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

by

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Approximately 25-40% of the general healthy population has a patent foramen ovale (PFO) (Woods *et al.*, 2010; Marriott *et al.*, 2013; Elliott *et al.*, 2013). Previous work by our lab has shown that after 16 days of exposure to 5260 m, subjects with a PFO (PFO+) had blunted ventilatory acclimatization to high altitude compared to subjects without a PFO (PFO–), such that PFO+ subjects had a lower partial pressure of arterial O_2, higher partial pressure of arterial CO_2, and lower O_2 saturation (Elliott *et al.*, 2015). However, in that study 7 / 11 PFO+ subjects were female, whereas only 2 / 10 PFO- subjects were female and thus potential sex differences were not accounted for and it is known that sex hormones can affect ventilatory responses to O_2 and CO_2 (Schoene *et al.*, 1986; Slatkovska *et al.*, 2006). Thus, it remains unknown if PFO+ subjects exhibit blunted acute ventilatory responses to hypoxia compared to PFO– subjects, independent of sex. Therefore, the purpose of this study was to determine if the presence of a PFO affects ventilatory responses during acute exposure to either poikilocapnic hypoxia or isocapnic hypoxia.

A total of 31 healthy, non-smoking subjects matched for height, weight, sex and age completed the entire study:

PFO+: age: 24 ± 5 yrs, height: 170 ± 11 cm, weight: 69 ± 14 kg, BSA: 1.8 ± 0.2 m^2

PFO-: age: 27 ± 9 yrs, height: 172 ± 10 cm, weight: 71 ± 16 kg, BSA: 1.8 ± 0.2 m^2

These 31 subjects included 15 PFO+ subjects (8 female) and 16 PFO – subjects (9 female). Subjects came to the lab and participated in two trials: poikilocapnic hypoxia (PH) and isocapnic hypoxia (IH). These trials were administered using the Dynamic End-tidal Forcing (DEF) breathing response system in a randomized and balanced order. The subjects were given a 40 min break between hypoxia trials. Acute Hypoxic ventilatory response (AHVR), calculated as the change in V_E divided by the change in SpO_2 ($\Delta V_E / \Delta SpO_2$), was done for both PH and IH trials. Hypoxic ventilatory decline (HVD), calculated as a percent decline with respect to the initial increase in V_E, was performed for IH trials only.

Despite differences in ventilatory acclimatization to high altitude, there were no difference in AHVR between PFO+ and PFO- subjects. PFO+ and PFO- showed no significant differences in hypoxic ventilatory decline HVD. There were also no differences in AHVR and HVD between males and females. Thus, our findings suggest differences in ventilatory acclimatization with chronic exposure to high altitude are likely not due to baseline differences in hypoxic chemosensitivity.

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Abbreviations and Terms

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

Acute Hypoxic Ventilatory Response (AHVR): initial, sharp increase in ventilation upon exposure to hypoxia

Basal metabolic rate (BMR): rate of energy expenditure at rest

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

F_ICO_2: fraction of inspired carbon dioxide

F_IO_2: fraction of inspired oxygen

Hypercapnia: condition marked by increased carbon dioxide in arterial blood

Hypoxia: condition marked by decreased oxygen in arterial blood

Hypoxic ventilatory decline (HVD): after 5-20 minutes of exposure to hypoxia, ventilation is expected to decrease and reach a plateau

Hypoxic ventilatory response (HVR): $\frac{\Delta V_E}{\Delta SpO_2}$ measures change in ventilation compared to change in arterial oxygen saturation to quantify an individual's response to hypoxic conditions

P_ACO_2: alveolar partial pressure of carbon dioxide

P_AO_2: alveolar partial pressure of oxygen

P_aCO_2: arterial partial pressure of carbon dioxide

P_aO_2: arterial partial pressure of oxygen

Patent foramen ovale (PFO): 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

SaO_2: arterial oxygen saturation

SpO_2: peripheral capillary oxygen saturation

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

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

Ventilatory depression: decrease in ventilation (V_E) with months to years of exposure to hypoxia. Findings on ventilatory depression have not been universal, and is likely largely dependent on various environmental and genetic factors.

Introduction

Since my second year at the University of Oregon, I have had the chance to work as an undergraduate research assistant in Dr. Andrew Lovering's Cardiopulmonary and Respiratory Physiology Lab of the Department of Human Physiology. One of the primary research focuses of the laboratory is on the effects of the patent foramen ovale (PFO), an intra-cardiac shunt between the right and left atria that exists in 25-40% of the general population (Woods et. al., 2010; Marriott *et al*., 2013; Elliott *et al*., 2013). Previous work by our lab has shown that: there is an increased gas exchange inefficiency at rest in PFO+ subjects likely due to the shunting (Lovering *et al*. 2011), individuals with a PFO (PFO+) have an ~0.4° C higher core body temperature when compared to those without a PFO (PFO-) (Davis *et al*., 2015), and after 16 days of exposure to 5260m, PFO+ subjects had a blunted ventilation compared to PFO- subjects (Elliot *et al*., 2015). Despite these existing studies, PFO research in healthy humans is still relatively new, and thus there is limited information and much more to be studied on the implications and clinical relevance of a PFO.

In contrast there has been significant investigation into the ventilatory response to hypercapnia and hypoxia across variety of conditions and populations. However, the effect of a PFO on these ventilatory responses have not been studied to date. Therefore, the primary focus of this thesis was the hypoxic ventilatory response (HVR), which compares the change in ventilation over the change in oxygen saturation in subjects with and without a PFO.

Purpose and Hypothesis

The purpose of this study was to determine if the presence of a PFO affects ventilatory responses during acute exposure to: 1) poikilocapnic hypoxia and 2) isocapnic hypoxia. Based on findings from the AltitudeOmics study (Elliot et al., 2015), the hypothesis of this study was that PFO+ individuals would have a blunted hypoxic ventilatory response breathing poikilocapnic and isocapnic hypoxia, compared to the PFO- individuals.

Background

Cardiopulmonary Physiology & Path of Blood flow

The cardiopulmonary system's primary function is the transport of oxygen (O_2) and nutrients to peripheral tissues while at the same time removing carbon dioxide (CO_2), a metabolic byproducts. Systemic venous, or deoxygenated blood enters the right atrium of the heart via the superior vena cava, inferior vena cava, and the coronary sinus. Through the right ventricle, deoxygenated blood travels to the lungs, where at the level of the alveoli, O_2 and CO_2 enter and exit the bloodstream, respectively. The oxygenated blood travels back to the left atrium of the heart, and to the rest of the body (**Figure 1**). The functions of the cardiopulmonary system are vital to cellular respiration and energy production, as O_2 is required for cellular respiration and CO_2 must be removed to maintain pH homeostasis.

Patent Foramen Ovale

The foramen ovale is a feature of the fetal heart. It is an interatrial opening that allows blood to flow directly from the right side of the heart to the left side of the heart, bypassing pulmonary circulation. The foramen ovale is vital to the fetus because in utero, a human embryo receives oxygenated blood from the mother and the developing lungs are not capable of 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 et al, 1998). When the baby is born, and starts to breath the atmospheric air, the increased left atrial pressure forcest the valve against the septal wall, which closes the foramen ovale. However, we and others

have observed that in 25-40% of the general population, a patent foramen ovale, or a PFO, exists (Woods et al., 2010; Marriott et al., 2013; Elliott et al., 2013).

