.5	69.6 ± 14.9	68.1 ± 8.4	72.5 ± 15.6	70 ± 7.7
	SpO_2 (%)	99.6 ± 0.8	99.4 ± 0.8	99.6 ± 0.7	99.4 ± 0.9	99.6 ± 0.6	99.4 ± 0.8	99.7 ± 0.6	99.4 ± 0.7
NH	V_E ($L \cdot min^{-1}$)	14.6 ± 1.0	16.0 ± 1.3	18.9 ± 1.4	19.0 ± 0.8	24.3 ± 1.7	22.5 ± 1.0	29.8 ± 2.5	27.1 ± 1.3
	V_T (L)	1.4 ± 0.1	1.4 ± 0.2	1.6 ± 0.2	1.6 ± 0.1	1.9 ± 0.2	1.8 ± 0.1	2.1 ± 0.2	2.0 ± 0.2
	RR ($breaths \cdot min^{-1}$)	12.1 ± 1.1	12.6 ± 0.9	12.9 ± 1.2	13.0 ± 0.9	14.5 ± 1.3	13.2 ± 0.8	15.3 ± 1.4	14.1 ± 0.7
	$P_{ET}O_2$ (mmHg)	100.6 ± 1.6	99.6 ± 1.8	101.0 ± 1.0	98.6 ± 1.7	100.3 ± 0.7	99.0 ± 1.5	100.1 ± 0.9	98.6 ± 1.6
	$P_{ET}CO_2$ (mmHg)	41.1 ± 0.8	41.9 ± 0.7	44.0 ± 0.7	45.2 ± 0.7	47.2 ± 0.7	48.1 ± 0.6	49.9 ± 0.7	51.0 ± 0.5
	HR (bpm)	67.9 ± 3.8	65.6 ± 2.2	68.9 ± 3.6	68.2 ± 2.2	71.7 ± 4.7	69.1 ± 2.4	72.7 ± 4.0	70.8 ± 2.2
	SpO_2 (%)	96.6 ± 0.5	96.2 ± 0.4	96.5 ± 0.5	96.3 ± 0.5	96.6 ± 0.4	96.6 ± 0.4	97.2 ± 0.4	97.1 ± 0.3

Table 2: Ventilatory and metabolic measures. Values are means ± standard deviation. No significant differences between groups ($p > .05$).

	All		Female		Male	
	PFO+ $n = 15$	PFO- $n = 16$	PFO+ $n = 8$	PFO- $n = 9$	PFO+ $n = 7$	PFO- $n = 7$
NH	1.4 ± 0.5*	2.0 ± 0.8	1.4 ± 0.5	1.9 ± 0.6	1.4 ± 0.5	2.0 ± 1.0
HH	1.3 ± 0.6*	1.8 ± 0.7	1.2 ± 0.4	1.7 ± 0.6	1.2 ± 0.8	1.8 ± 0.9

Table 3: Average HCVR slopes for all conditions and all groups. Values are means ± standard deviation. * indicates significant difference between PFO+ and PFO- groups ($p < .05$).

