COMPREHENISVE EXAMINATION OF THE DIFFERENCES IN

THERMOREGULATORY AND VENTILATORY RESPONSES

BETWEEN HUMANS WITH AND WITHOUT A PATENT

FORAMEN OVALE UNDER DIFFERENT

ENVIRONMENTAL CONDITIONS

by

JAMES THOMAS DAVIS

A DISSERTATION

Presented to the Department of Human Physiology
and the Graduate School of the University of Oregon
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

September 2016

DISSERTATION APPROVAL PAGE

Student: James Thomas Davis

Title: Comprehensive Examination of the Differences in Thermoregulatory and Ventilatory Responses Between Humans with and without a PFO Under Different Environmental Conditions

This dissertation has been accepted and approved in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Department of Human Physiology by:

Andrew T. Lovering	Chairperson
John R. Halliwill	Core Member
Hans C. Dreyer	Core Member
Matthew D. White	Core Member
Kryn Stankunas	Institutional Representative

and

Scott L. Pratt	Dean of the Graduate School

Original approval signatures are on file with the University of Oregon Graduate School.

Degree awarded September 2016

DISSERTATION ABSTRACT

James Thomas Davis

Doctor of Philosophy

Department of Human Physiology

September 2016

Title: Comprehensive Examination of the Differences in Thermoregulatory and
Ventilatory Responses Between Humans with and without a PFO Under Different
Environmental Conditions

The existence of a patent foramen ovale (PFO) has been known about for nearly 2,000 years. The prevalence of a PFO has been shown to be 25-40% in the general population. Despite the fact that blood flowing through a PFO acts as a shunt, there has been little research looking at the effect a PFO has on physiology in otherwise healthy humans.

In Chapter IV, the effect of a PFO on core temperature (T_{core}) prior to, and during exercise, was investigated. The design of this experiment included appropriate controls for a thermoregulatory study (i.e. measuring at same time of day, appropriate hydration and food intake, etc.). Results from this study indicate that subjects with a PFO (PFO+) have a T_{core} that is ~0.4°C higher at rest and during exercise than subjects without a PFO (PFO–). Additionally, this study showed that PFO– subjects do not increase T_{core} to the same extent breathing cold air as they do breathing ambient air during a 10-minute exercise bout, whereas there was no difference in T_{core} increase between these two conditions for PFO+ subjects. These findings suggest that the difference in T_{core} between PFO+ and PFO– subjects is potentially due to differences in respiratory heat loss.

The studies for Chapter V examined differences in thermoregulatory and

ventilatory responses during hot water (40°C) and cold water (20°C) immersion. This study found that compared to PFO– subjects, PFO+ subjects 1) increase T_{core} at the same rate during hot water immersion and 2) do not cool off as quickly during cold water immersion. Additionally, in subjects who reached a ventilatory threshold, PFO+ subjects had blunted ventilatory responses to increased T_{core} compared to PFO– subjects.

Finally, in Chapter VI it was shown that PFO+ subjects have blunted ventilatory responses during acute exposure to hyperoxic and normoxic hypercapnia. However, there were no differences in ventilatory responses between PFO+ and PFO– subjects during exposure to either isocapnic or poikilocapnic hypoxia. These findings suggest that PFO+ subjects have a blunted central chemoreflex.

This dissertation contains previously unpublished co-authored material.

CURRICULUM VITAE

NAME OF AUTHOR: James Thomas Davis

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon, Eugene, OR
California State University, Fullerton, Fullerton, CA
Brigham Young University, Provo, UT

DEGREES AWARDED:

Doctor of Philosophy, Human Physiology, 2016, University of Oregon
Master of Science, Kinesiology, 2011, California State University, Fullerton
Bachelor of Science, Exercise Science, 2000, Brigham Young University

AREAS OF SPECIAL INTEREST:

Patent Foramen Ovale
Thermoregulation
Pulmonary Physiology
Exercise Physiology

PROFESSIONAL EXPERIENCE:

Graduate Teaching Fellow, Department of Human Physiology, University of
    Oregon, September 2011 – June 2016

GRANTS, AWARDS, AND HONORS:

*Eugene & Clarissa Evonuk Memorial Graduate Fellowship in Environmental
    Physiology,* College of Arts and Sciences, June 2015

PUBLICATIONS:

Lovering, AT, Elliott JE, **Davis JT**. Physiological impact of patent foramen ovale
on pulmonary gas exchange, ventilatory acclimatization and thermoregulation. *J Appl
Physiol*; **121**, 512-517.

Duke, JW, **Davis JT**, Ryan B, Elliott JE. Beasley KM, Hawn JA, Byrnes W, Lovering, AT (2016). Decreased arterial PO2, not O2 content, increases blood flow through intrapulmonary arteriovenous anastomoses at rest. *J Physiol;* DOI: 10.1113/JP272211.

Lovering AT, Lozo M, Barak O, **Davis JT**, Lojpur M, Lozo P, Caljkusic K & Dujic Z (2016). Resting arterial hypoxemia in subjects with chronic heart failure, pulmonary hypertension and patent foramen ovale. *Experimental Physiology*; **101,** 657–670.

Davis JT, Ng C-YA, Hill SD, Padgett RC & Lovering AT (2015). Higher oesophageal temperature at rest and during exercise in humans with patent foramen ovale. *J Physiol* **593,** 4615–4630.

ACKNOWLEDGMENTS

effort to help me complete my final studies. It would have been impossible for me to accomplish as much as I have in the last year without the three of you.

Finally, I want to say thank-you to my family. While she might not understand how difficult this has been or how much she has helped, I am grateful for the encouragement and support my daughter Ellie has provided since I made the decision to go back to graduate school. I am also grateful to my parents Jim and Debbi Davis for their love and support. However, I have saved the best for last. I am forever indebted to my wife Michelle. You were crazy enough to marry me one month before we moved to Oregon, and you have been by my side the entire way. You are a truly amazing woman, and I will never know how I got lucky enough to find you. I love you and hope this makes for a great, belated 40th birthday present.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

A primary function of the cardiopulmonary system is to transport deoxygenated blood from the systemic circulation back to the pulmonary circulation where it is oxygenated and then returned to the systemic circulation. When blood reaches the pulmonary capillaries, oxygen binds to hemoglobin on red blood cells and is also dissolved into the plasma. At the same time carbon dioxide diffuses out of the plasma and into the airways. This oxygenated blood travels to the left side of the heart, where it is pumped to the systemic circulation so that oxygen can be delivered and carbon dioxide can be removed from the tissues. Deoxygenated blood leaving the tissues then returns to the right side of the heart where it is pumped into the pulmonary circulation, and the process starts over. An additional function of the cardiopulmonary system in humans is to dissipate heat via conduction and evaporation through the airways. This occurs when "hot blood" travelling through the pulmonary circulation dissipates heat into the alveoli and airways (Afonso *et al.*, 1962) where it is then exhaled into the environment.

The cardiopulmonary system is normally thought of as a closed system, such that all blood travels from the systemic circulation to the right heart, pulmonary circulation and left heart. However, there are a few known shunts in this system, such as intrapulmonary shunt pathways, bronchial and Thebesian circulation, and a patent foramen ovale (PFO), which allows blood to bypass part of this circuit. Of these three, the presence of a PFO is possibly the biggest break in this otherwise efficient system.

The foramen ovale, which is an opening between the right and left atria, is a normal part of the fetal circulation. This opening allows ~90% of the blood entering the

right atrium to bypass the pulmonary circulation and travel directly to the left atrium, where it is pumped into the systemic circulation (Murphy, 2005). While in utero the foramen ovale remains patent because right atrial pressure exceeds left atrial pressure. At birth pulmonary vascular resistance decreases due to the increased partial pressure of oxygen that occurs upon commencement of breathing. Decreases in pulmonary vascular resistance increase pulmonary blood flow, which reduces right atrial pressure causing left atrial pressure to exceed right atrial pressure, and the valvula foraminis ovalis to be forced against the left side of the septal wall. Normally, this valve fuses to the septal wall within the first two years of life (Geibel *et al.*, 2007). However, in ~35% of the population, this opening fails to close completely, and is referred to as a PFO (Hagen *et al.*, 1984; Marriott *et al.*, 2013; Elliott *et al.*, 2013).

Despite the relatively high prevalence of a PFO, little research has examined potential effects on physiology in healthy humans. Blood flowing through a PFO represents a right-to-left shunt that potentially affects the physiology of subjects who have this opening. For example, blood flowing through a PFO bypasses the pulmonary circulation, and therefore it does not undergo gas exchange or respiratory system cooling. This could reduce gas exchange efficiency, and the amount of respiratory heat loss (RHL) that occurs, which could result in decreased oxygen content and increased core body temperature (T_{core}), respectively. Consequently, changes to these physiological processes could in turn impact other physiological processes such as ventilation, heart rate, skin temperature (T_{skin}), and sweat rate. Accordingly, we sought to investigate the impact of PFO on several physiological processes in healthy humans.

The first objective, addressed in Chapter IV, was to determine if PFO+ subjects have a higher T_{core} than PFO– subjects. The second objective, addressed in Chapter V, was to determine if PFO+ subjects have altered thermoregulatory and ventilatory responses to passive heating and cooling. The third and final objective, addressed in Chapter VI, was to determine if a PFO affects ventilatory responses during acute exposure to hypoxic and hypercapnic environments.

Historical Perspective

"In the case of the other two (vena cava and pulmonary vein), which lie in contact with each other, there is a kind of orifice or fenestra (foramen ovale), common to both. At this orifice there is attached a membrane, like a lid or cover, opening toward the pulmonary vessel [left atrium], so that it will yield to the influx of blood from the vena cava, but will prevent its regurgitation into that vessel. So far, no doubt, we have much to admire in these contrivances of nature; but what surpasses them all is the way in which the foramen not long afterward becomes occluded. For soon after birth, either within a day or two, or, in some animals, after four or five days or a little longer, you will find the membrane at the foramen coalescing but not yet fully adherent. Looking at the same place in the adult animal, you would say there had never been a time when it was open; and, on the other hand, in a fetus, before or immediately after birth, when this membrane is attached, so to speak, only by its root, the rest of it hanging free in the vascular cavity, you would hardly believe in its ever becoming agglutinated."

– (Claudius Galen, ca 200 AD, from the Opera Omnia, vol. IV, p. 243; translation by Dalton, 1884, p. 69) (Dalton, 1884)

Embryologic development of the fetal heart begins as a single opening that eventually divides into the right and left atria and ventricles (Patten, 1938). As development of these chambers occur, one-way valves form which divide the right atrium and ventricle, as well as the left atrium and ventricle. These valves cause uni-directional blood flow through the heart. Conversely, the septal wall separates the right and left sides of the heart. This division forces deoxygenated blood entering the right atrium to go through the pulmonary circulation and undergo gas exchange before returning to the left side of the heart, where it is pumped into the systemic circulation.

While in utero, fetal gas exchange occurs at the maternal placenta, thus eliminating the need for gas exchange to occur in the fetal pulmonary circulation. Accordingly, prior to birth there is an opening in the septum that allows most blood entering the right atrium to go directly into the left atrium, with the remaining blood traversing the pulmonary circulation (Rasanen *et al.*, 1998; Murphy, 2005). This opening is referred to as a foramen ovale. Throughout fetal development, the foramen ovale changes from an opening between the right and left atria, to an incompletely formed septal wall.

Initially, the foramen ovale is an opening between the two atria. The septum primum then grows inferiorly from the superior portion of the heart until it connects with the endocardial cushion. Shortly after merging with the endocardial cushion, the superior portion of the septum primum dies, ostensibly due to apoptosis. This results in the foramen ovale consisting of an opening between the superior portions of the two atria. Shortly after the superior portion of the septum primum dies, the septum secundum

begins to grow from the superior wall of the right atrium, and to the right of where

formation of the septum primum began. By birth the septum secundum has descended

low enough that it is overlapping the septum primum. However, these two structures have

not merged together. This results in the overlapping portion of the septum primum acting

as a valve called the valvula foramina ovalis, which prevents left-to-right blood flow.

While in utero, right atrial pressure exceeds left atrial pressure in part due to high

pulmonary vascular resistance. Thus, 80-90% of in utero cardiac output bypasses the

pulmonary circulation (Rasanen *et al.*, 1998; Murphy, 2005). However, after birth

pulmonary blood flow increases due to decreased pulmonary vascular resistance, while at

the same time systemic vascular resistance gradually increases (Emmanouilides *et al.*,

1964). Once systemic vascular resistance exceeds pulmonary vascular resistance, mean

left atrial pressure surpasses mean right atrial pressure, and the valvula foramina ovalis is

forced against the septum secundum, thus preventing right to left blood flow across the

foramen ovale (Patten, 1938; Hagen *et al.*, 1984; Gao & Raj, 2010; Kerut *et al.*, 2013).

  Over the next several months, the valvula foramina ovalis progressively adheres

to the septum secundum, thus forming a permanent anatomical closure of the foramen

ovale and preventing right to left atrial blood flow. However, permanent anatomical

closure of the foramen ovale does not occur after birth in all humans, with the resulting

opening called a PFO. PFOs can occur due to the valvula foramina ovalis failing to

completely adhere to the septum secundum, fenestration of the valvula foramina ovalis,

or the valvula foramina ovalis being too small to cover the foramen ovale (Patten, 1938;

Aslam *et al.*, 2006; Kutty *et al.*, 2012).

A comprehensive publication by Patten (Patten, 1938) summarized PFO data in 9 autopsy studies which occurred from 1837 – 1934. This summary identified the presence of a probe patent PFO in 864/4083 subjects with prevalence ranging from 15% to 43% with an average of 21%. More recent autopsy studies (Hagen *et al.*, 1984; Kerut *et al.*, 2001) and studies using saline contrast echocardiography in living humans (Marriott *et al.*, 2013; Elliott *et al.*, 2013) support Patten's findings that PFO occurs in 25-40% of the population.

In addition to determining the overall prevalence of a PFO, Hagen *et al.* (Hagen *et al.*, 1984) found that the incidence of PFO decreases with age, declining from 34% in subjects between the ages of 0 and 29, to 25% in subjects between the ages of 30 and 79. While the incidence reportedly decreases, the average size of the PFO reportedly increases from 3.4 mm during the first decade of life to 5.8 mm during the 10th decade of life. These changes in incidence and size are likely due to the fact that complete adhesion of the valvula foramina ovalis and septum secundum can happen at any point, and that smaller PFOs are more likely to experience complete adhesion compared to larger PFOs.

Although a significant proportion of the population has a PFO, little research has examined its impact on physiological processes in healthy humans, presumably because it has been assumed to be of little significance. This is surprising as the normal model of cardiovascular physiology states that blood travels from the right side of the heart through the pulmonary circulation to the left side of the heart and then into the systemic circulation before it returns to the right side of the heart. This "normal model" of blood flow allows the lung to effectively perform several physiologically important roles including: pulmonary gas exchange, blood filter and respiratory system cooling.

However, PFO+ subjects have an opening between the right and left atria that can allow for varying, but potentially physiologically significant, amounts of cardiac output to bypass the pulmonary circulation in otherwise healthy humans. In order for a PFO to have a physiologically significant impact, right atrial pressure must exceed left atrial pressure for a time sufficient to allow for right-to-left blood flow across the foramen ovale. These conditions exist in the adult human at the end of a normal inspiration occurring concomitant with the end of ventricular diastole and beginning of ventricular systole (Strunk *et al.*, 1987; Aslam *et al.*, 2006; Fenster *et al.*, 2013*a*). Accordingly, right to left blood flow would likely occur to a greater degree under conditions of elevated right heart pressures such as exercise, high altitude, and in patients with pulmonary hypertension. Thus, blood that circumvents the pulmonary circulation will not undergo pulmonary gas exchange, be filtered in the pulmonary capillaries, or participate in respiratory system cooling, and there are certainly consequences that can occur as a result.

Interestingly, the foramen ovale and PFO have been studied for centuries. Dalton (Dalton, 1884), Patten (Patten, 1931) and Christie (Christie, 1930) have suggested that the scientific community has known about the existence of a PFO since the times of Galen (*ca* 200 AD), while Leonard Botali described the PFO in 1564 (Aslam *et al.*, 2006). Furthermore, as stated previously, 80 years ago the prevalence of a PFO was been estimated to be at least 25% (Patten, 1931). Although a significant proportion of the population has a PFO, little research has examined its impact on physiological processes in healthy humans because it has been assumed to be of a lower prevalence than actually exists, and therefore of little significance (Lovering *et al.*, 2011).

Recent work completed by Lovering et al. showed that during rest PFO+ subjects have decreased gas exchange efficiency, as measured by the alveolar-to-arterial oxygen difference ($AaDO_2$), compared to PFO–subjects (Lovering *et al.*, 2011). These findings are likely explained by the fact that blood flowing through the PFO does not undergo gas exchange in the pulmonary capillaries. Consequently, some deoxygenated blood enters the left ventricle, which reduces the arterial pressure of oxygen (PaO_2). Similarly, Elliott et al. showed that after 16 days of exposure to 5260 m PFO+ subjects have blunted ventilation responses, despite having lower PaO_2 and SaO_2, and an increased partial pressure of carbon dioxide ($PaCO_2$) than PFO– subjects (Elliott *et al.*, 2015). While the reasons for this blunted response are unknown, these findings demonstrate that the presence of a PFO affects physiologically relevant measures such as ventilatory acclimatization to altitude. Additionally, since blood flowing through a PFO does not undergo gas exchange or heat loss, it is plausible that in addition to the $AaDO_2$, a PFO may affect other measures such as T_{core}. If a PFO alters T_{core} then minute ventilation (V_E) and heart rate (HR) might also be affected. Reasons that a PFO could affect T_{core} include the fact that blood flowing through a PFO would not be able to dissipate heat into the airways, which could lead to a higher T_{core} in PFO+ subjects. Since PFO+ subjects have a widened $AaDO_2$, it is possible they could have a decreased PaO_2, which could stimulate an increased V_E. If PFO+ subjects have a lower PaO_2, this could decrease oxygen delivery, which could be offset by increasing heart rate, and therefore cardiac output.

The purpose of providing this historical perspective is to show that knowledge of a PFO is not new. However, despite the relatively large prevalence of PFO in the general population, very little research has been done to look at potential physiological effects of

a PFO. For example, although gas exchange has been studied extensively for over 100 years, it was not known until recently that a PFO affected gas exchange in otherwise healthy humans (Lovering *et al.*, 2011). Consequently, it is quite possible that some of the variation that is so often attributed to "normal biological variability" is caused by the presence or absence of a PFO.

Background and Significance

Patent Foramen Ovale

The cardiopulmonary system serves several important functions. Perhaps the most important of these functions is to transport oxygen from the inspired air and deliver it throughout the body, while eliminating carbon dioxide produced through metabolic processes. Additionally, the pulmonary circulation is generally thought of as a closed circuit. Deoxygenated blood enters the right side of the heart from the systemic circulation, where it is then pumped through the pulmonary circulation. Once blood reaches the pulmonary capillaries, oxygen diffuses from the alveoli into the blood, where it either binds to hemoglobin molecules on the red blood cells, or is dissolved into the plasma. Concomitantly, carbon dioxide in the plasma diffuses into the alveoli where it is exhaled into the ambient air. Oxygenated blood that returns to the left side of the heart is pumped into the systemic circulation. Despite the fact that the pulmonary circuit is generalized to be a closed circuit, there are multiple potential "holes." Perhaps the largest of these holes is a PFO. Blood flowing through a PFO is unable to participate in gas exchange. Consequently, this deoxygenated and carbon dioxide laden blood combines with blood returning from the pulmonary circuit, resulting in blood entering the systemic

circulation having lower oxygen and higher carbon dioxide concentrations than blood leaving the pulmonary capillaries. Despite the fact that there are certainly implications to these changes, and that the prevalence of PFO is ~25-40% among otherwise healthy humans (Hagen *et al.*, 1984; Marriott *et al.*, 2013; Elliott *et al.*, 2013; Fenster *et al.*, 2013*a*), little is known about the effect of a PFO on physiological responses in healthy humans.