Functions of the Respiratory System

The primary function of the respiratory system is gas exchange. The lungs facilitate exchange of O_2 and CO_2 between the body's external environment and the body's internal environment to maintain constant and favorable O_2 and CO_2 partial pressures. The exchange of O_2 and CO_2 occurs according to the partial pressure gradient of these gases. In the venous blood traveling to the alveoli, the partial pressure of O_2 (P_{O2}) is 40 mmHg and the partial pressure of CO_2 (PCO_2) is 46 mmHg. The alveolar partial pressure of O_2 (P_AO_2) is greater than the venous PO_2 at 100 mmHg and the alveolar CO_2 (P_ACO_2) is lesser than the venous CO_2 at 40 mmHg. Due to this pressure gradient, O_2 and CO_2 will move down their pressure gradients, into the blood stream and the alveoli, respectively. Similarly, as the oxygenated arterial blood travels to the tissues across the body, O_2 will be transported and dropped off and CO_2 will be picked up using the partial pressure gradient. Normal arterial PO_2 (P_aO_2) is 100 mmHg and normal arterial PCO_2 (P_aCO_2) is 40 mmHg, while the PO_2 and PCO_2 at the peripheral tissues would be 40 mmHg and 46 mmHg, respectively.

In the laboratory setting, gas exchange efficiency can be measured by calculating the alveolar-arterial oxygen difference, or $AaDO_2$. Simply put, a smaller $AaDO_2$ value, represents a minimal difference in oxygen content between the alveoli and arterial blood, would denote greater pulmonary gas exchange efficiency. As mentioned previously, Lovering *et al*. previously explored the effect of PFO on pulmonary gas exchange efficiency as measured by $AaDO_2$. The study showed that the

degree of right-to-left shunting caused by the PFO led to significantly higher $AaDO_2$ at rest, meaning that those with a PFO have decreased pulmonary gas exchange efficiency (Lovering *et al*., 2011)

One of the secondary functions of the respiratory system is respiratory cooling, which serves as a means for heat loss. It has been already established that ventilation increases significantly when core temperature reaches approximately 38.5°C (Cabanac & White, 1995). Though the mechanism behind this response is unclear, the increase in ventilation is thought to be the body's response to increase the amount heat dissipation through respiratory cooling.

Chemoreceptors and Chemical Control of Ventilation

O_2 and CO_2 partial pressures in the blood are the primary contributors to changes in ventilatory drives. P_aCO_2 is sensed mainly by central chemoreceptors located on the ventrolateral portion of the medulla of the central nervous system (CNS). Central chemoreceptors indirectly monitor arterial PCO_2 through changes in cerebrospinal fluid pH. The respiratory pattern generator within the medulla of the CNS is responsible for integrating the signals from central chemoreceptors and modulating a response based on indirect detection of PCO_2. The signal is then relayed to the diaphragm and accessory respiratory muscles to stimulate a change in ventilation (West *et al*., 2013). In conditions marked by high CO_2, or, hypercapnia, the increased firing from the central chemoreceptors would ultimately increase one's ventilation in efforts to bring the $PaCO_2$ back down to within normal range.

Similarly, peripheral chemoreceptors sense P_aO_2 and respond to conditions of low oxygen, or, hypoxia. Glomus cells of the carotid bodies sense the level of oxygen

and release transmitters in response to hypoxia (Prabhakar *et al.,* 2004). These chemoreceptors signal travels to the nucleus tractus solitarius (NTS) in the medulla, which relays information to the respiratory pattern generator, ultimately leading to the necessary change in ventilation. Thus, with decreased P_aO_2, peripheral chemoreceptor firing rate increases, resulting in increased ventilation to compensate for the body's need for O_2 (West *et al*., 2013).

It is important to note that, although we have identified hypoxia and hypercapnia as the two powerful drives to increased ventilation, what drives our ventilation at sea level is the rate of production of CO_2, represented by basal metabolic rate (BMR). Since the partial pressure of O_2 in atmospheric air at sea level is high, the need to exhale the CO_2 produced as a result of cellular metabolism serves as the primary drive for ventilation.

The Hypoxic Ventilatory Response

In general, hypoxic ventilatory response (HVR) refers to the increased ventilation due to low oxygen saturation. HVR compares the change in V_E to change in arterial oxygen saturation (SaO_2), measuring the response to hypoxic 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).

The time course of the HVR can be divided into four distinct phases (**Figure 2**.). First, the acute hypoxic response (AHVR) is expected occur and this refers to the initial, sharp increase in ventilation upon exposure to hypoxia due to rapid increase in peripheral chemoreceptor firing rate. After 5-20 minutes of exposure to hypoxia,

ventilation is expected to decrease and reach a plateau, and this phase is referred to as the hypoxic ventilatory decline (HVD). The exact mechanisms causing HVD are unclear; however, its occurrence could result from a decreased chemoreceptor sensitivity, elevations in cerebral blood flow and/or neural stimulus (Powell *et al*., 1998; Sato *et al*., 1994). Additionally, a study indicated that the development of HVD is caused, at least in part, by an increased peripheral chemoreflex threshold to the isocapnic hypoxic stimulus (Mahamed & Duffin, 2001). During the first month at altitude, ventilation is expected to increase steadily across time—a phase referred to as ventilatory acclimatization. Lastly, with months to years of exposure to hypoxia, there could be ventilatory depression (Ainslie *et al*. 2013). The ventilatory depression phase has been observed in some long-term residents of high altitude (Sato *et al*., 1994), but this finding has not been universal (Beall *et al*., 1997), and is likely largely dependent on various factors such as age, degree of altitude, along with environmental and genetic factors.

The Effect of Sex on Ventilation

In a study that explored the effects of the ovarian hormones progesterone and estrogen on effective alveolar ventilation and HVR, it was observed that subjects who had undergone an ovariectomy decreased ventilatory and carotid sinus nerve responsiveness to hypoxia, suggesting that the presence of physiological levels of ovarian hormones influences hypoxic chemosensitivity of peripheral chemoreceptors (Tatsumi *et al*., 1997; MacNutt *et al*., 2012). Furthermore, findings of a recent comprehensive study suggest that menstrual cycle phase may have an influence on V_E, as the results showed increased V_E at times of high progesterone and estrogen (MacNutt

et al., 2012). Although there is no clear consensus on the effect of biological sex on ventilation and ventilatory response, it was important to eliminate the sex affect to our best effort because we are interested in the possible effect of a PFO, alone, on ventilatory response.

The Effect of a Patent Foramen Ovale on Ventilation

In 2012, the Cardiopulmonary and Respiratory Physiology lab conducted a study initially designed to explore the effect of PFO on gas exchange efficiency at altitude, and encountered surprising findings that led the laboratory to further investigate the PFO's effect on ventilatory response and acclimatization to hypoxia. The AltitudeOmics expedition took place in Mt. Chacaltaya in Bolivia, ~5260 m (17,000 ft.) above sea level. The study population consisted of 21 healthy sea level residents. At day 1 in altitude, the PFO+ and PFO- group showed no significant differences in HVR. However, at day 16, PFO+ group showed HVR that was significantly less than that of the PFO- group (**Figure 3**). Therefore, we determined that subjects with a PFO showed blunted ventilatory acclimatization to high altitude (Elliott *et al*. 2015). This was a completely unexpected finding, because, under normal conditions, ventilation should increase over the 16 days in altitude, due to increasing sensitivity of the peripheral chemoreceptors to partial restoration of P_aO_2 and concomitant fall in P_aCO_2. Because PFO+ subjects would have blood bypassing pulmonary circulation and therefore gas exchange at the lungs, it was expected that these subjects would have decreased gas exchange efficiency compared to PFO- subjects. And due to this decreased efficiency, we expected the PFO+ subjects to increase ventilation to a greater degree to meet the

body's needs. Because the findings showed otherwise, the laboratory decided to further investigate the reasons for the blunted ventilatory acclimatization seen in PFO+ subjects.

Further investigation into the findings of the AltitudeOmics study was necessary due to several limitations in study design and sampling. Since the AltitudeOmics study was designed to explore questions regarding gas exchange, there existed some limitations that prevented us from drawing conclusions regarding the blunted HVR after 16 days in altitude. As mentioned in the section above, female sex hormones can cause their ventilatory responses to differ from those of males (MacNutt *et al*., 2012). However, the AltitudeOmics study was not designed to account for those differences, due to unequal numbers of males and females, making it hard for us to conclude whether the effects observed were due to the presence of a PFO or due to biological sex fifferences.