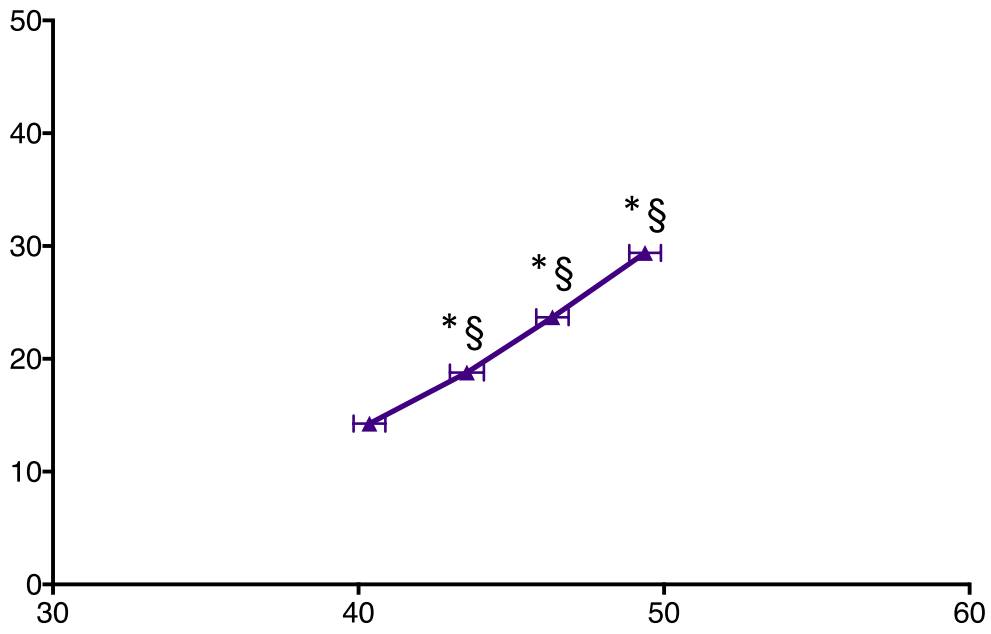

Figure 3A: The hypercapnic ventilatory response to acute normoxic hypercapnia for all subjects. Points and bars on figure are mean ± standard error. * indicates significant difference in $P_{ET}CO_2$ from resting baseline ($p < .05$). § indicates significant difference in V_E from resting baseline ($p < .05$).

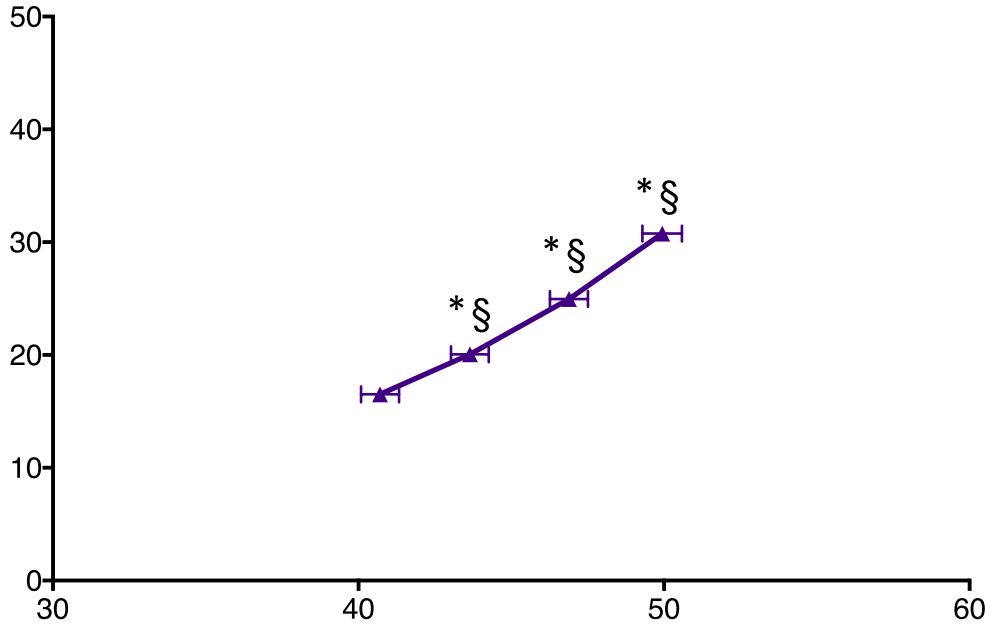

Figure 3B: The hypercapnic ventilatory response to acute hyperoxic hypercapnia for all subjects. Points and bars on figure are mean ± standard error. * indicates significant difference in $P_{ET}CO_2$ from resting baseline ($p < .05$). § indicates significant difference in V_E from resting baseline ($p < .05$).