Patent Foramen Ovale and Thermoregulation

Research completed by Lovering et al., demonstrated that PFO+ subjects have decreased gas exchange efficiency during rest as measured by the $AaDO_2$ (Lovering *et al.*, 2011). A surprise finding from that study was that PFO+ subjects had a higher T_{core} as measured by T_{esoph} than PFO– subjects. However, this study did not control for biological sex, ambient room temperature, time of day, or body surface area so it is difficult to definitively conclude, based on that study's design, if having a PFO results in a higher T_{core}. Despite these lack of controls, it is conceivable that PFO+ subjects have a greater T_{core}, as one of the functions of the lung and bronchial tree is to dissipate heat (Burch, 1945; Hanson, 1974). Blood flowing through a PFO would be unable to dissipate heat via respiratory system cooling, as it would not come in contact with the respiratory system, where up to ~10% of total body heat loss occurs (Burch, 1945).

The amount of heat retained could be affected by the amount of blood flowing through the PFO. It has been suggested that in some patients up to 21% of the cardiac output might flow through the PFO (Devuyst *et al.*, 2004). However, it is likely that in otherwise healthy, human subjects only 1-5% of the total cardiac output might flow through the PFO based on published resting $AaDO_2$ values (Devuyst *et al.*, 2004). PFO+

subjects who have blood flowing through the PFO could plausibly have a greater T_{core} due to decreased RHL.

Effect of a Patent Foramen Ovale on Heart Rate

In addition to finding that PFO+ subjects have worse gas exchange efficiency, findings from Lovering et al. suggest that PFO+ subjects might have different heart rates prior to exercise (Lovering *et al.*, 2011). While the 10-bpm difference between PFO+ and PFO− subjects was not significant, it is possible this was due to the study not having enough power to answer this question. The amount of oxygen delivered to the systemic circulation is determined by oxygen content and cardiac output. Since blood flowing through a PFO will reduce the overall oxygen content of arterial blood, delivery can be maintained by increasing cardiac output. This can be accomplished by increasing heart rate (HR). In addition to increasing HR to maintain oxygen delivery, HR might be greater in PFO+ subjects than PFO− subjects due to their higher T_{core}, because it is known that increasing T_{core} augments HR (Cabanac & White, 1995; Minson *et al.*, 1998).

Effect of a Patent Foramen Ovale on Ventilatory Responses to Hypercapnic and Hypoxic Environments

It has been well established that humans in hypercapnic or hypoxic environments increase ventilation in an effort to decrease carbon dioxide or increase oxygen in the body (Dempsey *et al.*, 1974; Powell, 2007; Duffin, 2007). However, until recently it was unknown if a PFO affected these responses. Elliott et al. recently demonstrated in a field study that PFO+ subjects have blunted ventilatory acclimatization to extreme altitude (5260 m) (Elliott *et al.*, 2015). In that study, there were no differences in ventilatory

11

responses after 1 day of exposure at 5260 m. It was expected that after several days of hypoxic exposure that ventilation would increase in both groups. However, they found that PFO+ subjects had a non-significant increase in ventilation compared to Day 1, while PFO– subjects had a larger, significant increase in ventilation. These findings were surprising since PFO+ subjects had a lower PaO_2 and SaO_2, while having a higher $PaCO_2$ than PFO– subjects. Although the physiological basis for this blunted response in PFO+ subjects is unknown, it provides some insight into the effect a PFO has on ventilatory responses. While Elliott et al. showed no differences in ventilatory responses during acute exposure to hypoxia, it is possible these findings were due to some unknown factor, such as a pyrogen, that couldn't be controlled for in a field study and/or wasn't measured. Furthermore, that study only provides information on poikilocapnic hypoxic exposure. To date there has been no research looking at the effect of PFO on ventilatory responses during acute exposure to normoxic hypercapnia, hyperoxic hypercapnia or isocapnic hypoxia.

Statement of Problem

The prevalence of a PFO is ~25-40% in the general population (Marriott *et al.*, 2013; Elliott *et al.*, 2013). However, to date, little research has examined the effect a PFO has on physiological processes in otherwise healthy humans. This is surprising as blood flowing through a PFO is a right-to-left shunt, which bypasses the pulmonary circulation. Blood flowing through the pulmonary circulation will undergo oxygenation and heat exchange. Thus, blood flowing through a PFO remains deoxygenated, and will not undergo heat exchange. It then follows that when blood flowing across a PFO combines

with blood returning from the pulmonary circulation, that the overall partial pressure of oxygen in the arterial blood will be reduced, while the temperature of the blood will be increased, which could then affect thermoregulatory and ventilatory responses.

Purpose and Hypotheses

The primary purpose of this dissertation is three fold: to determine if the presence of a PFO affects 1) core temperature during rest and exercise; 2) ventilatory and thermoregulatory responses to passive heating and cooling; and 3) ventilatory responses during acute exposure to hypoxic and hypercapnic environments.

Aim #1: Determine whether or not the presence of a PFO causes subjects to have a higher T_{core} as measured by T_{esoph} during rest and exercise in a thermoneutral environment due to reduced respiratory system cooling (~20°C).

To ascertain if a PFO is physiologically relevant as it relates to thermoregulation, we intend to determine if subjects with a PFO have a higher T_{core}, as measured by T_{esoph} before and during exercise. Previous research that provided a potential answer to this question was not designed to answer this question, as the main variable of interest was the alveolar-to-arterial oxygen difference (Lovering *et al.*, 2011). Consequently, appropriate controls were not in place for a temperature regulation study including the time of day, nutrition, fasting state and other factors known to influence thermoregulation (Sawka *et al.*, 2011). Therefore, it is still unknown if a PFO affects T_{core}. By establishing that a PFO plays a role in temperature regulation, it could cause a paradigm shift in our understanding of thermoregulation, as there would be two populations of humans that

13

thermoregulate in different ways. The experimental designs in this dissertation will allow us to determine if a PFO plays in thermoregulatory and ventilatory responses. Thus, the first aim of this dissertation is to establish whether or not a PFO actually plays a role in thermoregulation, specifically T_{core}, before and during exercise while breathing ambient and cold air.

Aim #2: Determine whether or not the presence of a PFO affects ventilatory and thermoregulatory responses to a) passive heating, and b) passive cooling.

With passive heating, ventilation increases once a threshold T_{core} is achieved (Cabanac & White, 1995; Lucas *et al.*, 2015). Potential explanations for this phenomenon include 1) increased metabolic activity and 2) the body making an effort to increase heat dissipation through respiratory system cooling in a two-phase panting response (White, 2006). This response also appears to be variable, and it is possible some of this variability might be explained by a PFO. If PFO+ subjects have a higher T_{core}, this might be due, in part, to reduced respiratory system heat loss. By having diminished respiratory system heat loss, PFO+ subjects would be unable to dissipate heat as effectively as PFO– subjects, which would result in PFO+ subjects increasing their T_{core} at a greater rate than PFO– subjects. Similarly, if PFO+ subjects are unable to dissipate heat as effectively as PFO– subjects, it is possible this will allow them to retain heat during cold exposure. Thus, the second aim of this dissertation was to determine if the presence of a PFO affects thermoregulatory and ventilatory responses to passive heating and cooling.

Aim #3: Determine if the presence of a PFO results in a greater hypoxic ventilatory response (HVR) and/or hypercapnic ventilatory response (HCVR) compared to PFO– subjects

Prior work done by Lovering et al. has shown that during resting conditions, PFO+ subjects have worse gas exchange efficiency as measured by the $AaDO_2$ than PFO– subjects (Lovering *et al.*, 2011). Additionally, Elliott et al. showed in a field study, that although there was no difference in HVR after 48 hours of exposure to 5260 m, over 16 days PFO+ subjects had decreased ventilatory acclimatization to altitude when compared to PFO– subjects despite a lower PaO_2 and higher $PaCO_2$ (Elliott *et al.*, 2015). Worsened gas exchange efficiency not only decreases oxygen content, but also augments $PaCO_2$. Increasing $PaCO_2$ has been shown to increase ventilation, but it is unknown if the response to elevated $PaCO_2$ is affected by the presence of a PFO. Therefore, the final aim of this dissertation is to determine if the presence of a PFO plays a role in the ventilatory responses during acute exposure to hypoxia or hypercapnia.

Chapter IV was published in the *Journal of Physiology* and Chi-Yan A. Ng, Sierra D. Hill, Dr. Richard C Padgett and Dr. Andrew T. Lovering are co-authors. I performed the experimental work, led the project, and the writing is entirely my own; Chi-Yan A. Ng and Sierra D. Hill assisted with data collection; and Dr. Richard C Padgett and Dr. Andrew T. Lovering helped develop the protocol, and provided guidance and editorial assistance

Chapter V will be submitted to the *Journal of Physiology* and Madeline W. Hay B.S., Alyssa M. Hardin, Dr. Matthew D. White and Dr. Andrew T. Lovering are co-authors. I performed the experimental work, led the project, and the writing is entirely my

15

own; Madeline Hay B.S. and Alyssa Hardin assisted with data collection and data analysis; and Dr. Matthew D. White and Dr. Andrew T. Lovering helped develop the protocol, and provided guidance and editorial assistance.

Chapter VI will be submitted to the *Journal of Physiology* and Lindsey Boulet B.S., Alyssa M. Hardin, Alex J. Chang, Dr. Glen Foster and Dr. Andrew T. Lovering are co-authors. I performed the experimental work, led the project, and the writing is entirely my own; Lindsey Boulet assisted with data collection, Alyssa M. Hardin and Alex Chang assisted with data collection and data analysis; and Dr. Glen Foster and Dr. Andrew T. Lovering helped develop the protocol, and provided guidance and editorial assistance.

CHAPTER II

REVIEW OF THE LITERATURE

Introduction

This literature review will sequentially follow the order of Chapters IV – VI, and the topic being addressed by each chapter. However, the patent foramen ovale is applicable to all chapters and will be addressed first. Additionally, Chapters IV – V address the topic of thermoregulation, although each chapter answers a different question related to thermoregulation. Therefore, thermoregulation will be addressed before delving into chapter-specific topics. Finally, ventilatory responses to hypoxia and hypercapnia will be addressed in the final section of this literature review as it relates solely to Chapter VI.

Patent Foramen Ovale

As stated previously, a PFO has been known to exist since the time of Galen (*ca* 200 AD) (Christie, 1930; Patten, 1931). However, despite this knowledge, little research has been done to investigate what effects the PFO might have on physiology in healthy humans. The most widely researched area with regards to a PFO is whether or not it contributes to the occurrence of stroke, transient ischemic attacks, or migraine, as blood clots can pass from the right atrium into the left atrium and then enter the systemic circulation. However, the literature is mixed, and debate continues as to whether or not PFO plays an important role in these issues (Kerut *et al.*, 2001; Meissner *et al.*, 2006; Aslam *et al.*, 2006; Geibel *et al.*, 2007; Kutty *et al.*, 2012).

Surprisingly, very little research has looked at the effects the presence of a PFO might have on other physiological processes. This is evidenced by a January 2016 search in the Journal of Applied Physiology only returning 12 articles that contain "patent foramen ovale" in the title or abstract, 9 of which were published since 2009. The pulmonary circulation is generally thought of as a closed circuit with blood entering the right side of the heart continuing thorough the pulmonary circulation, before entering the left side of the heart, where it is then pumped out to the systemic circulation. In PFO– subjects this is true, and the ability of the lung to perform its main functions of gas exchange and RHL is maximized. However, until recently, no research had looked at the effect of a PFO on gas exchange. Lovering et al. showed that during resting conditions in normoxic and hypoxic conditions, gas exchange, as measured by the alveolar to arterial oxygen difference ($AaDO_2$), was worse in PFO+ subjects (Lovering *et al.*, 2011). Furthermore, this study also suggested that a PFO might affect other physiological variables such as T_{core}. Similarly, Fenster et al., demonstrated that in 97 patients who underwent PFO closure, dyspnea and oxygen desaturation during exercise were reduced (Fenster *et al.*, 2013*b*). Elliott et al. has also shown that PFO+ subjects have an increased incidence of acute mountain sickness and blunted ventilatory acclimatization to altitude after 16 days at 5260 m (Elliott *et al.*, 2015). Taken together, these studies support the notion that the presence of a PFO can affect physiological responses in healthy humans.

Thermoregulation

Thermoregulation is vital to survival. One of the main functions of the body is to regulate T_{core}. According to the Glossary of Terms for Thermal Physiology, T_{core} is

defined as the mean temperature of the thermal core (Anon, 1987). T_{core} is monitored by the hypothalamus. This area of the brain receives afferent input from multiple places throughout the body, such as temperature sensitive neurons in the skin, muscle and abdomen. Additionally, the spinal cord is sensitive to changes in temperature (Satinoff, 1978). Since it is an integration center, the hypothalamus is the main regulator of T_{core}," with "normal" T_{core} being between 36.5 and 37.5°C under resting conditions, with some of this varability being attributable to Circadian rhythms. It must be noted that T_{core} can be measured by rectal and esophageal temperature in humans. Furthermore, there is inter- and intra-subject variability within this measure with studies demonstrating that resting T_{esoph} is 36-37°C (Curras *et al.*, 1991; Montain & Coyle, 1992; Cabanac & White, 1995; Griffin & Boulant, 1995; Altareki *et al.*, 2009; Lovering *et al.*, 2011).

While thermoregulation has been studied extensively for years, it is still disputed as to whether or not there a set-point temperature (T_{set}) or temperature null zone (T_{null}) (Bligh, 2006; Cabanac, 2006). A T_{set} implies that there is a specific T_{core} that is maintained and deviations from this temperature cause heat loss or conservation mechanisms to be activated. Thus, when T_{core} exceeds T_{set}, heat loss mechanisms, such as sweating and increased skin blood flow, are initiated. Conversely, when T_{core} falls below the T_{set}, heat generation and conservation mechanisms, such as shivering and increased central blood flow occur (Gonzalez-Alonso *et al.*, 1999; Coris *et al.*, 2004; Boulant, 2005). Alternatively, proponents of a T_{null} argue that there is a range of temperatures that exists, such that heat loss or conservation mechanisms are not initiated until T_{core} exceeds/falls below T_{null} (Mekjavić *et al.*, 1991). While T_{set} or T_{null} might not be the most accurate way to define how T_{core} is regulated, there is theoretically an operating point or

range ($T_{operate}$) that is regulated within the hypothalamus. Thus, T_{core} refers to the actual temperature of the hypothalamus which is estimated by rectal and esophageal temperature, and $T_{operate}$ refers to the temperature or null-zone of temperatures the brain is trying to maintain.

T_{core} can change for several reasons, including metabolic rate, illness or environmental conditions. Some of these conditions, such as when a person has a fever, cause the $T_{operate}$ to be shifted to a higher temperature. However, other circumstances, such as increased activity state, result in a change in T_{core} without a concurrent resetting of the $T_{operate}$. When T_{core} deviates without a concomitant change in $T_{operate}$, heat conservation or heat loss mechanisms are employed. Furthermore, as the difference between $T_{operate}$ increases, the response will be magnified. Multiple studies have shown that there are warm sensitive neurons that increase their firing rate, as T_{core} increases, as well as cold sensitive neurons that have increased firing rate as T_{core} decreases (Curras *et al.*, 1991; Griffin & Boulant, 1995; Noakes *et al.*, 2001). Additionally, $T_{operate}$ has a Circadian rhythm, such that T_{core} fluctuates throughout the day, with the lowest T_{core} occurring in the early morning hours, and the highest T_{core} happening during the late evening (Aschoff, 1983).

As mentioned previously, exercise results in T_{core} increasing above $T_{operate}$, initiating the onset of heat loss mechanisms including eccrine sweating and thermal hyperpnea (White, 2006). However, it is possible these mechanisms are not enough to return T_{core} back to $T_{operate}$. When heat loss mechanisms are unable to return T_{core} to $T_{operate}$, the priority of thermoregulation shifts from maintenance of a ~37°C T_{core}, to prevention of T_{core} from reaching a critical temperature ($T_{critical}$), which has been

hypothesized to be ~40°C (Nielsen *et al.*, 1993; 1997). However, it is unclear what physiological processes are employed to prevent T_{core} from reaching $T_{critical}$. One leading theory is that when T_{core} equals $T_{critical}$, the body begins to shutdown to prevent further damage from occurring (Gonzalez-Alonso *et al.*, 1999; Coris *et al.*, 2004). Another idea is that there is an anticipatory mechanism that prevents T_{core} from equaling $T_{critical}$ (Noakes *et al.*, 2001). As T_{core} rises due to increased metabolic rate arising from exercise, afferent feedback reaches the hypothalamus. This is interpreted to mean that $T_{critical}$ will be reached unless heat accumulation is reduced. Accordingly, exercise intensity decreases which leads to reduced heat production. While there is debate on how the body prevents T_{core} from reaching $T_{critical}$, it is generally accepted that the body is trying to prevent T_{core} from reaching a point that will cause damage (Gonzalez-Alonso *et al.*, 1999; Noakes *et al.*, 2001; Gonzalez-Alonso, 2007). Since maintenance of T_{core} in a physiological range is vital for normal human function, it would be important to understand why some people are better able to thermoregulate than others. If PFO+ and PFO– subjects have a different T_{core}, then it is conceivable that they would have different $T_{operate}$, otherwise they would have the same T_{core}. However, it is also possible that PFO+ and PFO– subjects have the same $T_{operate}$, despite differences in T_{core}. A possible explanation for this situation is selective brain cooling (SBC). SBC occurs when heat from: 1) the nasal cavity is lost in expired air, 2) the surface of the head and face is lost to the ambient air and 3) the internal carotid artery blood in the cavernous sinus is lost in countercurrent heat exchange in the carotid rete with the blood in the cooled venous drainage from the brain (Mariak *et al.*, 1999). Since SBC is affected by pulmonary ventilation, having a PFO would not inhibit SBC from occurring, as blood that flows through a PFO can travel to the brain.

Consequently, PFO+ and PFO− subjects could have different T_{core}, but have the same $T_{operate}$, if SBC occurs to a greater degree in PFO+ subjects than PFO− subjects. This could allow blood reaching the hypothalamus to be the same temperature in both groups. Thus, allowing PFO+ and PFO− subjects to have different T_{core}, but the same $T_{operate}$.

Some animals, such as canines, are known to dissipate large amounts of heat through a two phase panting response that includes a thermal tachypnea and a thermal hyperpnea. Humans in contrast only exhibit a thermal hyperpnea in an effort to dissipate heat (White, 2006). It has been suggested that up to 10% of heat loss from the body is carried away through expired air (Haldane, 1905; Burch, 1945; Hanson, 1974). As blood leaves the left ventricle and travels through the systemic circuit it picks up heat produced from the ongoing metabolic processes occurring within the body, resulting in an increased temperature of the blood and surrounding tissue (Afonso *et al.*, 1962). Blood flowing through the respiratory system is able to lose heat into the airways, which is then removed through the expired air. Once the blood reaches the pulmonary circulation, two things occur. First, carbon dioxide unloads from hemoglobin and oxygen loads onto hemoglobin. Both of these processes are exothermic (Haldane, 1905; Wyman, 1939; Hills, 1973), meaning that heat is produced which will further increase blood temperature. Second, assuming the airways have a lower temperature than pulmonary arterial blood, heat will be dissipated from the blood into the airways. This heat will then be lost to the environment as long as airway temperature is lower than environmental temperature. However, it is possible there is a perfusion component such blood flowing through a PFO, as well as a ventilation component to RHL. Thus perfusion dependent and ventilatory dependent RHL would be attenuated by increased blood flow through the

22

PFO and decreased V_E, respectively. Consequently, it is possible that in studies utilizing mouth breathing on a mouthpiece would essentially eliminate the amount of RHL that occurs in the upper airways. Thus, any differences seen would be attributable to RHL occurring in the lower airways.