Methods

This study received approval from the University of Oregon's Office for Protection of Human Subjects. Each subject was given documents outlining the study and provided written approval prior to participating in the study. All experimental procedures were conducted in accordance of the *Declaration of Helsinki*.

Participant Recruitment and Enrollment

62 total subjects were recruited for participation. The members of the research team at the cardiopulmonary and respiratory physiology laboratory described the nature of the study to all of the subjects, both orally and in writing. Of the 62, a total of 31 subjects (17 female) qualified and completed the entire study protocol. 15 subjects (8 female) were identified as PFO+, and 16 subjects (9 female) were identified as PFO-. Of the remaining 31 subjects who were not able to complete the study, 5 had poor pulmonary function, 11 withdrew before completion of the protocol for reasons no associated with the study, 3 could not get an IV placed for the screening process, 7 had late appearing bubbles (4 or more) after more than 3 cardiac cycles, and 5 were excluded to have even numbers of subjects in each group. In total, the PFO prevalence of the study was 46%, which is greater than what has been previously reported in various studies. Ultimately, 31 (15 PFO+) healthy, non-smoking subjects, age 25 ± 8 years, without history of cardiopulmonary pathology, completed the study.

Study Protocol—Day 1 & Day 2

The subjects were asked to visit the laboratory, located in the Center for Medical Education & Research at the University of Oregon, on two separate occasions. During

the first visit, subjects underwent ultrasound screening, pulmonary function and lung diffusion capacity testing, and the researchers took anthropometric measurements. During the second visit, subjects completed 2 separate hypoxia protocols: poikilocapnic hypoxia and isocapnic hypoxia, in a randomized order. Each trial was separated by a minimum of 40 minutes.

Ultrasound Screening

The transthoracic saline contrast echocardiography (TTSCE) method the laboratory utilizes for screening process has previously received validation for its sensitivity to accurately detect PFO in the general healthy population (Elliott *et al.*, 2013).

To screen for a presence of a PFO, the laboratory utilized TTSCE, and the procedure is thoroughly described by Lovering & Goodman (Lovering & Goodman, 2012). Along with the ultrasound technician, a researcher of the laboratory injects an agitated mixture of saline solution and air into a peripheral antecubital vein via an IV catheter. The suspension saline-air microbubble allows for excellent right-sided contrast in the ultrasound image.

In the absence of a PFO, there would be no immediate presence of the saline-air microbubble mixture crossing over from the right atrium into the left atrium. Thus, the criterion to identify a PFO is the appearance of 1 or more microbubble in the left heart in any frame during the subsequent 3 cardiac cycles (Freeman & Woods, 2008; Woods *et al.*, 2010; Marriott *et al.*, 2013; Elliott *et al.*, 2013). This procedure is performed in two different ways—during normal breathing and immediately after the release of a

Valsalva maneuver, which elevates right atrial pressure to create a physiological setting optimal for the detection of a right-to-left shunt, such as a PFO.

Pulmonary Function Test and Lung Diffusion Capacity

After obtaining the subject's height and weight (Ohaus Corporation, ES200L, Pinebrook, NJ), pulmonary function test, or PFT (Ultima PFX, MedGraphics, St. Paul, MN) was administered. PFTs were utilized to confirm normal lung function and screen for any signs of lung disease in our potential subjects.

PFTs measure for: forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), mid-expiratory flow rate (FEV_{25-75}), and slow vital capacity (SVC). Measurements are obtained using a spirometry system (Ultima PFX, MedGraphics, St. Paul, MN), and according to the standards established by the American Thoracic Society/European Respiratory Society (Macintyre *et al*., 2015). Measures such as total lung capacity (TLC), functional residual capacity (FRC), and residual volume (RV), can be obtained using whole-body plethysmography (Wagner *et al.*, 2005). Lastly, to measure lung diffusion capacity for carbon monoxide (DL_{CO}), the single-breath, breath-hold method (Knudson *et al.*, 1987), along with the method for timing and alveolar sample collection (MedGraphics Ultima PFX, Breeze v.6.3.006) were utilized.

Respiratory Measurements

In this study, we collected respiratory measures at 200 Hz using an analog-to-digital converter (Powerlab/16SP ML 880; ADInstruments, Colorado Springs, CO), and collected ventilatory and cardiovascular measures using commercially available software (LabChart V7.1, ADInstruments, Colorado Springs, CO). Subjects were

instrumented to breathe through an apparatus consisting of a mouthpiece, bacteriological filter, nose clip, and a two-way non-rebreathing valve (Hans Rudolph, Shawnee, KS), and this apparatus accounted for airflow resistance of 0.80 and 0.73 cm $H_2O \cdot l^{-1} \cdot sec^{-1}$ at flow rates of 1.5 and 3.0 l/sec, respectively. End-tidal gases (O_2 and CO_2) were analyzed using a system (ML206; ADInstruments, Colorado Springs, CO) that sample end tidal gases using respired gas pressures. Lastly, the combination of a pneumotachograph (HR 800L, Hans Rudolph, Shawnee, KS) and a differential pressure amplifier (ML141, ADInstruments, Colorado Springs, CO) was utilized to measure the respiratory flow near the mouth of the subject.

End-Tiding Forcing

A dynamic end-tidal forcing system (DEF) designed by Foster et al., which has been used previously to control end-tidal gases during physiological stressors (Querido *et al.*, 2013; Foster *et al., 2009*; Foster *et al.*, 2014), was utilized in this study to control and estimate arterial blood gases in place of an arterial line. The DEF system has shown its ability to change $P_{ET}O_2$ and $P_{ET}CO_2$ and maintain desired end-tidal values (**Figure 4**, Foster *et al.*, 2014).

The DEF system utilized gas solenoid valves for O_2, CO_2, and N_2, which serve to regulate the amount of each gas being delivered to the inspiratory reservoir through a mixing and humidification chamber. A software specifically designed to measure respiratory parameters (Labview 13.0, National Instruments, Austin TX) was utilized for determining $P_{ET}O_2$, $P_{ET}CO_2$, tidal volume (V_t), breathing frequency (*f*), and minute ventilation (V_E) for each expired breath. Using this information from the subject's expired breath, the DEF system adjusted the inspirate to clamp end-tidal O_2 and CO_2 at

desired levels. End-tidal steady state was defined as the time point when $P_{ET}O_2$ and $P_{ET}CO_2$ values remained within 1 mmHg of the desired target for 3 consecutive breaths.

Poikilocapnic Hypoxia (PH)

Once baseline measures were obtained, the F_IO_2 was adjusted, such that $P_{ET}O_2$ was clamped at 45 mm Hg. After steady-state $P_{ET}O_2$ was attained, subjects remained in this hypoxic condition for 20 minutes. Time points of interest were baseline and the time at which V_E was greatest in the last 5 minutes of hypoxic exposure.

Isocapnic Hypoxia (IH)

After establishing and determining baseline $P_{ET}O_2$ and $P_{ET}CO_2$ values, the F_IO_2 and F_ICO_2 were adjusted, such that $P_{ET}O_2$ and $P_{ET}CO_2$ were clamped at 45 mm Hg and resting values, respectively. Subjects remained in the hypoxic condition for 20 minutes after steady state in end-tidal gases was reached. Time points of interest were: baseline, the time at which V_E was greatest after first 5 minutes of hypoxic exposure, and the time at which V_E was the smallest in the last 5 minutes of hypoxic exposure.

Calculations & Statistical Analyses

In this study, we utilized the method of calculating AHVR and HVD previously used and outlined by Duffin (Duffin *et al*., 2007). In both PH and IH trials, AHVR values were calculated. To calculate for AHVR, we used: the one-minute average of baseline V_E before hypoxic exposure and the one-minute average around the peak V_E recorded in the first (IH) and last (PH) 5 minutes of hypoxic exposure, along with peripheral oxygen saturation values at those time points. Using these two points in the

subject's ventilatory response, AHVR was calculated as the difference in V_E of baseline and peak, divided by the difference in SpO_2 of baseline and peak.