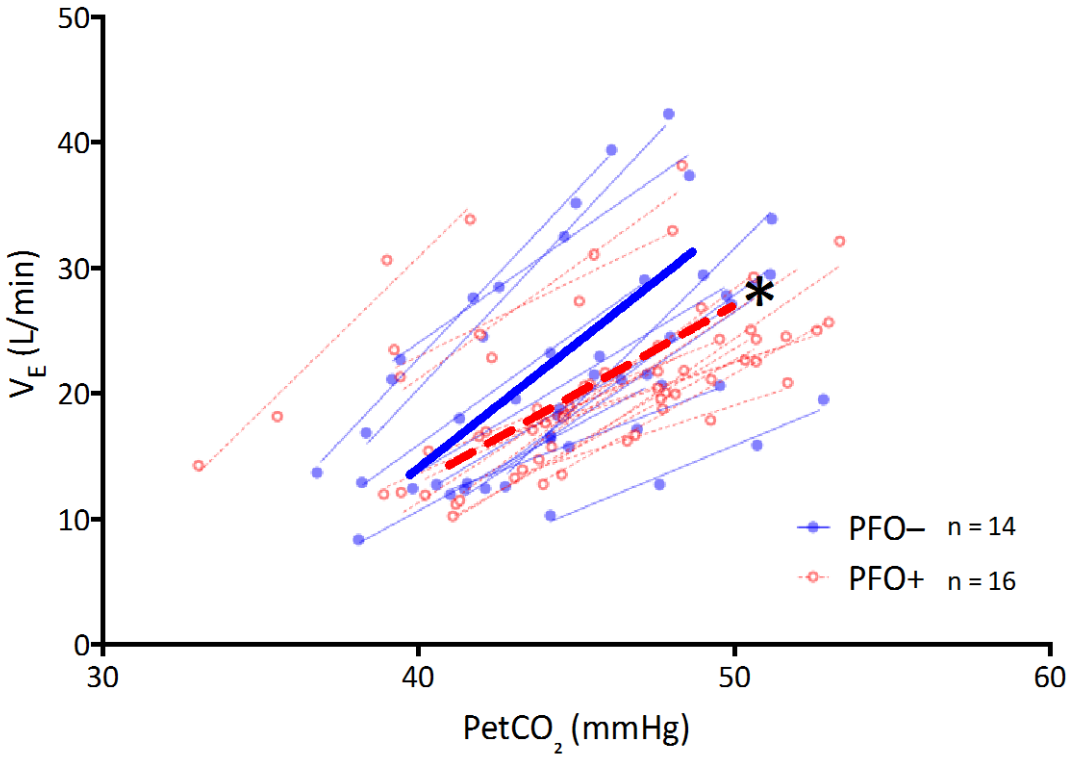

Figure 4A: The effect of a PFO on HCVR during acute exposure to normoxic hypercapnia for all subjects. Bold dashed line represents average HCVR for PFO+ subjects and bold solid line represents average HCVR for PFO- subjects. There was a main effect of PFO on HCVR. * indicates a significant difference in slope from PFO+ subjects ($p < .05$).