Having a PFO could potentially hinder thermoregulatory processes in hot environments. While a PFO is considered a right-to-left shunt as it relates to gas exchange (Lovering *et al.*, 2011), this could also be the case for temperature (Afonso *et al.*, 1962). This will presumably lead to blood leaving the left side of the heart having a higher temperature than blood entering the left side of the heart. It follows that PFO+ subjects who have this "heat shunt," will have a higher T_{core} than PFO− subjects. Additionally, in environments where ambient temperature exceeds T_{core}, only evaporative RHL occurs. Consequently, the amount of blood flowing through a PFO would likely have no effect on RHL, since the temperature gradient will go from the environment into the body. Under these circumstances the only way to modify RHL would be with changes in V_E. If PFO+ and PFO− subjects have similar ventilatory responses to heat it follows that they would experience the same amount of RHL. However, if PFO+ subjects had a blunted ventilatory response in a hot environment, they would have decreased RHL, which could cause them to have a higher T_{core}.

Conversely, when T_{core} drops below $T_{operate}$, as would happen during passive exposure to a cold environment the body employs heat conservation mechanisms. A main response to reduced T_{core} is redistribution of blood from the periphery to the core. This can be accomplished by vasoconstriction of the skin blood vessels, which helps prevent heat loss from the skin to the environment. The redistribution of blood to the core leads to

an increase in central venous pressure, which will increase right atrial pressure and stroke volume. These protection mechanisms could be doubly protective for PFO+ subjects, as the increased right atrial pressure increases the likelihood that blood will flow across the PFO. Increased blood flow across the PFO decreases the amount of blood that enters the pulmonary circulation, which is then able to dissipate heat into the airways. Consequently, it is plausible that PFO+ subjects do not lose as much heat through the airways as PFO– subjects. Therefore, having a PFO could possibly be beneficial in cold environments as it would take longer for PFO+ subjects, when compared to PFO– subjects, to lose a given amount of heat.

Ventilatory Responses to Passive Heating

The ability to dissipate heat is of vital importance. Humans can function and live in extremely hot environments up to ~50°C, because of this ability. In addition to conduction and convection, humans also dissipate heat through evaporation of sweat and radiation of heat from the body to the environment. However, there are limitations to these processes. Evaporation and heat loss from the upper airways only occurs when the atmosphere is not saturated with water vapor, and the ability to lose heat via radiation only occurs when the environment is cooler than the body. Therefore, if a subject is in an environment that is hot and/or extremely humid, the ability to dissipate heat will be severely hampered.

Up to 10% of total body heat loss can come from the airways (Burch, 1945). Additionally, Hanson demonstrated that evaporative heat loss through the lungs is dependent on T_{core}, while convective heat loss through the lungs is dependent on T_{core} and

ambient temperature (Hanson, 1974). Since 100% of the cardiac output goes through the pulmonary circulation in PFO– subjects, it follows that more RHL should occur in these individuals than PFO+ subjects. Furthermore, as ambient temperature decreases the difference in T_{esoph} between these groups will be augmented, since convective heat loss increases as ambient temperature decreases. Additionally, instead of using the phrasing "PFO+ subjects are hotter than PFO– subjects," it is likely more correct to say that PFO– subjects are in fact cooler than PFO+ subjects, because they experience more convective cooling than PFO+ subjects.

While evaporation of sweat, and radiation of heat from the skin to the environment are important cooling mechanisms, they are not the only means of heat dissipation. As mentioned previously, humans are able to dissipate small amounts of heat through expired air (Haldane, 1905; Burch, 1945; Hanson, 1974; Cabanac & White, 1995). Heat loss occurs through evaporation in the inner airways. The amount of RHL is dependent on several variables, including V_E, inspired and expired temperature and humidity (Hanson, 1974; Kenny & Jay, 2011). Increased V_E and/or a large gradient between inspired and expired temperature are the primary drivers in augmenting RHL.

The effect of increased T_{core} on hyperpnea has previously been examined (Haldane, 1905; Gaudio & Abramson, 1968; Cabanac & White, 1995; White & Cabanac, 1996; White, 2006). Bligh and Johnson defined hyperthermia as a 1°C increase in T_{core} above resting values (Bligh & Johnson, 1973). While a 1°C increase in T_{core} constitutes hyperthermia, this does not appear to be a great enough stimulus to cause thermal hyperpnea in all humans. The threshold for hyperthermia induced thermal hyperpnea occurs when T_{esoph} increases by ~1.5°C above resting values (Cabanac & White, 1995;

Fujii *et al.*, 2008; Lucas *et al.*, 2015). Additionally, this response is different than the

panting response which occurs in canines, as V_E increases due to an increase in V_t instead

of an increase in frequency (Gaudio & Abramson, 1968; Cabanac & White, 1995). It has

been hypothesized this increase in V_E occurs in response to an increased metabolic

demand or as a cooling mechanism, but the physiological basis for this response has yet

to be fully elucidated.

Normally V_E is primarily mediated by changes in $PaCO_2$ (Nattie, 1999).

However, during hyperthermic conditions it is unclear if $PaCO_2$ is the main determinant

of V_E. There are a number of hypotheses that attempt to explain increased V_E during

hyperthermic conditions. One notion is that increased T_{core} increases afferent signaling

from temperature sensitive neurons, thereby augmenting respiratory efferent activity in

an effort to reduce T_{core}. Research completed by Cabanac and White supports the idea

that thermal hyperpnea occurs in an attempt to defend T_{core} (Cabanac & White, 1995). In

their study, subjects were passively heated in a hot tub. V_E did not increase until T_{esoph}

reached ~38.5°C. They hypothesized the hyperpneic response occurred in an attempt to

specifically cool the brain.

While findings from Cabanac and White showed that increased T_{core} augments

V_E, it is possible this did not occur solely to cool to the brain. That study also showed that

oxygen uptake (VO_2), which is a measure of metabolism had a non-significant increase of

0.07 L•min^{-1} in hyperthermic conditions compared to normothermic conditions. This

suggests that the augmented V_E might be partially affected by an increase in metabolism.

Similarly, findings from Saxton (Saxton, 1981) showed when going from normothermic

to hyperthermic conditions, V_E increased by ~50%, while VO_2 only increased by ~18%.

While both of these studies demonstrated a thermal hyperpnea, which is when V_E increases out of proportion to VO_2 during hyperthermic conditions, they also suggest that the increase in V_E is not due solely to the increase in VO_2. Thus, it is plausible that some or all of the remainder in increased V_E occurs in an effort to maintain T_{core}.

Another hypothesis is that under hyperthermic conditions there is an increased sensitivity of ventilation to CO_2 (Cunningham & O'Riordan, 1957; Gaudio & Abramson, 1968). Gaudio and Abramson demonstrated that an T_{core} increase of 1°C resulted in a hyperventilatory response, as $PaCO_2$ decreased from 44 to 33 Torr. This appears to indicate that CO_2 sensitivity increases with a 1°C increase in T_{core} (Gaudio & Abramson, 1968). Additionally, Cunnigham & O'Riordan showed that for a given $PaCO_2$, V_E was greater in hyperthermic conditions (Cunningham & O'Riordan, 1957). This finding supports the idea that increased T_{core} might result in an increased sensitivity to changes in $PaCO_2$. Likewise the ventilatory response to hypoxia is greater during hyperthermic conditions than normothermic conditions both at rest (Curtis *et al.*, 2007), and during exercise (Chu *et al.*, 2007). While the mechanism between hyperthermia and increased ventilation has not been clearly elucidated, it seems apparent that when T_{core} increases, there is a concomitant rise in ventilation. Furthermore, it is plausible that the increased V_E during hyperthermic conditions is a combination of all three factors. However, it has yet to be determined if all these factors contribute to the increased V_E and how much each contributes to the overall response.

While the mechanisms for thermal hyperpnea are unclear, it is plausible the presence of a PFO could affect these responses. Since PFO+ subjects do not have all of their blood going through the pulmonary capillaries, it follows that they would be

dissipating less heat into the airways than PFO– subjects. However, it has yet to be determined if PFO+ subjects have less RHL than PFO– subjects.

Thermoregulatory Responses to Passive Cooling

As stated previously humans can live in environments that are several degrees different from the normal T_{core} of 36-37°C. This is done using heat conservation or production mechanisms. According to Young's Model of Acclimatization, there are three classifications of how body heat can be maintained during cold exposure, one of which is cold habituation (Young, 2011). Habituation is a desensitization or dampening of the normal response to a stressor that occurs when stimuli are repeated. Cold habituation results in a blunted shivering response and decreased subcutaneous vasoconstriction. Subjects that experience some degree of cold habituation are still able to maintain T_{core}. If PFO– subjects are colder than PFO+ subjects, it is plausible they shiver at a lower T_{core} and/or have reduced subcutaneous vasoconstriction. If either of these responses are evident in PFO– subjects, this would suggest they have a reduced degree of cold habituation compared to PFO+ subjects.

In cases where repeated exposure results in decreased T_{core}, metabolic or insulative acclimatization can occur. Metabolic acclimatization increases shivering and non-shivering thermogenesis. Shivering thermogenesis is the process where involuntary muscle contractions result in no useful work, but rather most of the energy produced is converted to heat, which can be used to maintain or increase T_{core}. Nonshivering thermogenesis occurs in brown adipose tissue, which contains uncoupling protein 1 (UCP-1). UCP-1 allows protons to cross the inner mitochondrial membrane back to the

matrix without rephosphorylating ADP. This results in no useful work occurring, and all energy is lost as heat. If PFO+ subjects have a greater T_{core} than PFO– subjects, it could be due in part to having a higher T_{core} at the onset of shivering.

Insulative acclimatization includes enhanced cutaneous vasoconstriction as redistribution of blood flow to the core. This results in a decreased amount of heat loss, as less skin blood flow results in less heat lost to the environment when T_{core} exceeds ambient temperature. Consequently, if PFO+ subjects have a greater T_{core} they might experience a different amount of insulative acclimatization, which would be evident by reduced skin blood flow that could contribute to them having a greater T_{core} than PFO– subjects.

One way to determine if there are differences in thermoregulatory responses to the cold is through the use of a passive cooling protocol. Using cold-water immersion allows determination of thermoregulatory responses to cold at a fixed T_{skin}. This is important as T_{skin} is an important determinant of the T_{core} at which shivering occurs (Cheng *et al.*, 1995). Cheng et al. showed an inverse relationship between the T_{core} at which shivering occurred and T_{skin}. Consequently, when trying to determine if there is a difference in the rate of heat loss between PFO+ and PFO– subjects, the water temperature needs to be cold enough that will allow body cooling to occur, but not so cold that shivering begins almost immediately. Tikusis et al. demonstrated that subjects could remain immersed in 18°C water for up to 90 minutes, while losing ~0.7°C in that time frame. However, it must be noted that subjects remained immersed even after they began shivering, and it is unclear at what T_{core} there was an onset of shivering (Tikuisis *et al.*, 2000). Hayward and Eckerson immersed subjects in 0°C water for 25-40 minutes. While it isn't stated how

long it took for shivering to start or the T_{core} at which shivering started, subjects did achieve maximal shivering metabolism ~15 minutes after immersion (Hayward & Eckerson, 1984).

When humans are subjected to cold water, the initial minute is characterized by a large increase in ventilation, followed by a rapid decrease. Over the course of ~5 minutes, steady-state is reached, but ventilation is still greater than pre-immersion values (Hayward & Eckerson, 1984; Cooper & Veale, 1986; Mekjavić & Bligh, 1989). Similarly, VO_2 has an initial spike during the first minute of immersion, followed by a decrease over the next several minutes before plateauing at a value greater than resting values (Mekjavić & Bligh, 1989).

If PFO+ subjects undergo less respiratory loss as we hypothesize, it follows then that they would be better able to retain heat in cold environments than PFO− subjects. Furthermore, it is plausible that PFO+ subjects have a higher $T_{operate}$ and therefore would employ heat conservation mechanisms sooner (i.e. higher T_{core}) than PFO− subjects. Additionally, if this hypothesis is supported, it would be reasonable to conclude that PFO+ subjects are better suited to cold environments than PFO− subjects.

Ventilatory Responses to Acute Hypoxia/hypercapnia

One of the main functions of the cardiopulmonary system is to deliver oxygen from the atmosphere to the body, and release carbon dioxide from the body into the atmosphere. In healthy humans at sea level this process is accomplished relatively easily, as the partial pressure of inspired oxygen (P_IO_2) is ~160 mm Hg, which results in a PaO_2 of ~100 mm Hg. At this PaO_2 the firing rate of the peripheral chemoreceptor is relatively

low (Prabhakar & Semenza, 2015). However, if PaO_2 drops to ~60 mm Hg firing rate

increases dramatically, which subsequently causes a rapid increase in ventilation. PaO_2 is

primarily sensed by the peripheral chemoreceptors. These chemoreceptors are located in

the carotid sinus and the aortic arch. The peripheral chemoreceptors sense PaO_2, $PaCO_2$,

and pH. While the peripheral chemoreceptors primarily sense PaO_2, it must be noted that

the firing rate of these chemoreceptors is affected by $PaCO_2$, such that for a given PaO_2

the firing rate will be augmented as $PaCO_2$ increases (Nielsen & Smith, 1952). Under

normocapnic conditions when $PaCO_2$ is ~40 mm Hg, peripheral chemoreceptor firing rate

significantly increases when PaO_2 drops below 60 mm Hg. When PaO_2 is greater than 60

mm Hg, any change to PaO_2 results in a relatively small change to SaO_2. However, when

PaO_2 is below 60 mm Hg, any decrease in PaO_2 is accompanied by a large reduction in

SaO_2. Consequently, the oxyhemoglobin dissociation curve, which shows the relationship

between PaO_2 and SaO_2, has two distinct portions (Kelman & Nunn, 1966). The point on

the curve where PaO_2 is ~60 mm Hg is referred to as the shoulder of the curve, and it is

the *de facto* separation of the two parts of the curve. When PaO_2 reaches the shoulder of

the curve, and drops below 60 mm Hg, the low partial pressure of oxygen results in the

inhibition of K^+ channels while stimulating Ca^{2+} channels. The influx of Ca^{2+} stimulates

acetylcholine release which activates afferent fibers of the glossopharyngeal and vagus

nerves, resulting in an increased V_E. In addition to monitoring PaO_2, peripheral

chemoreceptors also sense $PaCO_2$. As $PaCO_2$ increases, the firing rate of the peripheral

chemoreceptors for a given PaO_2 is augmented (Duffin, 2007; Smith *et al.*, 2010).

In addition to monitoring PaO_2, the body monitors $PaCO_2$, via the central

chemoreceptors which are located in the medulla, specifically the retrotrapezoid nucleus

While the peripheral chemoreceptors primarily sense PaO_2, the central chemoreceptors monitor $PaCO_2$ indirectly via interstitial brain $[H^+]$. Unlike H^+, $PaCO_2$ is able to cross the blood-brain barrier. Once CO_2 crosses this barrier it can combine with water to form carbonic acid. Furthermore, this reversible reaction is catalyzed by carbonic anhydrase. After formation, carbonic acid dissociates into bicarbonate and H^+, which is sensed by the central chemoreceptors. Consequently, a $PaCO_2$ greater than 40 mm Hg is analogous to a high interstitial brain $[H^+]$. Thus, when $PaCO_2$ and therefore interstitial brain $[H^+]$, is high, V_E increases in an effort to increase pH, which reduces $PaCO_2$. Unlike peripheral chemoreceptors, which monitor $PaCO_2$ and PaO_2, central chemoreceptors are not sensitive to changes in PaO_2. However, the firing rate of these chemoreceptors can be influenced by the other. For example increasing the firing rate of the peripheral chemoreceptor will increase the gain of the central chemoreceptor and vice versa (Blain *et al.*, 2009; 2010; Smith *et al.*, 2010).

As mentioned previously, when compared to PFO− subjects, PFO+ subjects likely have a greater $PaCO_2$ and lower PaO_2. Under normal, resting conditions at sea level, these differences are likely negligible without any resulting differences in V_E between these two groups. This idea is supported by research completed by Lovering et al and Elliott et al. In these two studies, under normoxic conditions PFO+ subjects trended towards having a PaO_2 that was 2-5 Torr lower, a $PaCO_2$ that was 2-3 Torr higher and a SaO_2 that was ~1% lower than PFO− subjects (Lovering *et al.*, 2011; Elliott *et al.*, 2015). Additionally, it is important to note that while PFO+ subjects had a greater stimulus to breathe due to the decreased PaO_2 and increased $PaCO_2$, they actually had a non-significant, lower V_E than PFO− subjects. One possible explanation for these findings is

that PFO+ subjects have an altered sensitivity to decreased PaO_2 and increased $PaCO_2$, such that for a given $PaCO_2$, PFO+ subjects will have a lower reduced V_E. Additionally, when PaO_2 is ~100 mm Hg, small decreases in PaO_2 do not significantly affect ventilation.

While small changes in PaO_2 during hypercapnic conditions don't have a large effect on V_E, this is not the case under hypoxic conditions. If placed in a hypoxic or hypercapnic environment, both groups of subjects will increase their V_E in an effort to compensate for the low PaO_2 or high $PaCO_2$. Due to blood flowing through the PFO acting as a shunt, increasing V_E will not affect PaO_2 or $PaCO_2$ of this shunted blood. This will ultimately cause PaO_2 to be lower and $PaCO_2$ to be greater in PFO+ subjects for a given V_E. Consequently, for a given increase in V_E, PFO+ subjects will be unable to increase PaO_2 or decrease $PaCO_2$ to the same amount as PFO– subjects. If PFO+ and PFO– subjects have the same set point for chemosensitivity, it follows that PFO+ subjects would have a greater HVR and HCVR when exposed to a hypoxic environment in an effort to increase PaO_2 and/or decrease $PaCO_2$.

Summary

This literature review aimed to show that the presence of a PFO might account for some of the variability that occurs with physiological responses (i.e. changes in T_{core}, shivering threshold, heart rate, ventilation) to different stressors (i.e. exercise, temperature, low oxygen, high carbon dioxide) in healthy humans. It would be important to know what impact if any a PFO has, due to PFO prevalence being at least 25% in

otherwise healthy humans. Consequently, this dissertation was designed in an effort to

determine what, if any, effect a PFO has on several physiological processes.

CHAPTER III

METHODS

Informed Consent

Prior to the start of any study, the University of Oregon's Research and

Compliance Services and the Committee for Protection of Human Subjects formally

approved the protocols that comprise this dissertation (Chapters IV – VI). Additionally,

all subjects met with me individually and we verbally discussed the procedures and risks

involved with each study. Furthermore, written informed consent was obtained from all

subjects prior to participation.

Echocardiographic Screening

Our laboratory has been collaborating with the Oregon Heart & Vascular Institute

for more than eight years. All echocardiographic screenings for this dissertation were

completed by one of two highly skilled (combined 40+ years of experience) registered

diagnostic cardiac sonographers from the Oregon Heart & Vascular Institute. Subjects

participating in any aim of this dissertation underwent a comprehensive

echocardiographic screening, performed by Randall Goodman, RDCS, or Eben Futral

MBA, RDCS and overseen by myself. These screenings involved placement of a three-

lead echocardiogram with subjects positioned in the left lateral decubitus position in a

reclining chair and with their head resting on their left arm. This position was utilized

because it allows the heart to move anteriorly and laterally against the subject's ribcage.

Additionally, it allows the ribs to spread apart, which enables a clear apical, four chamber

view of the heart to be obtained. During the screening, male subjects were asked to go

shirtless, while female subjects wore a loose fitting scrub top. This made it easier for the

sonographers to use the ultrasound probe.