HVD was only calculated for the IH trial. To calculate for HVD, included another time point of interest: the time at which V_E was smallest during the last 5 minutes of exposure to isocapnic hypoxia. Therefore, in addition to the one-minute average values for baseline and peak V_E used in the calculation of AHVR, we were concerned with the one-minute average V_E around the time at which V_E was smallest during the last 5 minutes of exposure to isocapnic hypoxia. Using those three time points, HVD was reported in terms of percent decline, using the equation: % HVD = 100* (V_E Peak – V_E Final) / (V_E Peak – V_E Baseline). For all other variables, a two-way mixed ANOVA (PFO x time point) with $\alpha = .05$ was utilized.

Data were analyzed using GraphPad Prism software (v 5.0b). Overall and group descriptive statistics (mean, standard deviation, and standard error of the mean) were calculated for all test variables. To determine significance between PFO+ and PFO− subjects, data were analyzed using a two-way mixed ANOVA with $\alpha = .05$.

Results

Anthropometrics and Lung Function

Anthropometric, pulmonary function, and DL_{CO} data for PFO+ and PFO- subjects are presented in **Table 1**. There were no significant differences between the PFO+ and PFO- ($p > .05$). However, we observed differences in height, weight, FVC, FEV_1, and DL_{CO} between male and female subjects (**Table 1**).

Cardiopulmonary measures

There were no differences in V_E, V_t, RR, or $P_{ET}O_2$ between PFO+ and PFO- subjects during both poikilocapnic and isocapnic hypoxia trials ($p > .05$, **Table 2**). Similarly, there was no effect of PFO on SpO_2 and HR during either of the trials ($p > .05$, **Table 2**).

Hypoxic Ventilatory Response

Poikilocapnic Hypoxic

There was no effect of PFO on the AHVR during the PH trial ($p > .05$, **Figure 5**). There was no significant difference in AHVR (mean ± SD) between PFO+ (0.08 ± 0.15) and PFO- (0.10 ± 0.21), $p > .05$ unpaired t-test.

Isocapnic Hypoxia

There was no effect of PFO on the Acute Hypoxic Ventilatory Response during the IH trial ($p > .05$, **Figure 6**). There was no significant difference in AHVR (mean ± SD) between PFO+ (0.85 ± 0.39) and PFO- (0.84 ± 0.41), $p > .05$ unpaired t-test.

Additionally, there was no effect of PFO on the Hypoxic Ventilatory Decline (HVD) during the IH trial between PFO+ (0.62 ± 0.22) and PFO- (0.68 ± 0.23) ($p > .05$, **Figure 7**) .

The Effect of Biological Sex on AHVR and HVD

We observed significant differences in V_t during the IH trial, along with V_t and V_E, SpO_2 during the PH trial. However, there were no significant differences in the ΔV_E ΔSpO_2 between male and female subjects, and thus there was no effect of biological sex on AHVR and HVD to both PH and IH ($p > .05$, **Figures 8 a,b** and **Figure 9**).

Discussion

In this study, we observed no significant differences in acute hypoxic ventilatory response (AVHR) and hypoxic ventilatory decline (HVD) between PFO+ and PFO- subjects. Thus, we suggest the differences in ventilatory acclimatization with chronic exposure to high altitude are likely not due to baseline differences in hypoxic chemosensitivity.

Presence of a PFO and the hypoxic ventilatory responses

Under normal conditions at sea-level, PaO_2 is 100 mmHg. As represented by the oxy-hemoglobin dissociation curve, peripheral chemoreceptor firing rate does not significantly increase until PaO_2 drops below ~60 mmHg (Prabhakar & Semenza, 2015). When PaO_2 drops below 60 mmHg, small changes in PaO_2 will cause drastic changes in SaO_2. Thus, during the two hypoxia trials, $P_{ET}O_2$ value was clamped at 45 mmHg to increase the likelihood that significant differences in V_E would occur if they were to exist.

The poikilocapnic hypoxia (PH) trial is a theoretical representation of high altitude. During the PH trial, $P_{ET}O_2$ was kept at 45 mmHg, and $P_{ET}CO_2$ was not controlled, meaning that the subjects would expire as much CO_2 as would result from simultaneous stimulation of the peripheral chemoreceptor and inhibition of the centeral chemoceptor. By decreasing the input from the central chemoreceptors, which is driven by increased P_aCO_2, the PH trial most closely stimulates acute high altitude conditions. This is evident in the results, as AHVR for both PFO+ and PFO- groups were significantly lower during the PH trial than the IH trial (PFO+: 0.08 ± 0.15 L min^{-1}

SpO_2^{-1}, PFO-: 0.10 ± 0.21 L min^{-1} SpO_2^{-1}). The findings, which suggest that there are no differences in AHVR between PFO+ and PFO- during poikilocapnic hypoxia, extend the day 1 findings of AltitudeOmics study (Elliott et al. 2015), which suggested that there are no differences in HVR upon initial exposure to 5260 m. This extension and confirmation of the findings of the AltitudeOmics study is vital since it was possible that the lack of differences seen in that study was caused by uneven numbers of females in each group. By studying AHVR during PH conditions with equal representation of males and females in each of the groups, the current study aimed to better explore the effect of PFO on HVR during acute PH.

During the isocapnic hypoxia (IH) trial, increase in ventilation is regulated by input from both the peripheral and central chemoreceptors. While reducing $P_{ET}O_2$ value to 45 mmHg to increase input from peripheral chemoreceptors, $P_{ET}CO_2$ value was also maintained at the subjects' resting values so that input was help constant. By maintaining the input from the central chemoreceptors, the IH trial aimed to create a unique condition where only the peripheral chemoreceptors are activated—thus, in this condition the contribution of the peripheral chemoreceptor is isolated from the central chemoreceptor. For the IH trial, we expected to see two distinct phases in the subjects' ventilatory profile: the first phase (0-5 min) of immediate increase in ventilation, referred to as AHVR; followed by a second phase (5-20 min) of slow decline in ventilation (Duffin, 2007), referred to as HVD.

We observed no significant differences in AHVR during the IH trial between the PFO+ and PFO- subjects. The AHVR observed in both groups (PFO+: 0.85 ± 0.39 L min^{-1} SpO_2^{-1}, PFO–: 0.84 ± 0.41 L min^{-1} SpO_2^{-1}) were similar to previous research of

similar design (Kolb et al., 2004), leading us to believe that there is no difference in baseline peripheral chemoreceptor sensitivity between PFO+ and PFO- subjects. Similarly, there were no differences in HVD during the IH trial between the two groups (PFO+: 62 ± 22 % decline, PFO-: 68 ± 23 % decline).

According to the findings of this study, it is likely the ventilatory profile to hypoxia could be redrawn for those with a PFO, where PFO- subjects represent the normal ventilatory profile to high altitude exposure. While PFO+ subjects appear to have a normal AHVR and HVD, as the ventilatory acclimatization process unfolds, their ventilation does not increase as much when compared to PFO- subjects. Based on the following findings, the differences in ventilatory acclimatization with chronic exposure to high altitude are likely not due to baseline differences in hypoxic chemosensitivity, but rather they are likely due to differences in the central chemoreceptor sensitivity.

Clinical Relevance of PFO and Chemosensitivity to O_2

The goal of research in applied physiology is to add to the growing knowledge of the human body and how it could benefit human lives. Thus, it is important to conclude by discussing the clinical relevance of PFOs and altered chemosensitivity to O_2. There have been observations regarding high prevalence of PFO in stroke patients (Bogousslavsky, et al. 1996). A PFO can allow for blood to bypass the pulmonary particulate filter, which could allow thromboemboli to enter arterial circulation, potentially leading to stroke. Additionally, a PFO could lead to impaired respiratory cooling system, as previous studies have reported PFO+ individuals to have ~0.4°

higher core temperature (Davis *et al*., 2015) but a blunted ventilatory response to passive heating (Davis *et al*., 2017 *in review*) when compared to PFO- individuals. These factors could potentially be linked with impaired tolerance for heat stress.