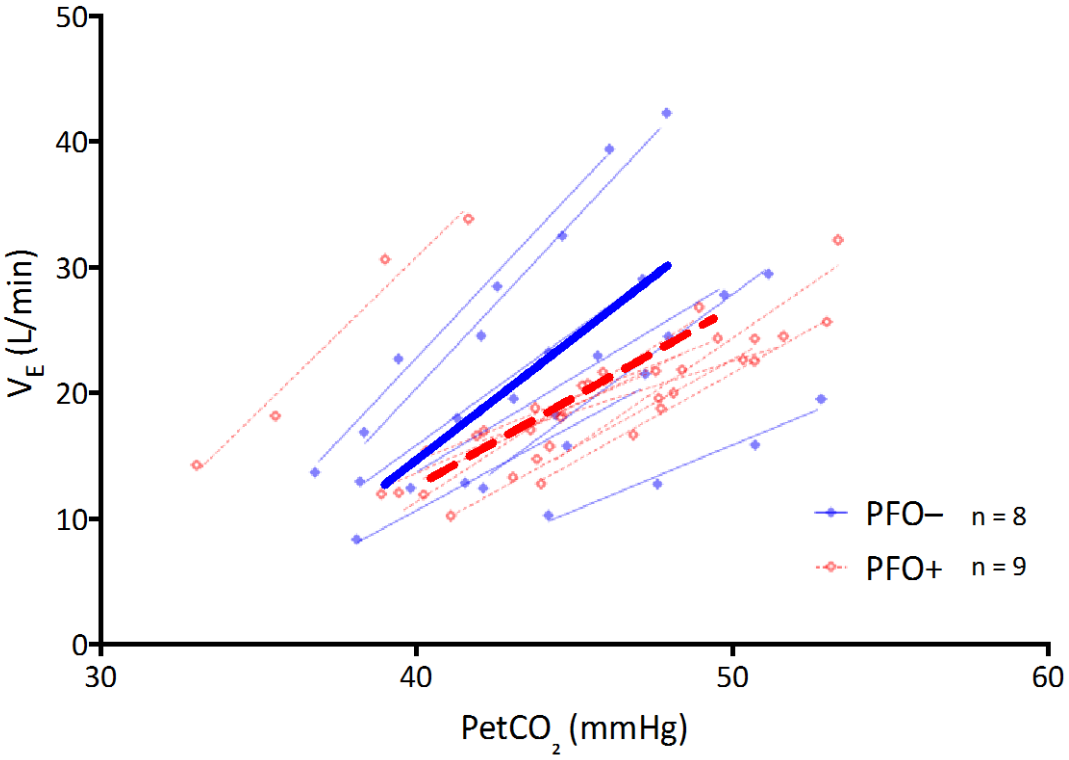

Figure 4B: The effect of a PFO on HCVR during acute exposure to normoxic hypercapnia for female subjects**.** Bold dashed line represents average HCVR for PFO+ subjects and bold solid line represents average HCVR for PFO- subjects. No significant differences between groups ($p > .05$).

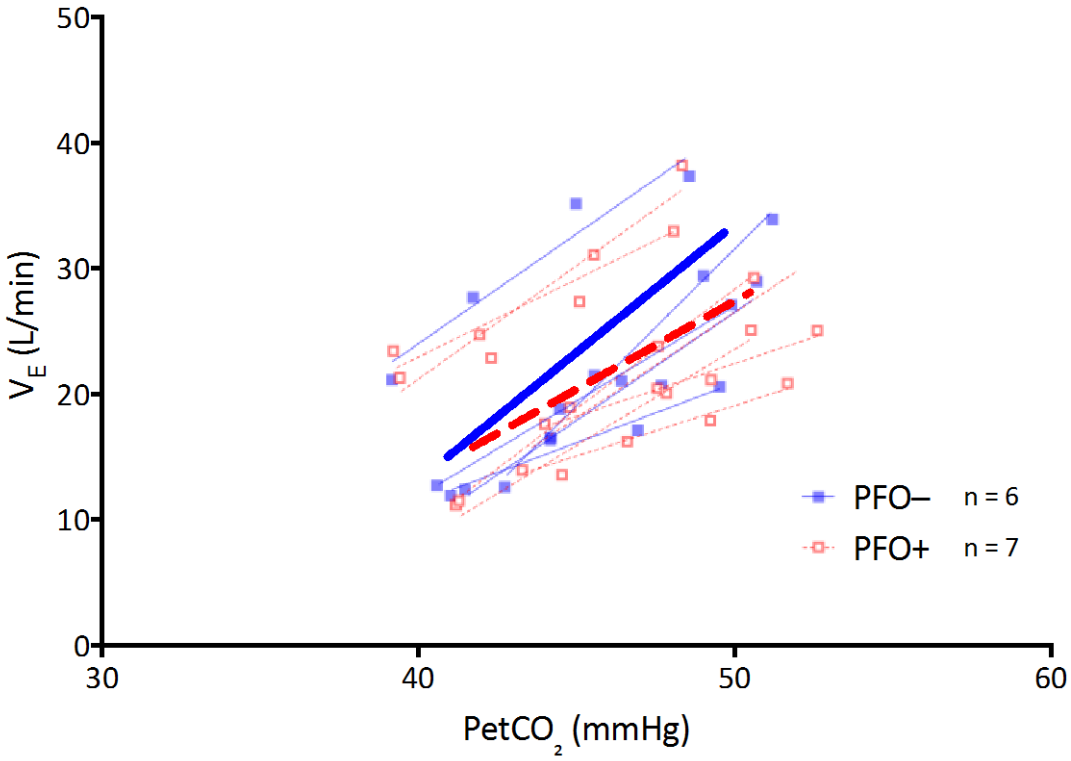

Figure 4C: The effect of a PFO on HCVR during acute exposure to normoxic hypercapnia for male subjects. Bold dashed line represents average HCVR for PFO+ subjects and bold solid line represents average HCVR for PFO- subjects. No significant differences between groups ($p > .05$).