Screenings began with the sonographer thoroughly examining cardiac structures

in an effort to rule out any cardiac abnormalities, including obstruction of the right

ventricular outflow tract or stenosis of the pulmonary artery and/or aorta. Left ventricular

function and all valves were inspected to rule out indicators of congenital heart disease,

while the pericardium was examined to rule out pericardial effusion. After confirming

normal heart function without signs of heart disease, identification of a PFO was the next

phase of the echocardiographic screening.

Patent Foramen Ovale Detection

The foramen ovale is an opening between the two atria of the heart. This opening

is a critical component of the fetal cardiopulmonary system as it allows ~90% of the

blood entering the right atrium to bypass the pulmonary circulation and enter directly into

the left atrium (Rasanen *et al.*, 1998). Consequently, in utero the foramen ovale is patent

in 100% of healthy humans. At birth, left atrial pressure increases above right atrial

pressure due to decreased pulmonary vascular resistance and pulmonary pressure. These

changes cause blood flow to be reversed through the foramen ovale. However, the

septum primum, which is a flap of tissue on the left atrial side of the foramen ovale, is

forced against the septal wall, thus preventing a left-to-right shunt from occurring. Over

time the septum primum will fuse with the atrial septal wall, thus completely closing off

the foramen ovale. However, in 25-40% of the population the septum primum fails to

completely close and the opening is then referred to as a patent foramen ovale (Hagen *et*

al., 1984; Marriott *et al.*, 2013; Elliott *et al.*, 2013). Even if a PFO is present, right-to-left atrial blood flow can only occur when right atrial pressure exceeds left atrial pressure, which forces the foramina ovalis away from the septal wall. This normally only occurs when inspiration coincides with either ventricular diastole (Fenster *et al.*, 2013*a*), or early ventricular systole (Strunk *et al.*, 1987).

In order to determine if a subject had a PFO, a technique called saline contrast echocardiography was utilized. This involved the placement of an intravenous catheter (refer to Subject Instrumentation) in the antecubital fossa, which was attached to an extension set and a three-way stopcock. Two 10 ml syringes were attached to the stopcock. One syringe contained 3 ml of sterile saline, while the other contained 1 ml of air. The contents of these two syringes were vigorously agitated for ~10 sec, which created a suspension of microbubbles that were then injected into a peripheral vein, while the solution was being agitated, the heart was visualized in the apical, four-chamber view, which allowed simultaneous visualization of the right and left side of the heart. Within 3-5 heartbeats after injection, microbubbles were visualized as a "cloud of echoes" in the right heart. Microbubbles appearing in the left heart within 3 cardiac cycles of initial appearance into the right heart constitute evidence of a PFO (Marriott *et al.*, 2013; Fenster *et al.*, 2013*a*).

The timing of when contrast appears in the left side of the heart is important. In addition to a PFO, blood flow through intrapulmonary shunts can allow contrast to appear in the left heart. However, it has been shown that during resting conditions in humans, pulmonary transit time is ~9 sec (Hopkins *et al.*, 1996), which would mean that in a person with a resting heart of 50 bpm, it would take 7-8 cardiac cycles to occur before

contrast could appear in the left heart. Consequently, any left-sided contrast that appears within 4 cardiac cycles is strong evidence of a PFO (Fenster *et al.*, 2013*a*). However, contrast that appears after 4 cardiac cycles would not be conclusive evidence of a PFO, because this contrast could have travelled through the pulmonary circulation.

Since right atrial pressure is generally lower than left sided pressure, saline contrast injections were performed under two conditions– normal resting conditions and upon the release of a Valsalva maneuver. Utilization of a Valsalva maneuver transiently increased right atrial pressure above left atrial pressure, which allowed right-to-left blood flow if a PFO existed.

Performing a Valsalva maneuver is straightforward. Subjects were instructed to take in a small breath of air and then hold their breath for 10-15 seconds while "bearing down." This increased intra-abdominal pressure while reducing blood flow from the inferior cava into the right atrium. Once the Valsalva maneuver was released a large, transient increase of blood flowed into the right atrium, causing right atrial pressure to exceed left atrial pressure. When performing a Valsalva maneuver in an effort to detect the presence of a PFO, the saline contrast was injected just prior to release of Valsalva. Thus, if a PFO existed, microbubbles would be able to cross the PFO and visualized in the left heart. Since correct performance of a Valsalva maneuver is key in detecting a PFO, subjects practiced its performance and timing prior to completion of a Valsalva maneuver in conjunction with saline contrast echocardiography. Only after the subject demonstrated the ability to correctly perform the maneuver was it completed with saline contrast echocardiography.

Lung Function Testing

The second phase of the initial screening of subjects was pulmonary function testing. These tests were performed to confirm that all subjects had normal pulmonary function without indication of lung disease. All tests were conducted according to the guidelines established by the American Thoracic Society/European Respiratory Society (ATS/ERS) (Macintyre *et al.*, 2005).

Forced Vital Capacity

The forced vital capacity (FVC) maneuver measures the maximal amount of air that can be exhaled after a maximal inspiration and is performed during a maximal expiratory effort. Subject instrumentation consisted of a low-resistance mouthpiece fitted over a MedGraphics PreVent pneumotachograph, which was connected to a MedGraphics Elite Series Plethysmograph, and a noseclip. For this maneuver, subjects were seated at a 90° angle with their feet flat on the floor. Subjects were instructed to breathe normally for several breaths. Once normal tidal breathing was established, subjects were instructed to exhale until residual volume (RV) was reached. At that point, subjects then inspired to total lung capacity (TLC) as rapidly as possible. Once TLC was achieved, subjects then exhaled maximally for ~6 sec until attainment of RV. Correct performance of this maneuver produces a flow-volume loop, which represents the subject's FVC and provides information about pulmonary function.

In addition to the total volume of air expired during this maneuver, the volume of air expired during the first second (FEV_1) is also an important measure. The ratio of FEV_1/FVC is ~0.80 in normal, healthy adults. An $FEV_1/FVC < 0.80$ is indicative of flow

limitation due to some obstruction. This can occur due to some pathological condition (i.e. asthma or chronic obstructive pulmonary disease) or with age (Fuhlbrigge *et al.*, 2006; Vestbo *et al.*, 2011).

According to ATS/ERS guidelines, there must be a minimum of 3 trials to ensure validity and repeatability. Repeatability is achieved when the difference between the largest and second largest FVC and FEV_1 is < 0.15 L. Additionally all parameters measured during the FVC have predicted values based on the subject's sex, age, height, weight and race. All subjects used in this dissertation had values, specifically FVC, FEV_1 and FEV_1/FVC, $\geq$ 85% of predicted values.

Slow Vital Capacity

After completion of the FVC maneuver, subjects performed a slow vital capacity (SVC) maneuver. Completion of a SVC is similar to the FVC. Subjects were seated in the same manner as the FVC maneuver while performing regular tidal breathing. After completion of 4 successive breaths that were similar in size and duration, subjects were instructed to inhale to TLC after reaching functional residual capacity (FRC) during normal exhalation. Once subjects reached TLC, they relaxed and allowed the elastic recoil of the lungs to force out the air. Subjects then exhaled all the way to RV, before inspiring back to TLC. Performance of a SVC reduces the rate of small airway collapse, which can allow for a value greater than the FVC.

Whole Body Plethysmography

While spriometric measures like FVC and SVC are clinically useful to identify lung diseases, they are unable to provide measures of lung volumes (i.e. TLC, FRC and RV), due to the limitations of spriometry. Despite this fact, whole body plethysmography can be used to measure thoracic gas volume (TGV) so that RV, FRC and TLC can be calculated, and it is considered the gold standard of measurement of TGV. Whole body plethysmography utilizes Boyle's Law:

$$P_1V_1 = P_2V_2$$

Equation 3.1. Boyle's Law

This law states that in a closed system under isothermal conditions, the volume-pressure product remains constant. During the test, subjects sat in a closed box (i.e. closed system) while breathing on a mouthpiece. Within the plethysmograph there were two pressure transducers, one that measured mouth pressure where the subject is breathing, and one that measured box pressure. Applying this to whole body plethysmography, the volume and pressure of the subject's thoracic cavity is related to the volume and pressure of the plethysmograph. Performance of this maneuver begins similarly to that of the SVC, however the subject placed their hands on their cheeks to prevent "puffing" of the cheeks, which would alter the measurement. After completion of 4 tidal breaths that were of similar size and duration, subjects were instructed to pant at a rate of 70-90 breaths per minute. These pants were very shallow breaths that corresponded to a specific mouth pressure generated by the panting maneuver and box pressure generated by the chest wall movement. After panting at the appropriate rate for ~5 sec, a shutter within the mouthpiece apparatus closed, and subjects would continue to pant for 2-3 sec. Once the shutter reopened, subjects completed a SVC maneuver.

Referring back to Boyle's Law (**Equation 3.1)** for box pressure

$$P_{InitialBox}V_{InitialBox} = P_{FinalBox}V_{FinalBox}$$

Where

$$V_{FinalBox} = V_{InitialBox} - \Delta V$$

Therefore

$$P_{InitialBox}V_{InitialBox} = P_{FinalBox}(V_{InitialBox} - \Delta V)$$

We can then solve for ΔV, which can be used in combination with the values

determined using mouth pressure. Therefore, by using Boyle's Law for mouth pressure

$$P_{InitialMouth}V_{InitialLungs} = P_{FinalMouth}V_{FinalLungs}$$

Where

$$V_{FinalLungs} = V_{InitialLungs} + \Delta V$$

Therefore

$$P_{InitialMouth}V_{InitialLungs} = P_{FinalMouth}(V_{InitialLungs} + \Delta V)$$

Taken together, this set of equations allows for determination of $V_{InitialLungs}$, which

is the volume at FRC. This value can then be subtracted from expiratory reserve volume

to determine RV. TLC can then be calculated by adding RV to VC (Levitzky, 2013).

Repeatability was achieved when at least 2 FRC values were within 5% agreement

(ATS/ERS).

Diffusion Capacity for Carbon Monoxide

While several methods exist for determining lung diffusion capacity for carbon

monoxide (e.g. intra-breath, rebreathing and steady-state), our laboratory uses the single-

breath, breath hold technique (Knudson *et al.*, 1987; Macintyre *et al.*, 2005) using a

MedGraphics Elite Series Plethysmograph. This technique began with subjects seated

upright in the plethysmograph and their feet on the ground. Subjects were instrumented

with a noseclip while breathing in a normal, relaxed manner through a mouthpiece and

pneumotachograph that was attached to the plethysmograph. Once there were four

consecutive breaths that had similar depth and duration, subjects were instructed to

breathe to RV. During exhalation, the expiratory outlet closed. Once RV was achieved,

subjects rapidly inhaled to TLC. During this inhalation subjects received gas from a test

cylinder (21% oxygen, 0.5% neon, 0.3% carbon monoxide, balance nitrogen). After TLC

was achieved, subjects held their breath for 8 sec, after which the expiratory outlet

opened and subjects were instructed to exhale rapidly. In order for accurate interpretation

to be possible, subjects needed to inhale >85% of their predicted vital capacity. This

exhaled gas was forced through a column of diatomaceous earth, which separates gases

based on their size, and then analyzed by a gas chromatograph. Neon, which is

biologically inert, was used as a tracer gas to help determine the initial alveolar carbon

monoxide fraction and the volume at which carbon monoxide uptake occurs. The

computer then compared the volume of carbon monoxide delivered and exhaled in order

to determine the amount of carbon monoxide that diffused from the alveolar air into the

blood. The volume of diffused carbon monoxide is standardized to the breath hold

duration (Knudson *et al.*, 1987; Macintyre *et al.*, 2005) and is reported as an absolute

diffusing capacity (DL_{CO}) and a diffusing capacity relative to alveolar volume

(DL_{CO}/V_A). The DL_{CO}/V_A value is used to account for differences in lung volume that

occur with height and sex.

Subject Instrumentation

Esophageal Temperature

Core body temperature (T_{core}) was measured for Chapters IV, V and VI. There are several ways to estimate T_{core} (e.g. rectal probe, intestinal pill), but there are limitations with each that are dependent upon the subject's activity (e.g. intensity, duration, etc.) (Sawka *et al.*, 2011). However, for the protocols used in the aforementioned chapters, esophageal temperature (T_{esoph}) was used as it approximates the temperature of the left ventricle and is much more quick to respond to changes, as compared to rectal or intestinal measures of T_{core} (Sawka *et al.*, 2011). This was an important factor as all protocols were less than 60 minutes and involved rapid temperature changes of up to 3.0°C in that time.

For all subjects in Chapters IV, V and VI, T_{esoph} was a main variable of interest. I placed all temperature probes (Mon-a-therm General Purpose Probe, 7fr with Thermes USB, Physitemp, Clifton, NJ). Prior to placement of the probe and in an effort to eliminate the gag reflex, subjects self-administered 1 ml of 2% Lidocaine gel into the nostril the probe was going to be placed, which anesthetized the nasal sinus cavity and the back of the throat. Two to three minutes after the lidocaine administration, subjects were seated in an upright position and then instructed to look up toward the ceiling. The probe was then placed into the nasal cavity and advanced until the tip of the probe was visualized in the back of the throat. Subjects were then provided a cup of water and a straw and were instructed to take and swallow small sips of water through the straw. The probe was advanced when the subject swallowed and this process continued until the esophageal probe was estimated to be at the level of the left ventricle, based on the subject's sitting height (Mekjavić & Rempel, 1990). Once the probe was at the

44

appropriate depth, it was affixed to the subject's nose for the duration of the study to prevent it from being accidentally swallowed.

Peripheral Oxygen Saturation and Heart Rate

For Chapters IV and V, heart rate (HR) and peripheral estimates of arterial oxygen saturation were recorded with a forehead pulse oximeter (Nellcor N600x, Covidien, Dublin, Ireland) and a finger pulse oximeter was used for Chapter VI (ADInstruments, MLT 321, Colorado Springs, CO). These data were recorded continuously by the metabolic data acquisition system. The pulse oximetry system was composed of an infrared light emitting diode (LED) and a red light LED. The forehead pulse oximeter and finger pulse oximeter was placed directly over the pupil and on the index finger, respectively, in an effort to estimate arterial hemoglobin saturation. The wavelengths of the infrared (940 nm) and red (660 nm) lights are reflected differently, dependent on whether or not the hemoglobin molecule is bound with oxygen. Under normal, resting conditions ~97% of hemoglobin is bound with oxygen (HbO_2) and ~2% of hemoglobin is bound with carbon monoxide (HbCO). While HbO_2 and HbCO are different molecules, the pulse oximeter interprets both as oxygenated hemoglobin. Consequently, pulse oximetry overestimates true arterial oxygen saturation by ~2%. In addition to estimating oxygen saturation, the pulse oximeter measures heart rate based on the pulsatile changes in color. Heart rate was measured using a 3-lead echocardiogram for Chapter VI.

3-Lead Echocardiogram

All subjects undergoing the echocardiographic screening, as well as all subjects in Chapter VI, had a 3-lead ECG placed to continuously monitor electrical activity of the heart. Electrodes were placed on the right clavicle, left iliac crest and left clavicle. This electrical activity was displayed and recorded in real-time for the echocardiographic screening (Phillips ie33) and the Chapter VI protocol (ADInstruments, Colorado Springs, CO).

Dynamic End-tidal Forcing System

In Chapter VI, an end-tidal forcing system (DEF) developed by Glen Foster, PhD (Querido *et al.*, 2013; Bain *et al.*, 2013; Foster *et al.*, 2014) was utilized in order to induce hypoxia and hypercapnia, in a manner that would allow for accurate measurements of HVR and HCVR. Swanson and Belville developed the dynamic end-tidal forcing technique as a way to maintain end-tidal oxygen ($P_{ET}O_2$), and carbon dioxide ($P_{ET}CO_2$) values (Swanson & Bellville, 1975). Prior to that, research examining HVR and HCVR had used stepwise changes in oxygen and/or carbon dioxide. An inherent flaw with use of a stepwise protocol is that it is difficult to maintain stable $P_{ET}CO_2$ levels. During poikilocapnic hypoxic exposure, when PaO_2 decreases, ventilation increases, resulting in less carbon dioxide, thus making it difficult to elucidate the actual effect that a specific PaO_2 has on ventilatory responses.

Use of a DEF system allowed $P_{ET}O_2$ and $P_{ET}CO_2$ values to be maintained at specific values, which permitted us to measure the responses to isocapnic and poikilocapnic hypoxia, as well as normoxic and hyperoxic hypercapnia. This was accomplished by analyzing $P_{ET}O_2$ and $P_{ET}CO_2$ on a breath-by-breath basis. Based on the

concentrations of oxygen and carbon dioxide, the gas concentration of the next inspired breath was adjusted in an effort to maintain or reach the target values. It must be noted that $P_{ET}O_2$ and $P_{ET}CO_2$ are only estimates of PaO_2 and $PaCO_2$, and under some conditions end-tidal values significantly differ from direct blood gas measurements, possibly due to V/Q mismatch, diffusion limitation, intrapulmonary shunt, intracardiac shunt, or even body position (Robbins *et al.*, 1990; Tymko *et al.*, 2015). Since blood flowing through a PFO does not go through the pulmonary circulation, it is possible that $P_{ET}O_2$ and $P_{ET}CO_2$ will overestimate and underestimate PaO_2 and $PaCO_2$, respectively. These differences would likely be magnified in subjects who have the greatest blood flow through a PFO. However, utilization of this technique will provide initial insight as to whether or not the presence of a PFO affects ventilatory responses during acute exposure to hypercapnia and hypoxia. Obtaining $P_{ET}O_2$ and $P_{ET}CO_2$ for each of these four conditions allowed us to determine the individual and combined ventilatory responses of the central and peripheral chemoreceptors to hypoxia and hypercapnia. This was important as stimulation of one chemoreceptor can affect the gain of the other chemoreceptor (See – Literature Review), and we wanted to determine how these responses might be affected by the presence or absence of a PFO.

The DEF system was used in conjunction with a personal computer that was interfaced with an analog-to-digital converter (Powerlab/16SP ML 880; ADInstruments, Colorado Springs, CO). Acquisition of the respiratory parameters occurred at 200 Hz and data were analyzed using commercially available software (LabChart V7.1, ADInstruments, Colorado Springs, CO). During all trials, subjects wore a noseclip while breathing through a mouthpiece attached to a biological filter and a two-way non-

rebreathing valve (7900 series, Hans Rudolph, Shawnee, KS). The pneumotachograph (HR 800L, Hans Rudolph) and differential pressure amplifier (ML141, ADInstruments, Colorado Springs, CO) were calibrated with a 3-liter syringe,

During each trial, various amounts of N_2, O_2 and CO_2 were administered to the pneumotach's inspiratory port. Independent solenoid valves connected to each of the three gases controlled inspired gas composition. After the gas was mixed it was sent through a humidifier, before being taken up by the subject. Software designed by Foster et al. (Querido *et al.*, 2013; Bain *et al.*, 2013; Foster *et al.*, 2014) operated in conjunction with PowerLab, determined the composition of each breath. Expired air was sampled at the mouth and allowed to flow through nafion tubing and a desiccant to remove any moisture. The volume and gas composition of each breath was determined by previous measures of $P_{ET}O_2$, $P_{ET}CO_2$, tidal volume (V_t), breathing frequency (f), and minute ventilation (V_E). Based on these values, the end-tidal forcing system adjusted the inspired gas composition to bring end-tidal gases to the desired target values. Feed-forward control of the inspired gas was based on estimates of baseline metabolic O_2 consumption and CO_2 production and employs the alveolar gas equation (**Equation 3.2**) to determine the required fraction of inspired oxygen (F_IO_2) and carbon dioxide (F_ICO_2). The DEF system prospectively adjusted inspired air to bring end-tidal gas to the desired level. Gas control was fine-tuned using a feedback control and error reduction algorithm.

$$P_AO_2 = F_IO_2 \, (P_{ATM} - P_{H2O}) - PaCO_2/RER$$

Equation 3.2. Alveolar Gas Equation

This equation states that the partial pressure of oxygen in the alveoli (P_AO_2) is affected by the F_IO_2 and the ratio of $PaCO_2$ and the respiratory exchange rate (RER).