Other studies have shown a possible relationship between PFO and increased risks for acute mountain sickness (AMS) (Elliott *et al.,* 2014), and others have demonstrated a relationship between PFO and high altitude pulmonary edema (HAPE), reporting a PFO frequency 4 times greater in HAPE-susceptible population due to severe hypoxemia and exaggerated pulmonary pressure (Allemann *et al*. 2006). Chronic hypoxia will lead to decreased P_AO_2, thus pulmonary vasoconstriction, which leads to exaggerated pulmonary arterial pressure, further increasing the potential for right to left shunt and worsening hypoxemia. This vicious cycle of hypoxemia could be the mechanism behind the more severe hypoxemia that PFO+ subjects experienced compared to PFO- subjects, ultimately leading to AMS and HAPE (**Figure 10**).

There also exists potential association between blunted chemosensitivity to hypoxia and increased risks for sudden infant death syndrome (SIDS) (Kinney *et al*., 2009). Thus, if there is a relationship between PFO and blunted chemosensitivity, a PFO could be associated with higher risks for SIDS. This is one of several possible areas for future research regarding the PFO.

Limitations

There are several limitations to this study. First, the study utilized end tidal gases as an estimate of arterial blood gas levels of O_2 and CO_2. Similarly, in calculating the AHVR values, the study utilized a finger pulse oximeter to measure for arterial

blood O_2 saturation. Thus, the study used SpO_2, an effective estimate of SaO_2, to estimate the HVR. Additionally, although the study recruited similar number of males and females, it failed to completely account for all the different phases of the menstrual cycle. If not limited by financial resources, the current study could have better controlled for potential biological sex differences by further categorizing female subjects into their respective current menstrual cycle phases, since there is evidence linking high female ovarian hormone levels and increased ventilation (MacNutt et al., 2012).

Lastly, the study took place over two total visits to the cardiopulmonary and respiratory physiology lab. Because most of the subject population had not previously participated in a study of this nature, some subjects expressed mild apprehension when breathing on the end-tidal breathing response system, which would contribute to some variability in the baseline resting values. Since it is well established that increased sympathetic response can lead to hyperventilation, the mild apprehension could have contributed to this variability by resulting in higher than normal ventilation values. To eliminate many of the external and mental factors that could lead to variation in ventilation, one possible direction for future study would be to measure ventilatory responses in subjects who are in non-REM, slow-wave sleep, when ventilation is solely driven by basal metabolic rate.

Summary of Findings

- Despite differences in ventilatory acclimatization to high altitude, there exists no difference in acute hypoxic ventilatory response (AHVR) between PFO+ and PFO- subjects.
- PFO+ and PFO- showed no significant differences in hypoxic ventilatory decline (HVD).
- There were no differences in AHVR and HVD between males and females.
- Differences in ventilatory acclimatization with chronic exposure to high altitude are likely not due to baseline differences in hypoxic chemosensitivity.

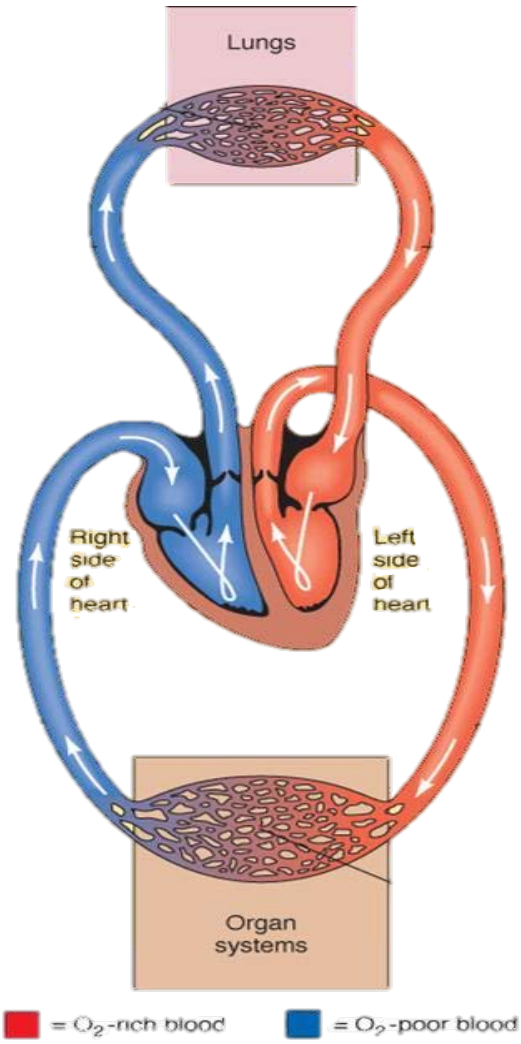

Figure 1. Overview of the Cardiopulmonary Physiology: Pathway of blood

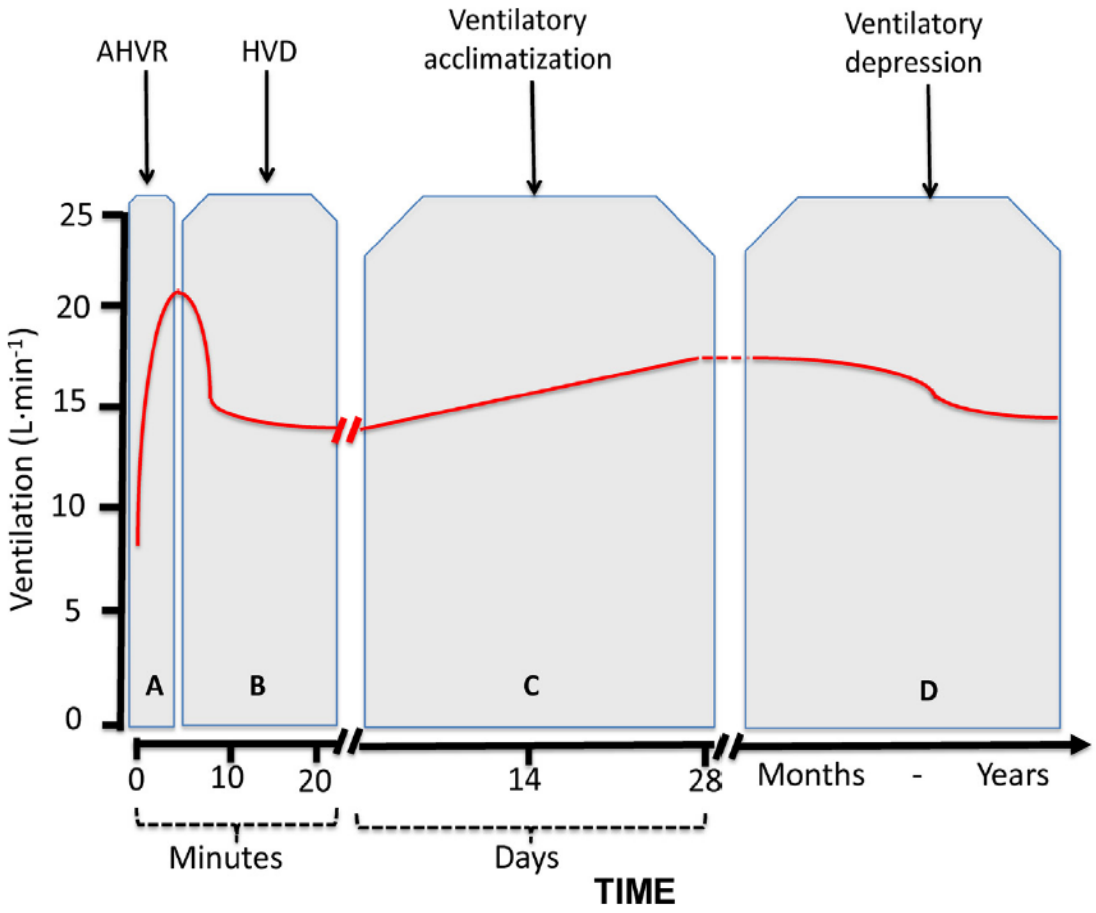

Figure 2. Changes in V_E upon acute & prolonged exposure to hypoxia; AHVR → HVD → ventilatory acclimatization → Hypoxic ventilatory depression (Ainslie *et al.* 2013)