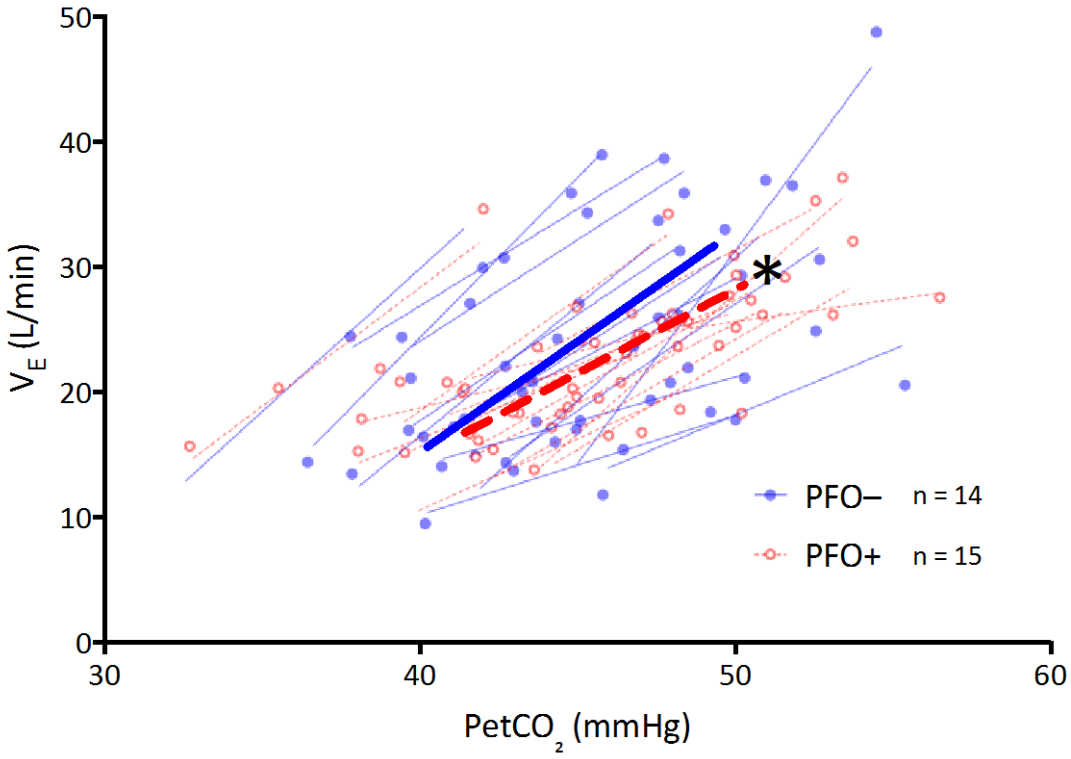

Figure 5A: The effect of a PFO on HCVR during acute exposure to hyperoxic hypercapnia for all subjects. Bold dashed line represents average HCVR for PFO+ subjects and bold solid line represents average HCVR for PFO- subjects. There was a main effect of PFO on HCVR. * indicates a significant difference in slope from PFO+ subjects ($p < .05$).