However, for the studies completed in this dissertation we did not measure arterial blood gases, consequently we used end-tidal gases, which as stated before can be significantly different from one another. Thus, this is a limitation of this approach.

CHAPTER IV

HIGHER OESOPHAGEAL TEMPERATURE AT REST AND DURING EXERCISE IN

HUMANS WITH PATENT FORAMEN OVALE

This chapter was published in the *Journal of Physiology* and Chi-Yan A. Ng,

Sierra D. Hill, Dr. Richard C Padgett and Dr. Andrew T. Lovering are co-authors. I

performed the experimental work, led the project, and the writing is entirely my own;

Chi-Yan A. Ng and Sierra D. Hill assisted with data collection; and Dr. Richard C

Padgett and Dr. Andrew T. Lovering helped develop the protocol, and provided guidance

and editorial assistance. All co-authors formally approved this manuscript prior to

submission.

Introduction

The foramen ovale has been studied for centuries and the scientific community

has known about the existence of a PFO since the times of Galen (*ca* 200 AD) (Christie,

1930; Patten, 1931). Autopsy studies (Hagen *et al.*, 1984; Kerut *et al.*, 2001) and studies

using saline contrast echocardiography in living humans (Woods *et al.*, 2010; Marriott *et

al.*, 2013; Elliott *et al.*, 2013) have demonstrated that a PFO is present in a substantial

proportion of the population with a prevalence of ~25-40% of the population. Individuals

with a PFO (PFO+) have an opening in the heart that can allow varying degrees of

cardiac output (Q_c) to bypass the respiratory system in otherwise healthy humans.

Accordingly, blood that circumvents the pulmonary circulation via the PFO will not be

filtered or undergo gas exchange, and there are certainly consequences that occur as a

result. Previous work by Lovering et al. investigating the impact of a PFO on pulmonary

gas exchange efficiency found, surprisingly, that core body temperature (T_{core}), measured by oesophageal temperature (T_{oeosph}), was higher in PFO+ subjects at maximal exercise compared to PFO– subjects. However the reasons for, and significance of, these findings were not determined in that study (Lovering *et al.*, 2011).

Respiratory system cooling, a well-known function of the lungs and airways, occurs via convective and evaporative heat loss at rest and during exercise (Burch, 1945; Hanson, 1974). Blood flowing through a PFO would not participate in respiratory system cooling and therefore would retain the heat that would otherwise be lost at the level of the respiratory system. Thus, in addition to affecting gas exchange and reducing the filtering ability of the pulmonary microcirculation, a PFO may also affect other physiological measures related to cardiorespiratory physiology, such as T_{core}. Additionally, any effect of the PFO would likely depend on the size of the opening between the atria.

While it is known that a PFO exists in a significant proportion of the population, there is little known on the potential impact of this intracardiac shunt on physiological processes outside of pulmonary gas exchange. Therefore, the purpose of this study was threefold. Our primary purpose was to determine whether or not having a PFO is associated with differences in T_{core}. Our secondary purpose was to ascertain whether or not the predicted size of the PFO is an important determinant in the association of PFO with T_{core}. Our tertiary purpose was to learn whether or not inhalation of cold and dry air influences T_{core} responses to exercise in subjects with and without a PFO. It was hypothesized that 1) PFO+ subjects would have a higher T_{oesoph} compared to PFO– subjects, 2) subjects with large PFOs would have the greatest T_{oesoph}, and 3) compared to breathing ambient air, when breathing cold and dry air prior to and during exercise,

subjects with large PFOs would increase T_{oesoph} to the same or greater degree whereas

PFO– subjects and those subjects with a small PFO would not increase T_{oesoph} by the

same amount.

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.

Participants

A total of 55 subjects were recruited for participation in this study. Researchers

described orally, and in writing, the nature of the study to all subjects, who subsequently

provided their written consent. A total of 30 subjects (15 PFO+, 15 PFO−) qualified and

completed the entire study. Of the remaining 25 subjects who did not complete the study,

5 did not pass pulmonary function due to forced vital capacity being less than 85%

predicted, 3 withdrew before completing the entire protocol for reasons not associated

with the study (time commitment, etc.), 8 individuals could not tolerate the oesophageal

probe, 7 individuals had late appearing contrast (>5 cardiac cycles after RV

opacification) and 3 individuals were identified as PFO+ after 15 PFO+ subjects had

already completed the study. In total we had a PFO prevalence of 52% which is greater

than what has been previously reported (Hagen *et al.*, 1984; Kerut *et al.*, 2001; Woods *et

al.*, 2010; Marriott *et al.*, 2013; Elliott *et al.*, 2013), because some subjects were invited to

participate that had been previously identified as being PFO+ or PFO−. However, none of

the subjects participating in this study and the study completed by Lovering et al (Lovering *et al.*, 2011). Ultimately, 30 (15 PFO+, 15 PFO) healthy, non-smoking male volunteers, age 24 ± 5 yr., without history of cardiopulmonary disease were recruited and, after written informed consent was given, agreed to proceed with the study.

Ultrasound Screening

Ultrasound screening has been previously described in detail (Lovering & Goodman, 2012). Initial agitated saline contrast studies were performed with subjects breathing room air and reclined at 45° in the left lateral decubitus position where a clear apical, four-chamber view was obtained. Care was taken to optimally visualize all four chambers, interatrial septum and delineate myocardial and valvular structures by individually adjusting the receiver gain settings. Each saline contrast injection was created by manually agitating 3 ml of sterile saline with 1 ml of room air for 15s between two 10 ml syringes connected in parallel to two 3-way stopcocks. The saline–air microbubble suspension was then immediately injected in a constant, forceful manner into a peripheral antecubital vein via an IV catheter (20–22 G). This mixture of saline and air provides excellent right-sided contrast. Following opacification of the right atrium and ventricle, the subsequent 20 cardiac cycles were recorded at >30 frames/s for further analysis.

The appearance of ≥1 microbubble in the left atrium or ventricle in any frame during the subsequent 20 cardiac cycles served as the criterion that subjects were either positive for an intracardiac right-to-left shunt (i.e. PFO) or demonstrated the transpulmonary passage of contrast (Freeman & Woods, 2008; Woods *et al.*, 2010;

Marriott *et al.*, 2013; Elliott *et al.*, 2013). Saline contrast injections were performed during normal breathing, as well as immediately following the release of a Valsalva manoeuvre in order to transiently elevate right atrial pressure and create conditions optimal for the detection of an intracardiac right-to-left shunt. Effective Valsalva manoeuvres were confirmed by a transient leftward shift of the interatrial septum. Valsalva manoeuvres do not increase left heart contrast in the absence of a PFO. An intracardiac right-to-left shunt was suspected if microbubbles appeared in the left heart ≤3 cardiac cycles following right heart opacification. Subsequently, these subjects were classified as PFO+, while all others without left sided contrast were categorized as PFO−. Using this approach we have shown that we have the sensitivity to accurately detect PFO in the general healthy population (Elliott *et al.*, 2013).

It has recently been demonstrated that the degree of left-sided contrast visualized upon release of a Valsalva manoeuvre in PFO+ subjects correlates with the size of the PFO (Fenster *et al.*, 2013*a*). Accordingly, those with more contrast likely have a larger PFO compared to those with less contrast. Taking a similar approach to that by Fenster and colleagues, in this study, subjects who had ≤12 bubbles upon release of Valsalva manoeuvre in the left ventricle in any one frame were classified as having a small degree of shunt, while subjects with ≥13 bubbles upon release of Valsalva manuever were classified as having a large degree of shunt. During the screening process, within the final PFO+ group of 15 subjects, 8 subjects were categorized as having a large degree of shunt upon the release of a Valsalva and 7 subjects were categorized as having a small degree of shunt upon the release of a Valsalva so there was a nearly equal distribution of large and small PFO sizes within the PFO+ group.

54

Pulmonary Function and Lung Diffusion Capacity

Prior to the pulmonary function testing, using an electronic scale (Ohaus Corporation, ES200L, Pinebrook, NJ) researchers obtained the subject's weight while wearing shorts as well as the subject's standing and sitting height. Baseline pulmonary function testing included measures of forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and forced midexpiratory flows (FEF25–75). Measurements were made with a computerized spirometry system (Ultima PFX, MedGraphics, St. Paul, MN) according to American Thoracic Society/European Respiratory Society (ATS/ERS) standards (Macintyre *et al.*, 2005). Lung volumes and capacities were determined using whole-body plethysmography (Wanger *et al.*, 2005). Lung diffusion capacity for carbon monoxide (DL_{CO}) was determined by the single-breath, breath-hold method (Knudson *et al.*, 1987; Macintyre *et al.*, 2005) using the Jones and Meade method for timing and alveolar sample collection (MedGraphics Ultima PFX, Breeze v.6.3.006) . Predicted values for DL_{CO} were calculated as previously described (Gutierrez *et al.*, 2004).

Exercise Protocols

Subjects completed three different exercise protocols, each separated by a minimum of 48 hours. After obtaining the subject's weights, experimenters placed the oesophageal probe through the nostril to a specified depth beyond the nasal flare based on the subject's sitting height, as before (Mekjavić & Rempel, 1990). Once instrumentation was completed, subjects sat on the exercise bike and remained seated in a forward leaning position until the protocol was completed. For each exercise trial, subjects

breathed through a low-resistance two-way nonrebreathing mouthpiece (model 2400, Hans Rudolph, Kansas City, MO), and pneumotachograph (MedGraphics PreVent). This inspiratory side of the mouthpiece was connected to a custom made large bore (~5 cm diameter) corrugated stainless-steel tube that contained ~8 L of air [designed by Kris Johnson and the University of Oregon Scientific Instrument Fabrication and Engineering Shop after that previously described (Muller *et al.*, 2011)].

During the first trial (Trial #1), participants completed an incremental cycle to exhaustion to measure VO_{2MAX}. Subjects completed the test on an electronically braked cycle ergometer (Lode Excalibur Sport). Following the 10-minute pre-exercise period, subjects completed a 2 minute warm-up period at 50 W. Immediately after the warm-up period, the exercise protocol began with subjects beginning at 75 W. Power output increased 25 W every 60 seconds until subjects achieved volitional exhaustion. Researchers obtained all measures every 60 seconds during the entire protocol. Measures obtained included oesophageal temperature (T_{oesoph}), inspired air temperature (T_{insp}), expired air temperature (T_{exp}) (Thermes USB, Physitemp, Clifton, NJ) heart rate (HR) (Tyco, Nellcor Oximax N-600, Mansfield, MA), predicted arterial saturation (SpO_2)(Tyco, Nellcor Oximax N-600, Mansfield, MA), rating of perceived exertion for lungs and legs ($RPE_{dyspnea}$ and $RPE_{leg\ discomfort}$, respectively) (Borg, 1973), oxygen uptake (VO_2), minute ventilation (V_E) (Medgraphics Ultima PFX, Breeze v6.3.006). Environmental measures (temperature [T_{air}], humidity and pressure) were also obtained every minute (Davis, Perception II, Hayward, CA).

During the remaining two trials (Trials #2 & 3), subjects followed the same pre-exercise and warm-up procedures except that the warm-up procedure was completed at

20% of the maximum workload achieved during the VO$_{2MAX}$ test. These two trials were

designed so that we could examine the effect of respiratory cooling on T$_{core}$ whereby

subjects either breathed ambient air, or cold and dry air. The two conditions were

randomly assigned for Trials #2 and 3. The exercise protocol consisted of four stages,

each 150 seconds in duration. These stages were completed at 25, 50, 75 and 90% of the

maximum power output attained during the first protocol **(Table 4.1)**. Researchers

obtained the same measures during Trials #2 & 3 as described above for Trial #1.

However, during the exercise protocol measures were obtained at the midpoint and

endpoint of each stage. Measures were obtained every 60 seconds during all other phases

of the experimental trial. For all exercise trials subjects breathed through the large bore

corrugated stainless-steel tube. During the cold and dry air breathing trial, subjects

breathed air, which was cooled by immersing the corrugated stainless-steel tube in a −

13.0 ± 4.3 °C (PFO+ −13.5 ± 4.5 °C vs. PFO− −12.5 ± 4.2 °C) saltwater slurry, which

resulted in an average T$_{insp}$ during the course of the entire exercise trial of 2.0 ± 3.5 °C

(PFO+ 2.0 ± 3.8 °C vs. PFO− 1.9 ± 3.1 °C). However, T$_{insp}$, which had an inverse

relationship with V$_E$, decreased as workload intensity increased **(Table 4.1)**.

Confounder Controls

To minimize confounding variability, investigators directed participants to

maintain their nutrition, hydration, and sleep schedules for each trial. Researchers

instructed subjects to maintain their physical activity throughout the study to minimize

changes in aerobic capacity. Participants refrained from physical activity 36 hours before

each trial and fasted for 12 hours preceding each trial. Similarly, subjects drank 1 L of

Table 4.1. Workload intensity and inspired air temperature during cold air trial

Intensity	PFO+		PFO–		Overall	
	Workload (Watts)	T_{insp} (°C)	Workload (Watts)	T_{insp} (°C)	Workload (Watts)	T_{insp} (°C)
25% of Maximum Workload	75 ± 12	4.0 ± 3.5	79 ± 10	3.7 ± 2.5	77 ± 11	3.8 ± 3.0
50% of Maximum Workload	149 ± 24	2.5 ± 3.4	158 ± 20	2.3 ± 2.5	154 ± 22	2.4 ± 3.0
75% of Maximum Workload	224 ± 36	0.8 ± 3.4	238 ± 30	0.8 ± 2.9	231 ± 33	0.8 ± 3.1
90% of Maximum Workload	269 ± 44	–0.8 ± 3.4	285 ± 36	–0.6 ± 3.3	277 ± 40	–0.7 ± 3.3

Values are mean ± standard deviation. There were no significant differences between groups or workload intensities.

Table 4.2. Environmental conditions

	Room Temperature (°C)	Relative Humidity	Barometric Pressure (mm Hg)
VO_{2MAX}	21 ± 1	37 ± 10	753 ± 4
Relative Workload – Ambient air	21 ± 1	36 ± 10	753 ± 4
Relative Workload – Cold and dry air	20 ± 1	36 ± 12	754 ± 3

Values are mean ± standard deviation. There were no significant differences among trial days.

Table 4.3. Anthropometric, VO_{2MAX} and pulmonary function data

	PFO– (n = 15)	PFO+ (n=15)	Overall (n = 30)
Age (years)	23 ± 5	24 ± 5	24 ± 5
Height (cm)	178.4 ± 5.6	176.3 ± 5.1	177.4 ± 5.4
Weight (kg)	76.0 ± 9.1	78.2 ± 9.6	77.1 ± 9.3
BSA (m^2)	1.94 ± 0.13	1.95 ± 0.14	1.95 ± 0.13
Maximal watts	317 ± 40	298 ± 49	308 ± 45
VO_{2MAX} (ml•kg^{-1}•min^{-1})	50.3 ± 6.9	46.0 ± 7.2	48.1 ± 7.2
FVC (L)	5.47 ± 0.52	5.19 ± 0.53	5.33 ± 0.53
FEV$_1$ (L)	4.51 ± 0.45	4.27 ± 0.52	4.39 ± 0.49
DL_{CO} (ml•min^{-1}•Torr^{-1})	32.6 ± 3.0	30.5 ± 4.4	31.5 ± 3.9

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

Table 4.4. Respiratory data during VO_{2MAX} trial

	PFO– (n = 15)	PFO+ (n=15)	Overall (n = 30)
Pre-exercise V_t (L)	0.84 ± 0.27	0.98 ± 0.34	0.91 ± 0.31
Pre-exercise RR (br/min)	16 ± 4	15 ± 5	15 ± 4
Pre-exercise V_E (L/min)	12.6 ± 3	13.1 ± 3.2	12.9 ± 3.1
Pre-exercise SpO_2	99 ± 1	99 ± 1	99 ± 1
Max V_t (L)	2.49 ± 0.24	2.35 ± 0.36	2.42 ± 0.31
Max RR (br/min)	53 ± 9	56 ± 9	54 ± 9
Max V_E (L/min)	130.4 ± 23.7	127.7 ± 22.1	129.0 ± 22.5
Max SpO_2	97 ± 2	97 ± 2	97 ± 2

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

water the night before and 500 ml the morning of each trial. Participants self-reported that they obtained at least 7 hours of sleep the night before each trial. Subjects completed trials at the same time ± 1 hour to minimize circadian variability in temperature (Little & Rummel, 1971).

Environmental Conditions

All trials were completed in an environment that was 21 ± 1 °C with a relative humidity of 13 ± 4 % and a barometric pressure of 753 ± 4 Torr (**Table 4.2**).

Statistical Analyses & Power Calculation

The number of subjects required to see a significant difference in the T_{oesoph} between PFO+ and PFO− subjects was calculated based on the findings from Lovering et al. (Lovering *et al.*, 2011) and unpublished findings. Accordingly, we used the mean T_{oesoph} from these two sources (PFO+ 36.8 ± 0.5 vs. PFO− 36.3 ± 0.5), an alpha level of 0.05 and a power of 0.80 to determine that at least 12 subjects in each group were needed. Retrospective calculations from our findings revealed a power of 0.86.
Researchers analysed data 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 analysed using a two-way mixed ANOVA (PFO x workload) with α = .05. In the event of a significant F ratio, specific pairwise differences were examined with Bonferroni's post hoc test. Additional *a priori t*-tests were also completed. An alpha of .05 was used for the level of significance.

Results

Lung Function and Maximal Oxygen Uptake

Anthropometric, pulmonary function, DLco, and VO$_{2MAX}$ data for PFO+ and PFO− subjects are presented in **Table 4.3**. There were no significant differences between the two groups, except for pre-exercise HR, which was higher in PFO+ subjects compared to PFO–, 72 ± 13 vs. 62 ± 10 (**Figure 4.1**). Additionally, during the VO$_{2MAX}$ trial, PFO+ subjects had a slightly higher HR of ~5 bpm throughout the exercise portion (not significant, $p = .27$, see Appenidx).