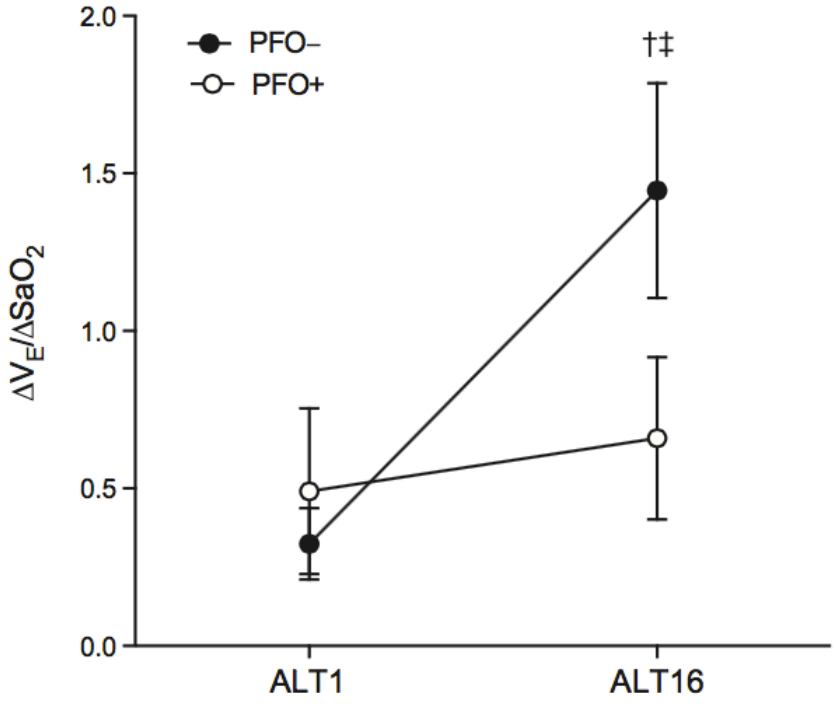

Figure 3. Observation of blunted HVR in those with PFO, when comparing day 1 HVR to day 16 HVR (Elliott *et al.*, 2015)

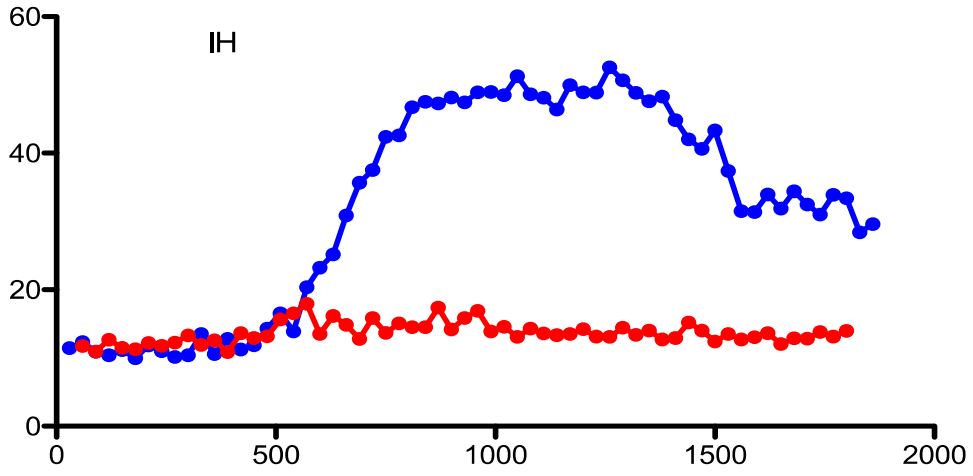

Figure 4. Example V_E Trace of a Subject (PFO-); Time points of interest (V_E Baseline, V_E Peak, V_E Decline).

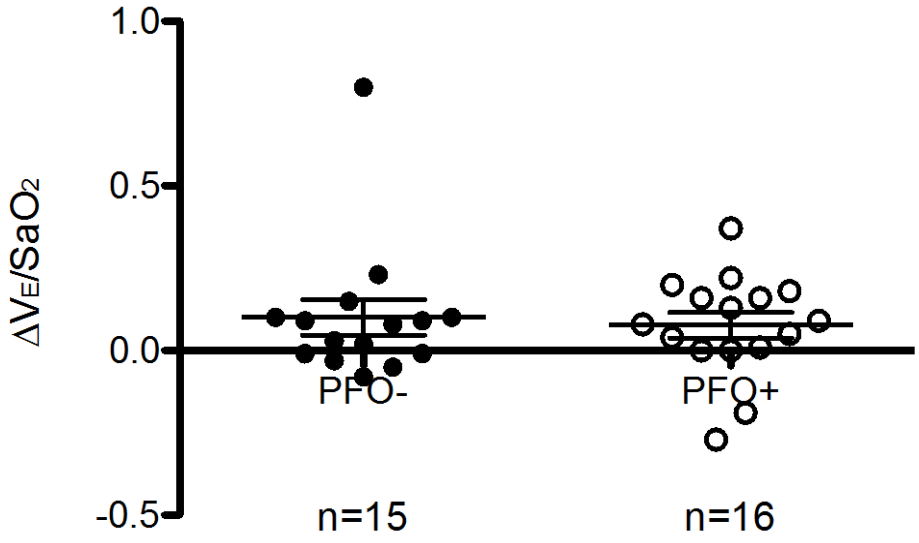

Figure 5. Effect of PFO on AHVR to Poikilocapnic Hypoxia. There was no significant difference in AHVR (mean ± SD) between PFO+ (0.08 ± 0.15) and PFO- (0.10 ± 0.21), p >.05 unpaired t-test.

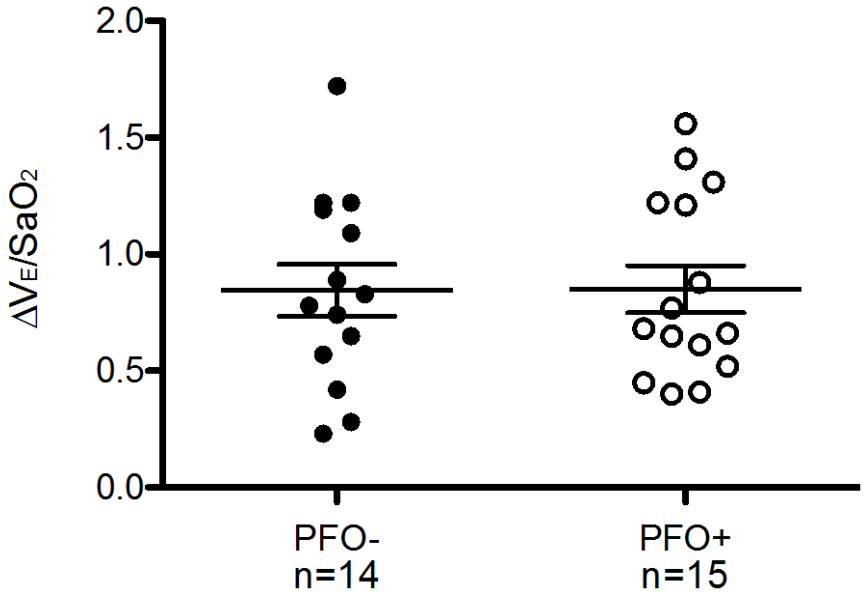

Figure 6. Effect of PFO on AHVR to Isocapnic Hypoxia. There was no significant difference in AHVR (mean ± SD) between PFO+ (0.85 ± 0.39) and PFO- (0.84 ± 0.41), $p > .05$ unpaired t-test.