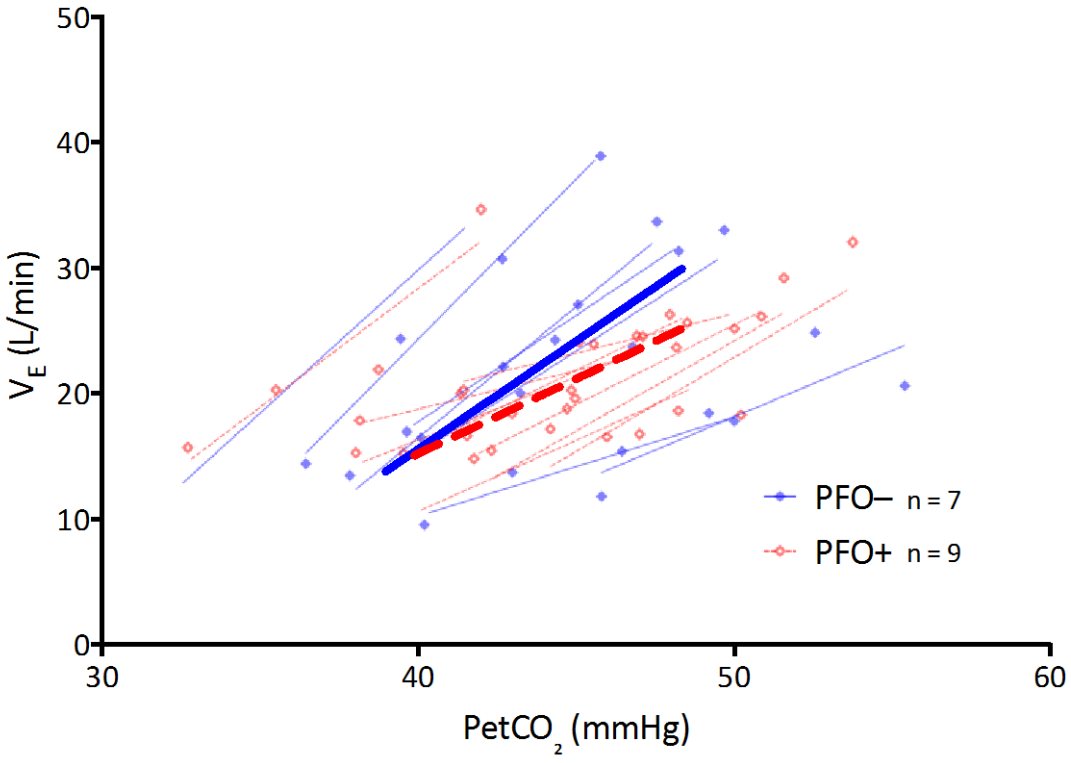

Figure 5B: The effect of a PFO on HCVR during acute exposure to hyperoxic hypercapnia for female subjects. Bold dashed line represents average HCVR for PFO+ subjects and bold solid line represents average HCVR for PFO- subjects. No significant differences between groups ($p > .05$).