Environmental Conditions and Exercise Intensities

There were no differences in ambient temperature, relative humidity or barometric pressure among the three trials **(Table 4.2)**. There were no significant differences in maximum workload achieved during the VO$_{2MAX}$ test between the two groups (PFO+ 298 ± 49W vs. PFO– 317 ± 40W). Additionally, there were no differences in workload intensity for the relative workload trials **(Table 4.1).**

Respiratory Measures and RPE

Respiratory measures made during pre-exercise and at VO$_{2MAX}$, including tidal volume (V$_t$), respiratory rate (RR), minute ventilation (V$_E$) and peripheral arterial saturation (SpO$_2$) are presented in **Table 4.4**. There were no significant differences between the two groups at any time point measured for V$_t$, RR or SpO$_2$. During the VO$_{2MAX}$ trial, PFO+ subjects had a greater V$_E$ than PFO− subjects with specific differences occurring at 200 W **(Figure 4.2)** but no specific differences in V$_E$ occurred during pre-exercise conditions or at VO$_{2MAX}$. There were no differences in V$_E$ between relative workload trials for either group. No differences existed between groups for

61
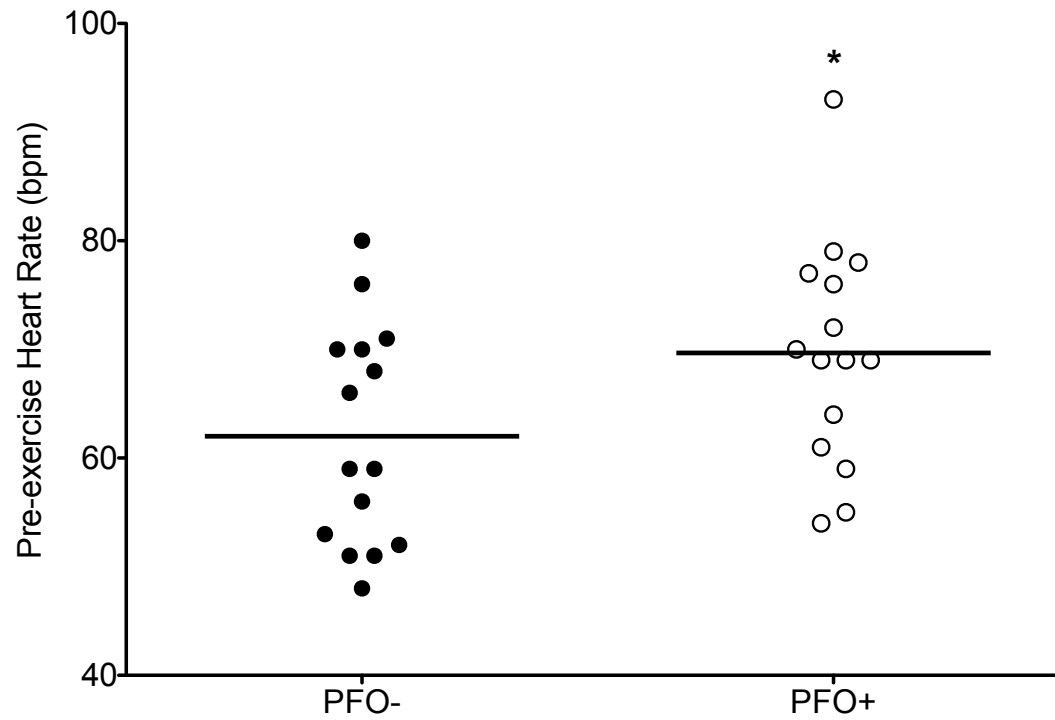
Figure 4.1. Effect of PFO on HR during pre-exercise conditions. Line indicates mean for each group. PFO+ subjects had a higher HR than PFO− subject as indicated by * ($p < .05$).
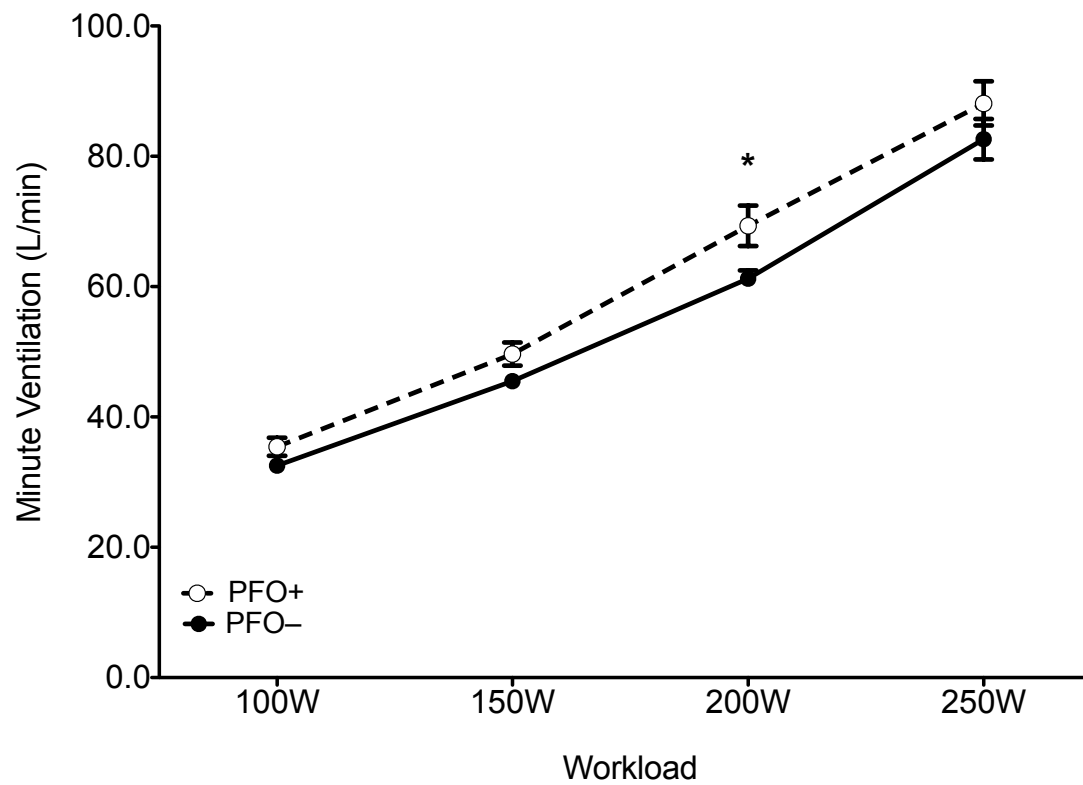
Figure 4.2. Effect of PFO on VE during VO2MAX test. Values are mean ± standard error. There was a main effect of PFO on V_E. Specific pairwise differences are indicated by * ($p < .05$).

RPE$_{\text{leg discomfort}}$ or RPE$_{\text{dyspnea}}$ at any time point during any of the exercise trials (data not shown).

Metabolic Measures

Metabolic measures made during pre-exercise and at VO$_{2MAX}$, including oxygen uptake (VO$_2$), carbon dioxide elimination (VCO$_2$) and respiratory exchange ratio (RER)

are presented in **Table 4.4**. There were no statistically significant differences between the two groups at any time point measured. However, throughout the VO_{2MAX} trial, PFO+ subjects had a VO_2 that was ~5% slightly greater than PFO− subjects (not significant, p = .25, see Appendix).

Oesophageal Temperature at Rest and During VO₂MAX Test

During the VO_{2MAX} test trial, there was a main effect of PFO on T_{oesoph}, with specific pairwise differences occurring at 150, 200 and 250 W ($p < .05$). Generally, PFO+ subjects had a higher T_{oesoph} of ~0.4°C at each time point **(Figure 4.3)**. Between groups, there were no differences in the absolute change in T_{oesoph} from pre-exercise conditions (p = .36, see Appendix).

Comparison of Oesophageal Temperature Change Between Relative Workload Trials

There were no differences in the absolute increase of T_{oesoph} from pre-exercise to 90% of maximal workload during the ambient air trial between PFO+ (0.8 ± 0.4°C) and PFO− subjects (0.9 ± 0.4°C). There were no differences in the absolute increase of T_{oesoph} from pre-exercise conditions to 90% of maximal workload when comparing the ambient trial to the cold and dry air trial within either group, PFO+ (p = .98) or PFO− (p = .12). However, during the cold and dry air trial, T_{oesoph} in PFO+ subjects remained unchanged compared to the ambient air trial (Δ = 0.0 ± 0.3°C), whereas PFO− subjects achieved a T_{oesoph} during the cold and dry air trial that was ~0.3°C lower than the ambient air trial (Δ = −0.3 ± 0.4°C) **(Figure 4.4).**

Effect of PFO Size

There was no effect of PFO size on data listed in **Tables 4.1-5**. There was a main effect of PFO size (small vs. large) on T_{oesoph} pre-exercise and during the VO_{2MAX} test
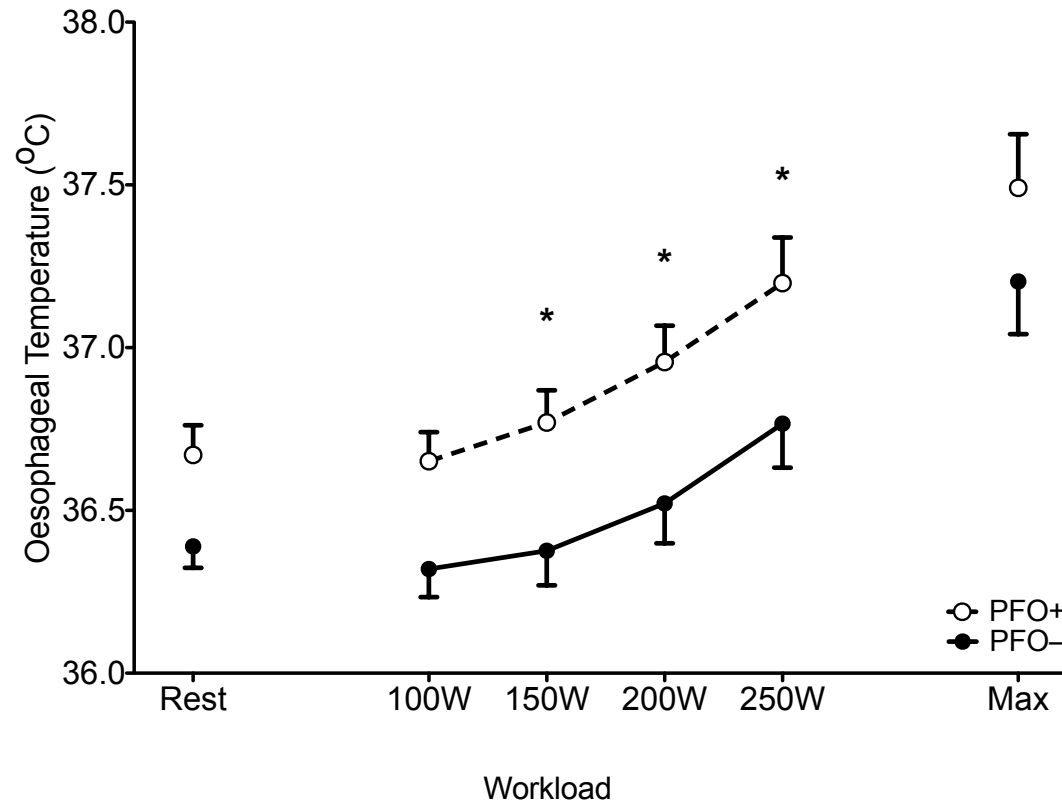
Figure 4.3. Effect of PFO on T$_{oesoph}$ during VO$_{2MAX}$ test. Values are mean ± standard error. There was a main effect of PFO on T$_{oesoph}$. Specific pairwise differences are indicated by * ($p < .05$).
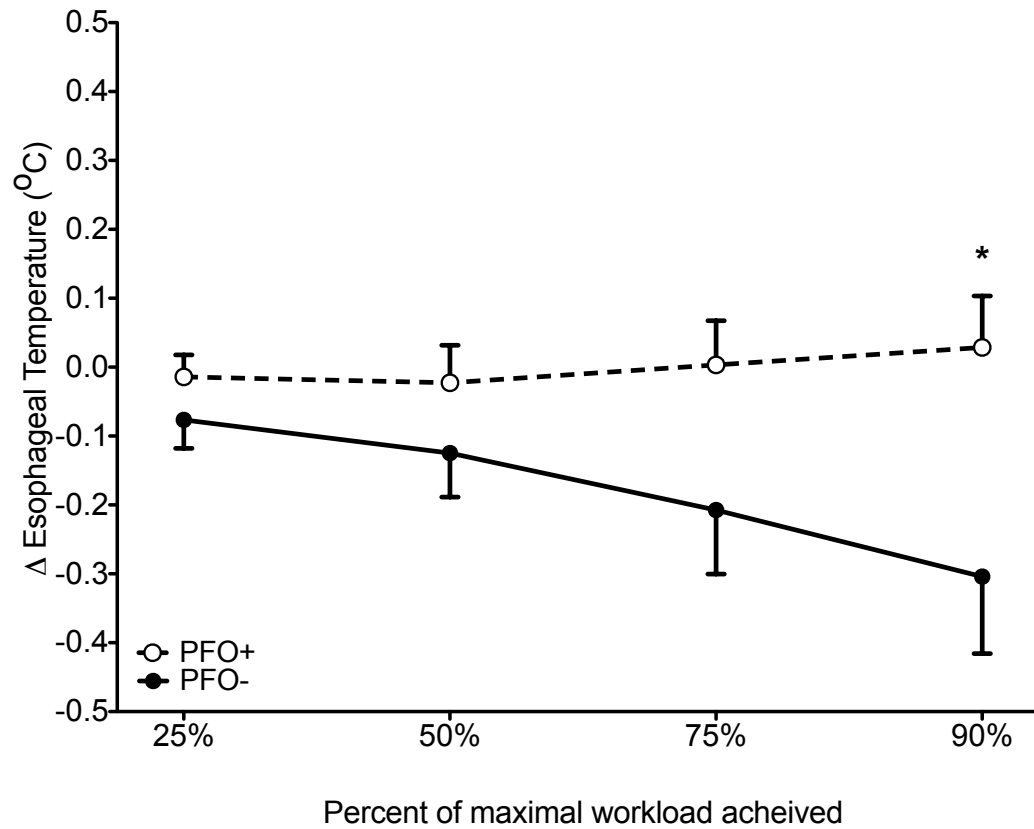
Figure 4.4. Effect of PFO on differences in absolute change of T_{oesoph} during relative workload tests between breathing cold and dry air and ambient air. Values are mean responses ± standard error. There was an effect of temperature of PFO occurring during the 90% of max workload ($p < .05$).

when comparing large PFO ($\geq$13 bubbles upon release of Valsalva manoeuvre), small PFO ($\leq$12 bubbles upon release of Valsalva manoeuvre) or PFO$-$ subjects **(Figure 4.5).** Specific pairwise differences showed that subjects with a large PFO had a significantly higher T_{oesoph} than PFO$-$ subjects at 150, 200 and 250 W ($p < .05$). However, during the cold and dry air trial, T_{oesoph} in subjects with a large PFO had a slight increase in T_{oesoph} compared to the ambient air trial ($\Delta = 0.2 \pm 0.3$°C), while subjects with a small PFO achieved a T_{oesoph} during the cold and dry air trial that was ~0.1°C lower than the ambient air trial ($\Delta = -0.1 \pm 0.1$°C) **(Figure 4.6).**

Ventilatory Equivalent for Oxygen (V_E/VO_2)

There was not a significant interaction or main effect of PFO on V_E/VO_2 during the VO_{2MAX} trial. However, during both relative workload trials there was both a significant interaction and main effect for the presence of a PFO on V_E/VO_2 ($p < .05$). Specific pairwise differences occurred during exercise at 90% of maximum workload during both conditions. When ambient air was breathed, the plot for both groups fell along the same trajectory **(Figure 4.7A).** However, during the cold and dry air trial the plot for PFO$-$ subjects **(Figure 4.7B, closed circles)** was left-shifted compared to PFO$+$ subjects **(Figure 4.7B, open circles).**

Discussion

In this study we determined that PFO$+$ subjects have a higher T_{core}, as measured by T_{oesoph}, during pre-exercise and exercise conditions, compared to PFO$-$ subjects. We
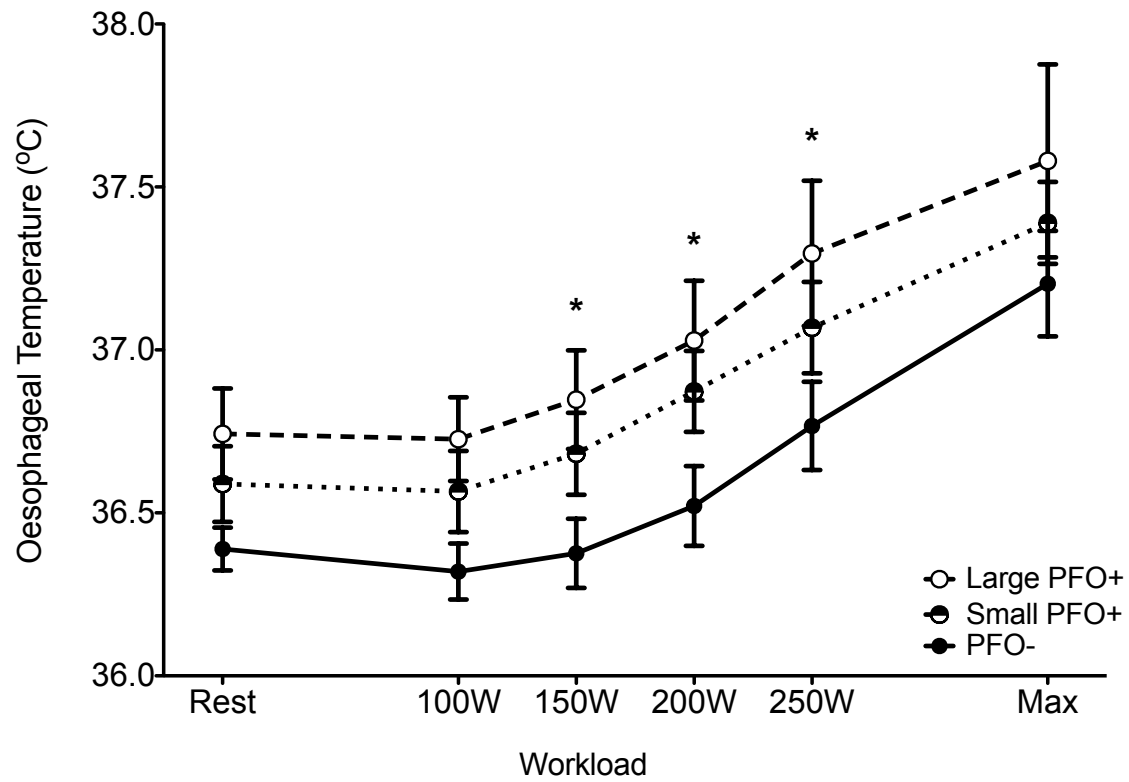
Figure 4.5. Effect of size of PFO on T$_{oesoph}$ during VO$_{2MAX}$ test. Values are mean ± standard error. There was a main effect of PFO size on T$_{oesoph}$. Significant differences from PFO– are indicated by * ($p < .05$).
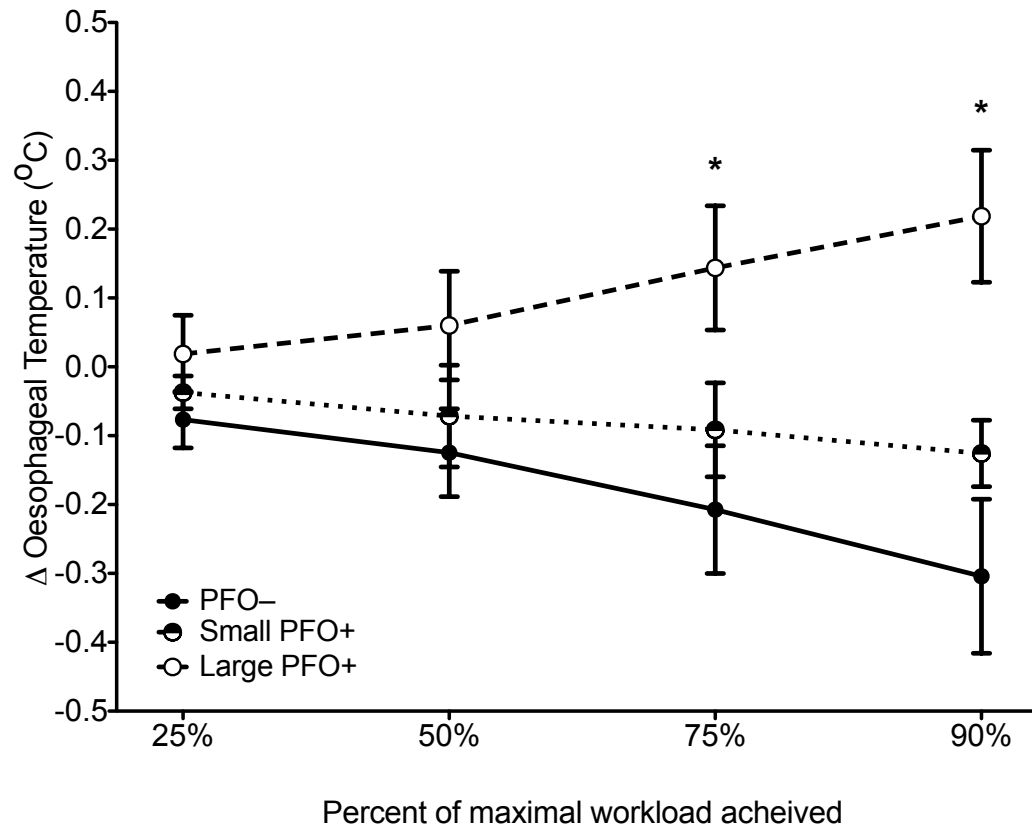
Figure 4.6. Effect of the size of PFO on differences in absolute change of T_{oesoph} during relative workload tests between breathing ambient and cold air. Values are mean responses ± standard error. There was a main effect PFO size on differences in T_{oesoph}. Significant differences from PFO– are indicated by * ($p < .05$).
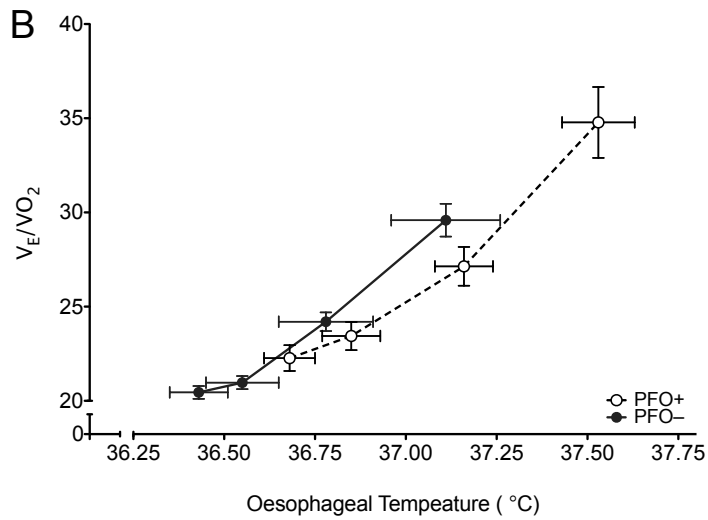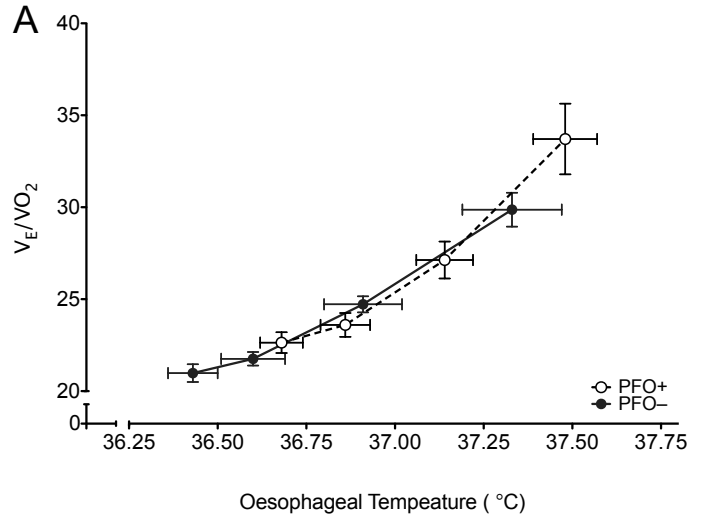
Figure 4.7. Effect of T_{oesoph} on V_E/VO_2 in PFO+ and PFO− subjects. Panel A shows the relationship during the relative workload trial while breathing ambient air. Panel B shows the relationship during the relative workload trial while breathing cold and dry air. PFO+ subjects did not show any change in the relationship between the two trials, while PFO− subjects showed a left shifted curve when breathing cold and dry air. Values are mean responses ± standard error.