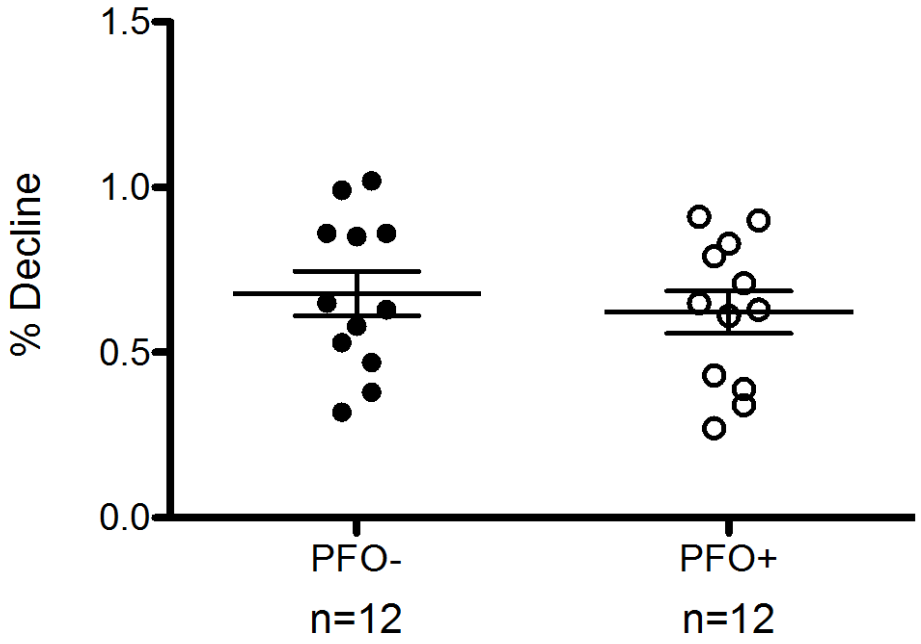

Figure 7. Effect of PFO on HVD during Isocapnic Hypoxia. There was no significant difference in HVD (mean± SD) between PFO+ (0.62 ± 0.22) and PFO- (0.68 ± 0.23), p >.05 unpaired t-test.

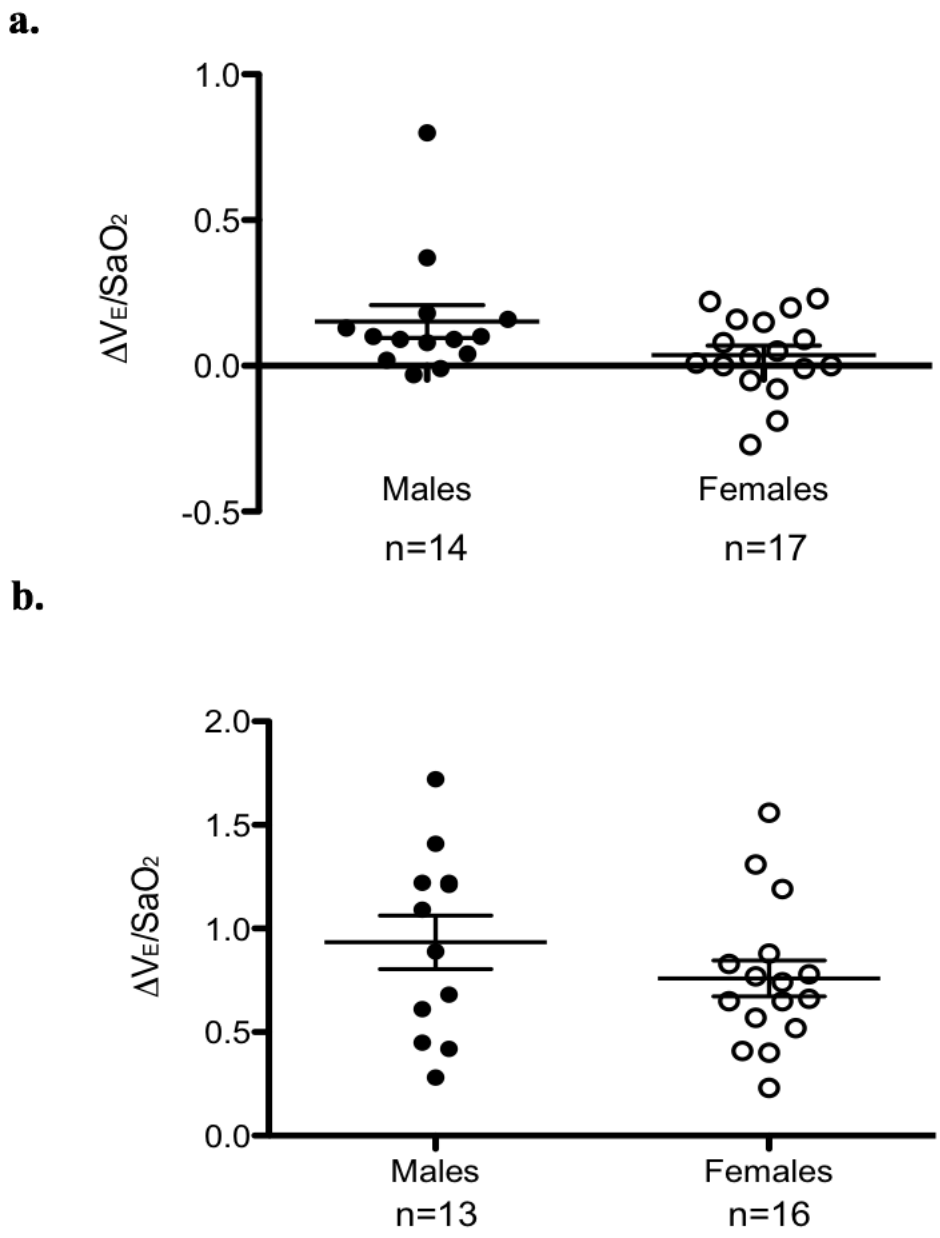

Figure 8. Effect of Biological Sex on AHVR to PH (**8.a**) and IH (**8.b**). There were no significant differences in AHVR to PH (mean± SD) between males (0.15 ± 0.21) and females (0.04 ± 0.14) nor to IH (mean± SD) between males (0.93 ± 0.45) and females (0.76 ± 0.35), p >.05 unpaired t-test.

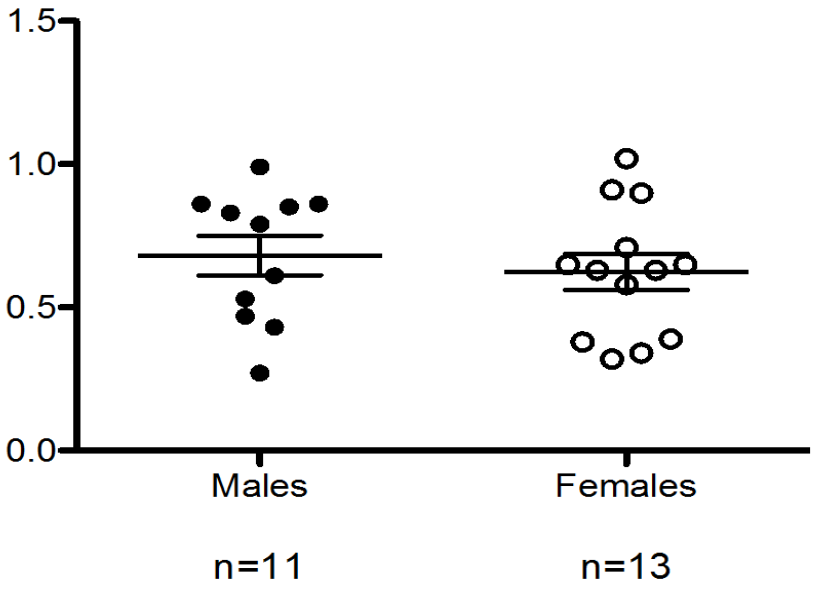

Figure 9. Effect of Biological Sex on HVD. There was no significant difference in HVD (mean± SD) to IH between males (0.68 ± 0.23) and females (0.62 ± 0.23), p >.05 unpaired t-test.