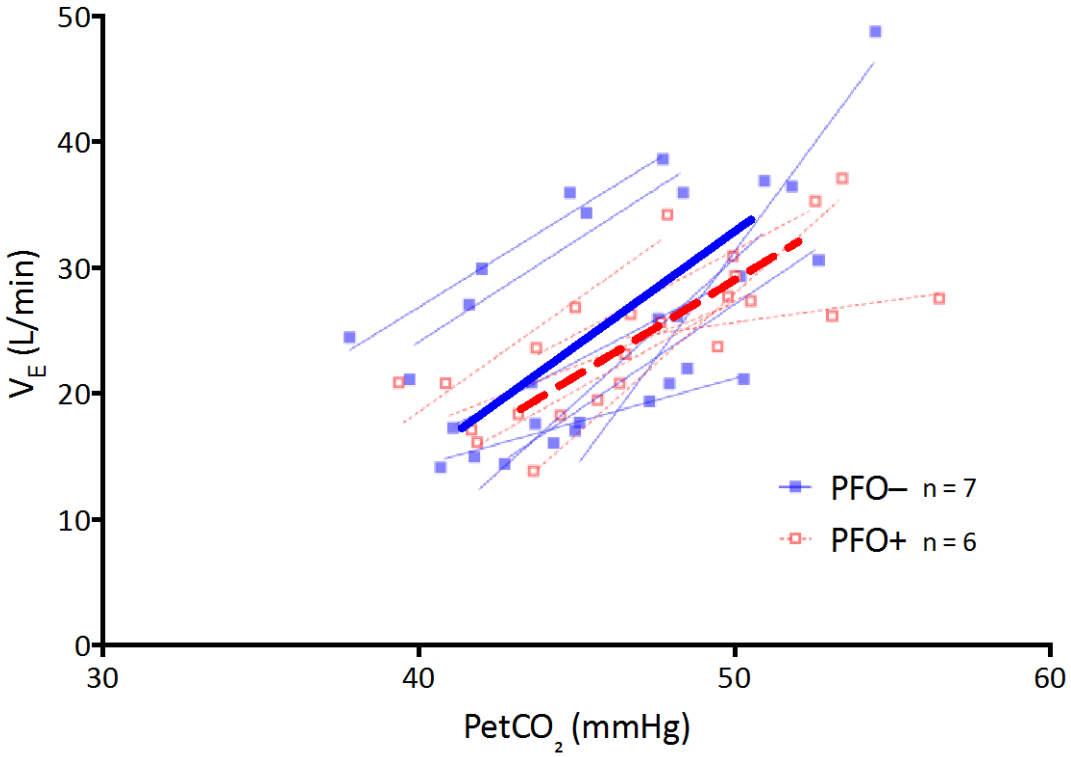

Figure 5C: The effect of a PFO on HCVR during acute exposure to hyperoxic hypercapnia for male subjects. Bold dashed line represents average HCVR for PFO+ subjects and bold solid line represents average HCVR for PFO- subjects. No significant differences between groups ($p > .05$).

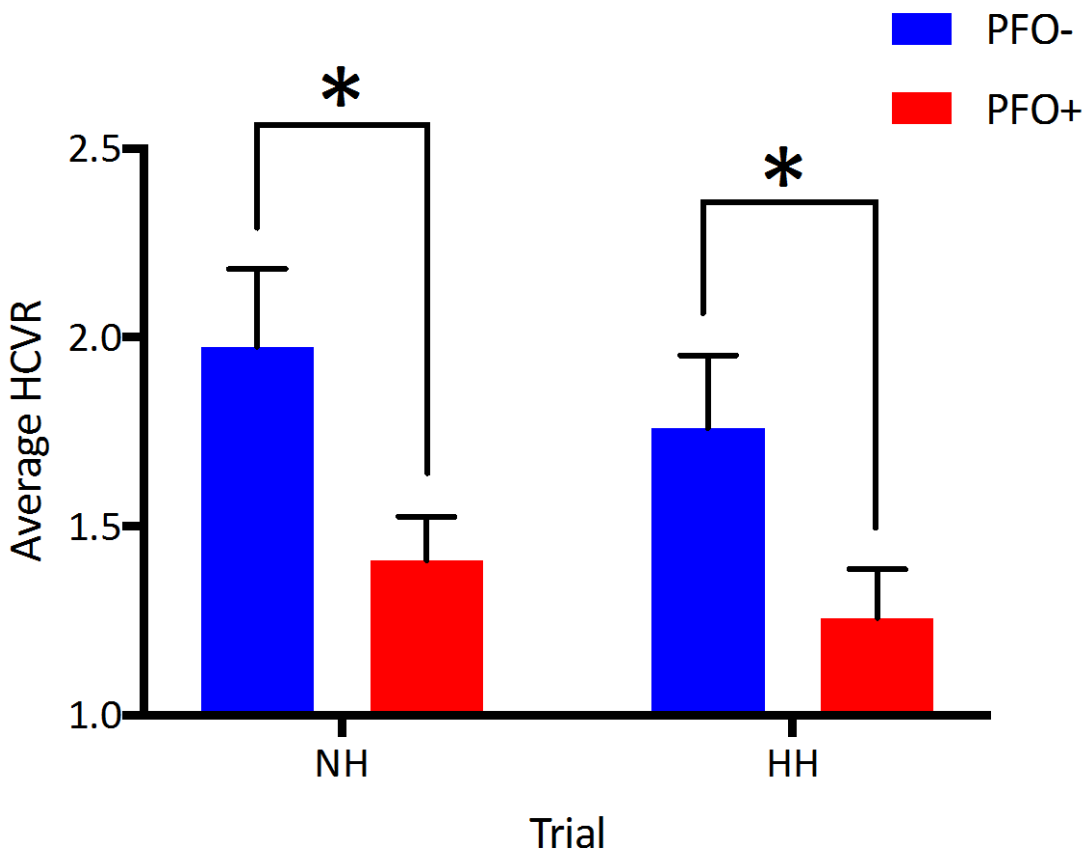

Figure 6: Comparison of average HCVR slopes for PFO+ and PFO- subjects between normoxic hypercapnia and hyperoxic hypercapnia trials. There was a main effect of PFO on HCVR slope during the normoxic hypercapnia and hyperoxic hypercapnia trials. Values are means ± standard deviation. * indicates a significant difference in slope between PFO+ and PFO- subjects ($p < .05$).