71

also determined that the difference in T_{oesoph} was associated with the estimated size of the PFO. Additionally, the current study suggest that respiratory system cooling was impaired during exercise breathing cold and dry air in subjects with a PFO and the impact of this impairment was associated with the size of a PFO.

Presence of PFO and Oesophageal Temperature Pre-exercise and During Exercise Breathing Room Temperature Air

While investigating the effect of a PFO on pulmonary gas exchange efficiency, Lovering et al., reported the surprising finding that a small group of PFO+ subjects (n = 8) had a greater T_{oesoph} at maximal exercise compared to a small group of PFO− subjects, (*n* = 8) (Lovering *et al.*, 2011). In that study, PFO+ subjects tended to have a greater T_{oesoph} than PFO– subjects at submaximal exercise intensities, although this was not significantly different. Of note, that study was neither well designed nor appropriately powered to examine the differences in T_{core} between two groups since the primary outcome variable in that study was the alveolar-to-arterial oxygen difference. More specifically, that study did not control for ambient temperature, studies were conducted at different times of day, subjects included men and women, and there were no controls for food intake, hydration status, or menstrual cycle, and their study had a relatively small sample size (*n* = 8 PFO+, 8 PFO–). Additionally, in that study only 2 subjects would have been classified as subjects with a large PFO based on the reported bubbles scores.

The present study was primarily interested in T_{oesoph} between PFO+ and PFO– subjects. Therefore, we designed the current study to have the appropriate controls and power to accurately determine whether or not PFO+ subjects have a higher T_{oesoph} than

PFO– subjects. The current study shows that in thermoneutral conditions pre-exercise and during exercise conditions, PFO+ subjects do in fact have a greater T_{oesoph} of ~0.4°C compared to PFO– subjects. In addition to showing that PFO+ subjects have a greater T_{oesoph} than PFO– subjects, we have also demonstrated that the estimated PFO size is also important. Specifically, subjects with a large PFO (≥13 bubbles with release of Valsalva manoeuvre) had a significantly higher T_{oesoph} pre-exercise and during exercise than PFO– subjects, however there was no difference in T_{oesoph} between subjects with a small PFO (≤12 bubbles with release of Valsalva manoeuvre) and PFO– subjects.

It has been established that exercise increases T_{core} (Galloway & Maughan, 1997; Maughan *et al.*, 2007; Altareki *et al.*, 2009; Kenefick *et al.*, 2009). In resting humans ~70% of the metabolic heat comes from internal organs and viscera whereas during exercise ~90% of the metabolic heat comes from exercising muscles (Sawka *et al.*, 2011). The heat balance equation states T_{core} increases when heat production is greater than heat loss. Conversely, T_{core} decreases when heat production is less than heat loss. The variables in the heat balance equation include metabolic rate, mechanical work, rate of radiant and convective heat exchange, rate of conduction and rate of evaporation. Since, PFO+ subjects have a higher T_{oesoph} than PFO– subjects, it follows that there are differences in these variables between these two populations.

PFO+ and PFO– subjects maintained the same differences in T_{oesoph} prior to and during exercise (**Figure 4.3**). Thus, whatever is responsible for the differences in T_{oesoph} between subjects with and without a PFO before exercise is not likely changing during exercise. One explanation for these findings is differences in respiratory system cooling. It has been suggested that ~10% of total body heat loss occurs through the respiratory

system via convective and evaporative heat loss (Burch, 1945). Additionally, Hanson demonstrated that within the lungs, evaporative heat loss is dependent on T_{core}, while convective heat loss is dependent on T_{core} and T_{insp} (Hanson, 1974). Blood flowing through the PFO does not go through the pulmonary circulation, and is unable to dissipate heat into the airways, thus diminishing the amount of heat that can be lost via respiratory system cooling. Since PFO+ subjects may have up to 5% of the cardiac output bypass the pulmonary circulation, and PFO– subjects have none of the cardiac output circumventing the pulmonary circulation, it is plausible that PFO+ subjects experience up to 5% less respiratory system cooling than PFO– subjects. Consequently, PFO– subjects might be able to dissipate more heat through respiratory system cooling than PFO+ subjects. Although, the present study was not designed in a manner to accurately measure the amount of respiratory heat loss, the current data suggest that less respiratory heat loss occurs in PFO+ subjects than PFO– subjects when breathing cold and dry air **(Figure 4.6)**.

      While it was not possible to determine the magnitude of respiratory heat loss occurring in each group, it is possible to calculate the increase in temperature that would occur when "warm blood" is shunted from the right heart across the PFO and combines with "cool blood" entering the left heart from the pulmonary circulation. Assuming that 5% of the total cardiac output (Q_{TOT}) bypasses the pulmonary circulation under resting conditions in PFO+ subjects and the temperature gradient from the right side of the heart to the left side of the heart was 1°C, then one would predict this would result in T_{core} being ~0.1°C greater in PFO+ subjects (Appendix).

Since the loading of oxygen onto haemoglobin and the dissolution of oxygen into plasma are exothermic processes (Roughton, 1935; Roughton *et al.*, 1936; Wyman, 1939), heat is likely produced in the left atrium and ventricle of PFO+ subjects when shunted, deoxygenated blood combines with oxygenated blood returning from the pulmonary circulation. These processes could result in an increased T_{core}. However, it is possible that some or all of the heat produced is eliminated by endothermic processes, such as the unloading of carbon dioxide from haemoglobin or the evolution of carbon dioxide from the blood (Kernohan & Roughton, 1968). Consequently, while it is conceivable that the mixing of oxygenated and deoxygenated blood may increase T_{core}, it is unknown if this actually occurs, and to what extent T_{core} would be affected. If T_{core} were to increase as a result of these processes, PFO– subjects would not increase T_{core} in this manner, since their entire cardiac output passes through the pulmonary circulation, meaning there would be no mixing of oxygenated and deoxygenated blood from the PFO in the left atrium and ventricle.

It is also possible that PFO+ subjects have blunted skin blood flow and/or sweating responses to increases in T_{core}. However, the present study was not designed in a manner that would detect differences in skin blood flow or sweat rate. It must be noted that despite differences in T_{oesoph} before exercise, PFO+ subjects were not sweating, despite having a T_{oesoph} that was 0.4°C greater. Additionally, there might be differences in metabolic rate between PFO+ and PFO– subjects. While there were not significant differences, PFO+ subjects had a metabolic rate that was ~4% greater prior to exercise, and ~3% greater throughout the VO_{2MAX} test (through 250 W). Therefore, it is plausible

that the higher T_{oesoph} in PFO+ subjects could be attributed to the non-significant increases in metabolic rate.

Thus, while we have shown a ~0.4°C difference in T_{oesoph} between PFO+ and PFO– subjects, the design of the present study prevents us from accurately determining what is causing this entire difference in T_{core}. We can only estimate how much T_{core} would increase when "warm" shunted blood combines with "cool" blood. While it is likely that the shunted blood might account for up to 0.1°C of the T_{core} difference between PFO+ and PFO– subjects during pre-exercise and exercise conditions breathing ambient air, it does not explain all off the differences in T_{core}, and there are other likely contributors to the observed differences, such as respiratory heat loss, skin blood flow, or metabolic differences. Additionally, there may be other unknown factors which could cause differences in the ability to store, produce or lose heat, all of which should be investigated in subjects with PFO in the future.

Interestingly, we found that the estimated size of the PFO was associated with the differences in T_{core} with a larger PFO associated with a higher T_{core} than a smaller PFO. On the surface, this finding could be explained by the fact that more blood flow can occur through a large PFO than through a small PFO (Fenster *et al.*, 2013*a*), thereby resulting in potentially more blood bypassing the respiratory system, which would decrease the amount of heat lost through the respiratory system. Accordingly, subjects with a small PFO appear to have a physiologically insignificant amount of blood flowing through it, as their temperature responses to exercise are more similar to PFO– subjects than PFO+ subjects **(Figure 4.5).** However, our arguments above suggest that the blood flow across

the PFO cannot explain the entire difference in T_{core} between these two groups of subjects.

Alternatively, the reason that the foramen ovale fails to close in all subjects is not known. Based on our data, it is tempting to speculate that one reason there are differences in temperatures associated with PFO size is because higher core body temperatures, or something related to the higher core body temperatures, play a role in preventing the closure of a PFO. Accordingly, higher core body temperatures are associated with large PFOs and lower core temperatures are associated with smaller PFOs or PFO closure. However, this remains to be directly determined and could not be determined by the data collected in this study.

Impact of Breathing Cold Dry Air on Core Temperature in Subjects With and Without a PFO

Previous work has demonstrated that breathing cold and dry air reduces the increase in temperature that occurs during exercise, demonstrating a significant effect of cold air breathing on respiratory system heat loss (Geladas & Banister, 1988). The present study showed that during a 10-minute exercise trial breathing cold and dry air, subjects with a PFO increased T_{oesoph} by the same amount regardless of T_{insp}. However, in PFO– subjects T_{oesoph} did not increase as much when breathing cold and dry air compared to ambient air **(Figure 4.4).** As mentioned previously, both T_{core} and T_{insp} affect respiratory convective heat loss. Additionally, there is an inverse relationship between T_{insp} and respiratory convective heat loss, so more convective heat loss occurs when T_{insp} is lower. Furthermore, McFadden and colleagues have demonstrated that the degree of

airway cooling increases with increasing ventilation and reduced inspired air temperatures such that the temperature gradients were 8-9°C greater when breathing frigid air (-18.6°C) compared to breathing room temperature air (26.7°C) (McFadden *et al.*, 1985). Since 100% of the cardiac output undergoes convective heat loss in the airways in PFO– subjects, but not PFO+ subjects, convective heat loss will be greater in PFO– subjects while breathing cold and dry air than PFO+ subjects. Although our data support this idea, we did not directly measure respiratory system heat loss between our subjects.

When we examined the differences in T_{oesoph} between subjects with and without PFO based on the estimated size of the PFO, we found that subjects with a small PFO did not increase their T_{oesoph} as much as they did during exercise breathing ambient air when breathing cold and dry air **(Figure 4.6).** Interestingly, the subjects with a large PFO actually achieved a greater increase in temperature breathing cold and dry air. One explanation for this increase in temperature in subjects with a large PFO is that breathing cold air has been shown to increase sympathetic nerve activity (Heindl *et al.*, 2004), which may cause peripheral vasoconstriction and reduced skin blood flow. Thus, assuming similar regulation of skin blood flow between those with and without PFO, if breathing cold air reduced skin blood flow, the ability to lose heat at the skin would have been reduced in both groups equally. Nevertheless, the subjects with a large PFO achieved a higher T_{core} when breathing cold dry air, whereas subjects with a small PFO and without a PFO achieved a lower T_{core}. In combination, these data support the idea that when breathing cold and dry air, respiratory system cooling occurs to a greater degree in

subjects with the greatest amounts of blood flowing through the lungs, i.e. subjects with either a small PFO or no PFO.

Effect of PFO on Minute Ventilation and Ventilatory Equivalent for Oxygen (V_E/VO_2)

During the VO_{2MAX} test, PFO+ subjects had a significantly higher V_E than PFO– subjects, with specific pairwise differences occurring at 200 W. However, there were no differences in V_E between groups during either of the relative workload trials. Since PFO+ subjects had a greater V_E at the same absolute workload, it follows they should experience more respiratory cooling than PFO– subjects. However, PFO+ subjects had a higher T_{oesoph} and there was no difference between groups in how much T_{oesoph} increased from pre-exercise conditions. This suggests that, despite having a greater V_E, PFO+ subjects did not have as much respiratory system cooling as PFO– subjects. During the relative workload trials, PFO+ subjects had a greater V_E/VO_2 than PFO– subjects with specific differences found at 90% of maximum workload during both conditions. These findings are in line with previous work by done by Sun et al. (Sun *et al.*, 2002) which compared healthy controls to two groups of subjects with primary pulmonary hypertension: those with right-to-left shunt and without right-to-left shunt. With pulmonary hypertension it would be expected that increased right heart pressure would facilitate intracardiac shunting across a PFO because of the differences in right and left heart atrial pressure gradients favouring right-to-left blood flow across the PFO. Accordingly, pulmonary hypertension subjects with intracardiac right-to-left shunt had a greater V_E/VO_2 than the healthy controls and hypertension subjects without right-to-left shunt.

In the present study, when comparing the ambient and cold and dry air trials within PFO+ and PFO– subjects, there was no difference in V_E/VO_2 between trials for either group. However, as stated previously, PFO– subjects had a decreased T_{oesoph} at 90% of maximal workload while breathing cold and dry air compared to ambient air; PFO+ subjects had no such difference. While it has been shown that increased T_{oesoph} augments V_E/VO_2 at rest (Cabanac & White, 1995; White, 2006), our findings suggest that PFO+ subjects might have a decreased ventilatory sensitivity to increases in T_{core}. Consequently, PFO+ subjects might have impaired control of breathing that could result in a decreased amount of respiratory system cooling compared to PFO– subjects. Ultimately this could also contribute to PFO+ subjects having an elevated T_{core} compared to PFO– subjects.

Effect of PFO on Heart Rate

Despite the fact that ~35% of the population has a PFO (Marriott *et al.*, 2013; Elliott *et al.*, 2013), we have been unable to find any previous work examining the effect of a PFO on HR. It has been established that increased T_{core} leads to an augmented HR (Cabanac & White, 1995; Minson *et al.*, 1998). Our findings show that during pre-exercise conditions, PFO+ subjects have a higher HR than PFO– subjects **(Figure 4.1)**. However, there were not any significant differences between the two groups during exercise (see Appendix). The differences observed pre-exercise might be explained by pre-exercise HR to a greater degree by T_{core} (Cabanac & White, 1995; Minson *et al.*, 1998) and metabolic rate (Spurr *et al.*, 1988), while maximal HR during exercise is affected to a greater degree by age (Fox & Haskell, 1970; Fox *et al.*, 1971) and with

lesser contributions from T_{core} or metabolic rate. Data from the present study support

these findings as significant differences in HR were only found under pre-exercise

conditions and not during exercise. However, HR was ~5 bpm higher in PFO+ subjects

than PFO− subjects throughout the entire VO_{2MAX} test.

Although not statistically significant, PFO+ subjects had a slightly greater pre-

exercise VO_2 than PFO− subjects among all three trials (PFO+ 0.40 ± 0.08 vs. PFO− 0.39

± 0.07). This 4.2% difference in metabolic rate could be attributed to some of the

differences in $T_{oesoph,}$ since it has been shown that increasing T_{core} by ~1.0°C can increase

metabolic rate by ~10% (Saxton, 1981; Cabanac & White, 1995). An elevated metabolic

rate would lead to an increased demand for oxygen, which could be compensated for by

augmenting HR. However, calculating the Q_{10} effect during pre-exercise conditions for

both VO_2 and HR resulted in values (5.4 and 144.5, respectively) that were well above

the typical biological values of 2-3, which would be expected for a Q_{10} effect. Although it

is likely that increased T_{oesoph} and the resulting changes in VO_2 contribute to the greater

HR seen in PFO+ subjects, it does not fully account for all of these differences. However,

this study was not designed to detect differences in HR, and it is possible under longer

duration steady-state exercise conditions that PFO+ subjects might have a significantly

higher HR than PFO− subjects. During this study, a steady state was not attained during

any of the exercise protocols, as each workload lasted no more than 2 ½ minutes.

Therefore, HR was likely increasing at the end of each workload in an effort to match the

metabolic demand. Consequently, any differences in HR that might exist due to increased

T_{oesoph} in PFO+ subjects might be masked by HR increasing to help maintain oxygen

delivery during times of increased metabolic demand. The current study cannot determine

if a PFO has an effect on any measures, such as HR, that would be affected under steady-state exercise conditions. Consequently, future studies should be designed to determine if the presence of a PFO affects HR during steady-state exercise. Of note, the four classic indicators of heat acclimitzation are lower heart rate, lower core temperature, reduced sweating rate and improved aerobic exercise capacity during heat stress (Sawka *et al.*, 2011). Interestingly, our PFO+ subjects had a higher core temp and higher HR during pre-exercise under thermoneutral conditions suggesting that these subjects have characteristics opposite those of subjects who are heat acclimated.