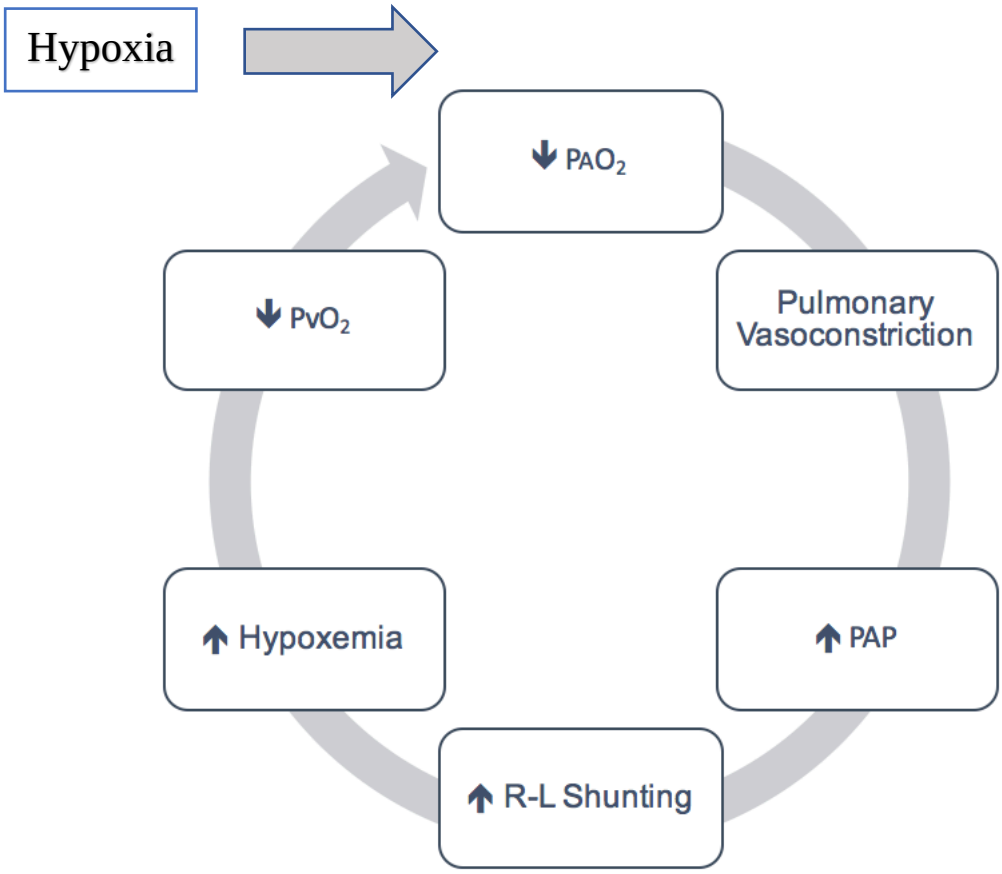

Figure 10. Mechanism behind PFO's association to worsening hypoxemia in high altitude, leading to increased susceptibility to AMS and HAPE

Table 1. Anthropometric and pulmonary function data

	PFO+			PFO–			Overall		
	Females $n = 8$	Males $n = 7$	Overall $n = 15$	Females $n = 9$	Males $n = 7$	Overall $n = 16$	Females $n = 17$	Males $n = 14$	Overall $n = 31$
Age (years)	25 ± 10	28 ± 6	24 ± 5	21 ± 2	28 ± 9	27 ± 9	23 ± 7	27 ± 9	25 ± 8
Height (cm)	165 ± 6	180 ± 9	170 ± 12	162 ± 7	180 ± 6	172 ± 10	163 ± 7	172 ± 10	171 ± 11
Weight (kg)	59 ± 5	82 ± 10	70 ± 15	59 ± 8	82 ± 15	71 ± 16	59 ± 7	71 ± 16	70 ± 15
BSA (m^2)	1.6 ± 0.1	2.0 ± 0.2	1.8 ± 0.2	1.6 ± 0.1	2.0 ± 0.2	1.8 ± 0.2	1.6 ± 0.1	1.8 ± 0.2	1.8 ± 0.2
FVC (L)	3.9 ± 0.5	5.6 ± 1.1	4.5 ± 1.3	3.6 ± 0.4	5.4 ± 0.5	4.6 ± 0.9	3.8 ± 0.5	5.5 ± 0.8	4.7 ± 1.1
FEV_1 (L)	3.3 ± 0.4	4.7 ± 0.4	4.0 ± 0.8	3.3 ± 0.3	4.6 ± 0.3	4.0 ± 0.7	3.3 ± 0.3	4.7 ± 0.4	4.0 ± 0.8
D_{LCO} ($ml \cdot min^{-1} \cdot Torr^{-1}$)	32.1 ± 4.3	45.1 ± 7.9	37.3 ± 11.5	27.0 ± 4.4	39.5 ± 6.3	36.1 ± 6.5	29.5 ± 5.0	42.1 ± 7.4	36.6 ± 8.9
D_{LCO}/V_A ($ml \cdot min^{-1} \cdot Torr^{-1} \cdot L^{-1}$)	6.2 ± 0.5	6.1 ± 0.4	6.0 ± 0.5	6.0 ± 0.6	5.7 ± 0.8	5.9 ± 0.7	6.1 ± 0.5	5.9 ± 0.6	6.0 ± 0.6

Values are mean ± standard deviation. No significant differences between groups

Table 2. Ventilatory and metabolic measures during isocapnic and poikilocapnic hypoxia

	Isocapnic Hypoxia				Poikilocapnic Hypoxia			
	Rest		Max V_E		Rest		Max V_E	
	PFO–	PFO+	PFO–	PFO+	PFO–	PFO+	PFO–	PFO+
V_E ($L \bullet min^{-1}$)	12.7 ± 1.2	11.6 ± 1.2	35.9 ± 2.9	34.4 ± 3.0	12.5 ± 0.9	11.9 ± 1.1	23.3 ± 2.6	22.4 ± 2.4
V_t (L)	1.2 ± 0.1	1.0 ± 0.1	2.2 ± 0.2	2.1 ± 0.2	1.1 ± 0.1	1.0 ± 0.1	1.9 ± 0.2	1.6 ± 0.2
RR ($breaths \bullet min^{-1}$)	11.8 ± 1.1	12.5 ± 0.7	17.7 ± 1.7	17.4 ± 1.1	12.4 ± 1.1	12.8 ± 0.8	13.4 ± 1.2	15.0 ± 1.1
$P_{ET}O_2$ (mmHg)	99.3 ± 1.2	99.4 ± 1.2	43.3 ± 1.1	44.1 ± 1.1	102 ± 1.5	101.9 ± 1.2	61.8 ± 10.6	51.5 ± 2
$P_{ET}CO_2$ (mmHg)	37.8 ± 0.9	38.2 ± 0.7	40.6 ± 0.7	39.4 ± 1.0	36.0 ± 1.4	38.3 ± 0.8	29.7 ± 2.3	28.5 ± 2.6
HR (bpm)	62.7 ± 3.3	60.7 ± 2.1	80.4 ± 3.9	82.9 ± 2.4	60.2 ± 3.3	67.4 ± 4.5	73.0 ± 4.2	76.9 ± 3.1
SpO_2 (%)	97.3 ± 0.4	97.4 ± 0.4	78.6 ± 1.0	78.9 ± 1.2	97.8 ± 0.3	97.3 ± 0.3	79.6 ± 1.4	78.9 ± 1.2

Values are mean ± standard deviation. No significant differences between groups.

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