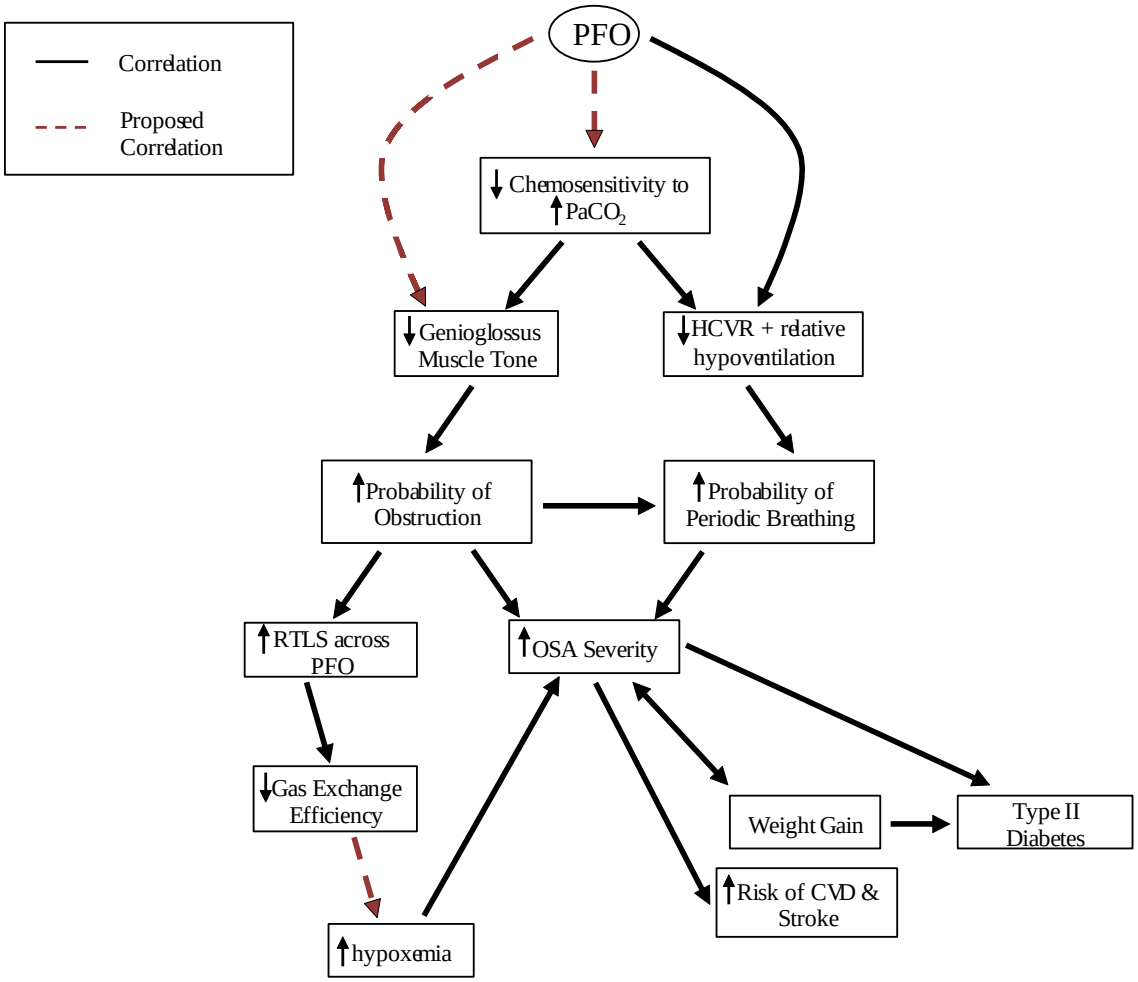

Figure 7: Flow chart demonstrating proposed correlations between PFO and OSA.

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