Limitations

We did not measure differences in sweat rate, skin blood flow, or skin temperature between PFO+ and PFO− subjects. Increasing T_{core} by ~0.1-0.2°C induces sweating (Kenny *et al.*, 1997; 2003). However, during pre-exercise conditions in a thermoneutral environment none of the PFO+ subjects in the present study were sweating, despite having a T_{oesoph} that was ~0.4°C higher than PFO− subjects. It is possible that PFO+ subjects have decreased thermoregulatory responses to increases in T_{core}. However, another explanation is that both PFO+ and PFO− subjects are maintaining T_{core} within their own "normal range." In order to determine if one group or another has altered thermoregulatory responses, future research should look at sweat rates and/or skin blood flow between PFO+ and PFO− subjects. Additionally, since skin temperature was not measured, it was not possible to calculate mean body temperature. Thus, while differences in T_{core} existed, it is possible that PFO+ and PFO− subjects had the same mean body temperature.

Trials were completed in thermoneutral (~20°C) and dry conditions (relative humidity < 40%). The effectiveness of respiratory cooling depends on the temperature and humidity of the inspired air (Hanson, 1974). It is possible that if this study was conducted in an environment that was not conducive to respiratory heat loss (i.e. hot and/or humid environment), that the differences in T_{core} between PFO+ and PFO– would not be significant. Furthermore, we were unable to accurately measure the amount of respiratory heat loss that occurred. Future research should be designed in a manner that will allow respiratory heat loss to be quantified.

Additionally, we measured SpO_2, which only estimates SaO_2. There were not any differences in SpO_2, which is not surprising because it is not a sensitive enough measure to detect minor differences in arterial saturation that would occur with an ~0.4°C difference in T_{oesoph} in subjects breathing room air. However, SaO_2 was not a primary outcome variable for this study.

We classified PFO+ subjects as those with large PFOs and those with small PFOs, but we did not directly measure the size of the PFO. We estimated the size of the PFO using saline contrast echocardiography to determine the amount of microbubbles that were visualized in the left ventricle upon release of a Valsalva manoeuvre, which demonstrates the potential for blood flow to occur across a PFO. Knowing the exact size of the PFO might have provided us with a better estimate of the potential for blood flow across the PFO, because it is possible that the amount of blood flowing through the PFO during the release of a Valsalva manoeuvre would be different than what flows across it at rest and during exercise, particularly as right and left atrial pressure increase with exercise intensity. However, research by Fenster et al. has shown that the amount of left-

sided contrast correlates with blood flow across the PFO (Fenster *et al.*, 2013*a*). Additionally, the fact that we saw differences between the two groups suggests that our size classification is valid in otherwise healthy humans. Nevertheless, conditions might exist where subjects with a pathophysiologic condition, such as pulmonary hypertension, and a small PFO have more blood flowing across the PFO than otherwise healthy adults who have a large PFO due the increased right atrial pressure and resulting pressure gradient. Thus, in addition to the size of the PFO, the driving pressure gradient across the right and left atrium would also be important in determining the amount of (or potential for) blood flow across the PFO.

Summary

This study has demonstrated that 1) the presence of a PFO is associated with a ~0.4°C higher T_{oesoph} than PFO– subjects prior to and during a VO_{2MAX} test, 2) the size of a PFO is associated with T_{oesoph} where subjects with a large PFO have a greater T_{oesoph} than PFO– subjects and subjects with a small PFO, and 3) compared to breathing ambient air, when breathing cold and dry air prior to and during exercise, subjects with a large PFO increased T_{oesoph} by a greater amount, whereas PFO– subjects and those subjects with a small PFO did not increase T_{oesoph} to the same degree. These findings are important because PFO+ subjects may be better able to maintain T_{oesoph} when breathing cold and dry air during exercise since they appear to lose less heat than subjects without a PFO. For this reason, research examining exercise performance in, and/or tolerance to, cold and hot environments may want to consider the effect of a PFO on T_{core} and respiratory system cooling as we have demonstrated here the association of a PFO with

84

T_{core}. Lastly, while normal T_{core} has been generally accepted to be ~37.0°C, there is some amount of variability in this measure. The present study has demonstrated that the presence or absence of a PFO may explain some of the biological variability observed in T_{core}.

CHAPTER V

THE EFFECT OF A PATENT FORAMEN OVALE ON THERMOREGULATORY

AND VENTILATORY RESPONSES TO PASSIVE HEATING AND COOLING

This chapter will be submitted to the Journal of Physiology and Madeline W. Hay B.S.,

Alyssa M. Hardin, Dr. Matthew D. White and Dr. Andrew T. Lovering are co-authors. I

performed the experimental work, led the project, and the writing is entirely my own; Madeline

Hay B.S., and Alyssa Hardin assisted with data collection and data analysis; and Drs. Matthew

White and Andrew T. Lovering helped develop the protocol, and provided guidance and editorial

assistance. All co-authors will formally approve this manuscript prior to submission.

Introduction

The scientific community has known of the existence of a patent foramen ovale (PFO)

since the times of Galen (*ca* 200 AD) (Christie, 1930; Patten, 1931). Autopsy studies (Hagen *et

al.*, 1984; Kerut *et al.*, 2001) and studies using saline contrast echocardiography in living humans

(Woods *et al.*, 2010; Marriott *et al.*, 2013; Elliott *et al.*, 2013) have demonstrated that a PFO is

present in a substantial proportion of the general population with a prevalence of ~25-40%.

Individuals with a PFO (PFO+) have an opening in the heart that can allow varying degrees of

cardiac output (Q_c) to bypass the respiratory system in otherwise healthy humans. Previous work

by Davis et al. (Davis *et al.*, 2015) and Elliott et al. (Elliott *et al.*, 2015), who investigated the

impact of a PFO on both core body temperature (T_{core}), and pulmonary gas exchange efficiency

respectively, have shown that PFO+ subjects have an increased T_{core} of ~0.4°C, decreased gas

exchange efficiency at rest, and blunted ventilatory acclimatization to 5260 m. While it has been

shown that the presence of a PFO affects these physiological measures in thermoneutral

environments, it is unknown if the presence of a PFO affects thermoregulatory and ventilatory responses to passive cooling and heating.

Cabanac and White previously demonstrated that thermal hyperpnoea during passive body heating occurs when T_{core} reaches ~38.5°C (Cabanac & White, 1995). However, it is presently unknown if the presence of a PFO affects the magnitude of the ventilatory response or the T_{core} threshold at which this ventilatory response occurs. Conversely the shivering response allows heat liberation from stored macronutrients (Daniels & Baker, 1961; Mekjavić *et al.*, 1991; Cheng *et al.*, 1995; Wadhwa *et al.*, 2005). Similar to thermal hyperpnoea, there is variability associated with these responses, and it is unknown if presence of a PFO can explain some of this variability as it relates to the rate at which T_{core} decreases during whole body cooling or the T_{core} threshold for the initiation of shivering. Therefore, the purpose of this study was fourfold, does the presence of a PFO affect 1) the rate at which T_{core} increases during passive heating, or decreases during passive cooling), 2) the T_{core} threshold at which shivering occurs, 3) the T_{core} threshold at which thermal hyperpnoea occurs, and 4) the magnitude of the increase in V_E during hyperthermia. It was hypothesized that during passive cooling PFO+ subjects would 1) shiver at a higher T_{core} than PFO− subjects, and 2) would take longer to reach a critical T_{core} than PFO− subjects. Additionally, during passive heating PFO+ subjects 1) would reach a critical T_{core} sooner than PFO− subjects, and 2) would experience thermal hyperpnoea at a higher T_{core} threshold than PFO− subjects.

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 with the Declaration of Helsinki.

Participants

A total of 41 subjects were recruited for participation in this study. Researchers described orally, and in writing, the nature of the study to all subjects. A total of 27 subjects (13 PFO+, 14 PFO−) qualified and completed the entire study, 7 (4 PFO+) of which participated in a prior study looking at the effect of a PFO on oesophageal temperature (Davis *et al.*, 2015). Of the remaining 14 subjects who did not complete the study, 4 did not pass pulmonary function due to forced vital capacity being less than 85% predicted, 7 withdrew before completing the entire protocol for reasons not associated with the study, and 3 subjects could not tolerate the placement of an oesophageal probe. In total we had a PFO prevalence of 48% in our subject pool which is greater than reported previously (Hagen *et al.*, 1984; Kerut *et al.*, 2001; Woods *et al.*, 2010; Marriott *et al.*, 2013; Elliott *et al.*, 2013), because some subjects were invited to participate that had been previously identified as being PFO+ or PFO−. Additionally, 9 subjects (5 PFO+) in this study also participated in the work previously done by Davis et al (Davis *et al.,* 2015). Ultimately, 27 (13 PFO+, 14 PFO) healthy, non-smoking male volunteers, age 26 ± 8 yr., without history of cardiopulmonary disease were recruited and, after written informed consent was given, agreed to proceed with the study.

Ultrasound Screening

Ultrasound screening has been previously described in detail (Lovering & Goodman, 2012). Initial agitated saline contrast studies were performed with subjects breathing room air and reclined at 45° in the left lateral decubitus position where a clear apical, four-chamber view was obtained. Care was taken to optimally visualize all four chambers, interatrial septum and delineate myocardial and valvular structures by individually adjusting the receiver gain settings. Each saline contrast injection was created by manually agitating 3 ml of sterile saline with 1 ml of room air for 15 s between two 10 ml syringes connected in parallel to two 3-way stopcocks. The saline–air microbubble suspension was then immediately injected in a constant, forceful manner into a peripheral antecubital vein via an IV catheter (20–22 G). This mixture of saline and air provides excellent right-sided contrast. Following opacification of the right atrium and ventricle, the subsequent 20 cardiac cycles were recorded at >30 frames/s for further analysis.

The appearance of ≥1 microbubble in the left atrium or ventricle in any frame during the subsequent 20 cardiac cycles served as the criterion that subjects were either positive for an intracardiac right-to-left shunt (i.e. PFO) or demonstrated the transpulmonary passage of contrast (Freeman & Woods, 2008; Woods et al., 2010; Marriott et al., 2013; Elliott et al., 2013). Saline contrast injections were performed during normal breathing, as well as immediately following the release of a Valsalva manoeuvre in order to transiently elevate right atrial pressure and create conditions optimal for the detection of an intracardiac right-to-left shunt. Effective Valsalva manoeuvres were confirmed by a transient leftward shift of the interatrial septum. Valsalva manoeuvres do not increase left heart contrast in the absence of a PFO. An intracardiac right-to-left shunt was suspected if microbubbles appeared in the left heart ≤3 cardiac cycles following right heart opacification. Subsequently, these subjects were classified as PFO+, while all others

were categorized as PFO−. Using this approach we have shown that we have the sensitivity to accurately detect PFO in the general healthy population (Elliott et al., 2013).

Pulmonary Function and Lung Diffusion Capacity

Prior to the pulmonary function testing, using an electronic scale (Ohaus Corporation, ES200L, Pinebrook, NJ) researchers obtained the subject's weight while wearing shorts as well as the subject's standing and sitting height. Baseline pulmonary function testing included measures of forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and forced midexpiratory flows (FEF$_{25-75}$). Measurements were made with a computerized spirometry system (Ultima PFX, MedGraphics, St. Paul, MN) according to American Thoracic Society/European Respiratory Society (ATS/ERS) standards (Macintyre et al., 2005). Lung volumes and capacities were determined using whole-body plethysmography (Wanger et al., 2005). Lung diffusion capacity for carbon monoxide (DL$_{CO}$) was determined by the single-breath, breath-hold method (Knudson et al., 1987) using the Jones and Meade method for timing and alveolar sample collection (MedGraphics Ultima PFX, Breeze v.6.3.006) . Predicted values for DL$_{CO}$ were calculated as previously described (Gutierrez et al., 2004).

Protocols

Subjects completed two different trials, each separated by a minimum of 48 hours, with a randomized order. Experimenters placed the oesophageal probe through the nostril to a specified depth beyond the nasal flare based on the subject's sitting height, (Mekjavić & Rempel, 1990) as before (Davis *et al.*, 2015). Once oesophageal probe placement was completed, nude weight was obtained. Subjects subsequently put swim trunks on and then sat upright in a chair while

breathing through a low-resistance two-way non-rebreathing valve (model 2400, Hans Rudolph, Kansas City, MO), and pneumotachograph (MedGraphics PreVent) for 15-minutes. At the end of the 15-minute period, subjects entered either the hot tub that was filled with water ($40.5 \pm 0.2°C$) or the cold tub that was filled with water ($19.5 \pm 0.9°C$), where they remained for the duration of the study. At the completion of both trials, nude weight was obtained and then the oesophageal probe was removed. Whole body water loss was calculated as the difference in weight before and after immersion. Measures including oesophageal temperature (T_{oesoph}), water temperature (T_{water}) (Thermes USB, Physitemp, Clifton, NJ), inspired air temperature (T_{insp}), expired air temperature (T_{exp}), inspired relative humidity (Hum_{insp}), expired inspired relative humidity (Hum_{exp}) (Vaisala HMT 337, Boston, MA) heart rate (HR) (Tyco, Nellcor Oximax N-600, Mansfield, MA), peripheral arterial saturation (SpO_2) (Tyco, Nellcor Oximax N-600, Mansfield, MA), rate of oxygen uptake (VO_2), rate of carbon dioxide elimination (VCO_2), and minute ventilation (V_E) (Medgraphics Ultima PFX, Breeze v6.3.006) were continuously obtained. Thermal sensation (Young *et al.*, 1987) and environmental measures (temperature [T_{air}], humidity and pressure) were obtained every five minutes (Davis, Perception II, Hayward, CA). Due to the rapid cooling of air from the mouth to the expired temperature and humidity sensors, which were placed in the expired port of the Hans Rudolph non-rebreathing valve, we measured air temperature at the mouth in a subset of 5 subjects (2 PFO+). These data were then used to create a regression model which was used to estimate expired air temperature at the mouth, which would provide a more accurate measure of respiratory heat loss.

While in the hot tub, subjects sat immersed to the level of the clavicles. Additionally, a fur-lined hat with earflaps was worn to minimize heat loss through the head. Subjects remained

in the hot tub until one of the following criteria occurred: 1) T_{oesoph} reached 39.5°C, 2) subjects had been immersed for 30-minutes, or 3) subjects requested to exit the tub.

While in the cold tub, subjects were in a reclined position immersed to the level of the nipple line. Subjects remained in the cold tub until one of the following criteria occurred: 1) T_{oesoph} dropped to 35.5°C, 2) subjects had been immersed for 60-minutes, 3) subjects displayed sustained shivering for 5 consecutive minutes as determined by a 25% increase in VO_2 (Doufas *et al.*, 2003; Wadhwa *et al.*, 2005) or 4) subjects requested to exit the tub.

Confounder Controls

To minimize confounding variability, investigators directed participants to maintain their nutrition, hydration, and sleep schedules for each trial. Participants fasted for 12 hours preceding each trial. Similarly, subjects drank 1 L of water the night before and 500 ml the morning of each trial. Participants self-reported that they obtained at least 6 hours of sleep the night before each trial. Subjects completed trials at the same time ± 1 hour to minimize circadian variability in T_{core} (Little & Rummel, 1971).

Environmental Conditions

All trials were completed in an environment that was 24 ± 1 °C with a relative humidity of 29 ± 7 % and a barometric pressure of 752 ± 5 Torr (**Table 5.1**).

Statistical Analyses & Power Calculation

Data were analysed 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 analysed using a two-way mixed ANOVA (PFO x Time Point) with $\alpha = .05$. Since all subjects spent differing amounts of time immersed in the hot/cold tubs, data were analysed by comparing three specific time points for the cold tub, which were: 1) just prior to immersion, 2) the time at which T_{oesoph} was the highest, and 3) the time just prior to when subjects exited the tub. The two specific time points analysed during the hot tub trial were 1) just prior to immersion, and 2) the time just prior to when subjects exited the tub. Data collected during the first 5 min of cold water immersion were not used because of the reflex hyperventilation caused by cold-water immersion (Tikuisis *et al.*, 2000).

An additional two-way mixed ANOVA (PFO x Time Point) with $\alpha = .05$ was run on T_{oesoph} and V_E during hot water immersion from subjects who reached ventilatory threshold. For this ANOVA the ventilatory threshold was used as a third time point. This time point was determined as the instance where the end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) dropped 5 Torr lower than resting values (Lucas *et al.*, 2015). In the event of a significant F ratio, specific pairwise differences were examined with Bonferroni's post hoc test. Additional *a priori unpaired 2 tailed t*-tests were completed for means comparisons. An alpha of .05 was used for the level of significance.

Results

Lung Function

Anthropometric, pulmonary function, and DL_{CO}, for PFO+ and PFO− subjects are presented in **Table 5.2**. There were no significant differences between the two groups.

Environmental Conditions and Water Temperature

There were no differences in ambient temperature, relative humidity or barometric pressure between the two trials ($p > .05$, **Table 5.1**). Additionally, there was no difference in ambient temperature, relative humidity or barometric pressure between subject groups for either trial ($p > .05$, **Table 5.1**). Similarly, there were no differences in water temperature between the two groups during the cold tub (PFO+ = 19.6 ± 0.7 °C; PFO– = 19.4 ± 0.8 °C, $p > .05$) and hot tub (PFO+ = 40.5 ± 0.1 °C; PFO– = 40.5 ± 0.0 °C, $p > .05$) trials.

Core Temperature

During the cold tub trial, there was an interaction effect between PFO Group and Time ($p < .05$) on T_{oesoph}. Also, there was a main effect of PFO Group on T_{oesoph} with specific differences occurring at the pre-immersion, peak T_{oesoph} and final time points. PFO+ subjects had a higher T_{oesoph} than PFO– subjects that was ~0.4°C greater both prior to immersion and at peak T_{oesoph}, and this difference increased to ~0.7°C at the end of immersion (Figure 1A).

There were 9 subjects (6 PFO+) who shivered during cold water immersion. In these subjects there was no interaction between PFO Group and time, but there was a main effect of PFO Group on T_{oesoph} with specific differences occurring at all time points (Figure 1B). Similar to the analysis looking at all subjects, shivering PFO+ subjects had a higher T_{oesoph} than shivering PFO– subjects of ~0.4°C prior to immersion and at peak T_{oesoph} (see Appendix), that increased to ~0.5°C at the end of immersion. The onset of sustained shivering occurred at a higher T_{oesoph} in PFO+ subjects (36.3 ± 0.3°C) compared to PFO– subjects (35.8 ± 0.1°C, $p < .05$).

During the hot tub trial, there was no interaction effect between PFO Group and Time on T_{oesoph} ($p > 0.05$). However, there was a main effect of PFO Group on T_{oesoph} with a significant

Table 5.1 Environmental conditions for cold water and hot water immersion

	Ambient Temperature (°C)	Relative Humidity (%)	Barometric Pressure (mm Hg)
Cold water immersion	23 ± 1	30 ± 8	752 ± 5
Hot water immersion	23 ± 1	33 ± 6	752 ± 5

Values are mean ± standard deviation. There were no significant differences among trial days.

Table 5.2. Anthropometric, and pulmonary function data for cold water and hot water immersion

	PFO+ (n=13)	PFO– (n = 14)	Overall (n = 27)
Age (years)	25 ± 8	26 ± 8	26 ± 8
Height (cm)	181.6 ± 6.3	179.0 ± 7.5	180.3 ± 6.9
Weight (kg)	83.1 ± 10.1	80.9 ± 7.8	82.1 ± 9.0
BSA (m^2)	2.04 ± 0.14	2.00 ± 0.13	2.03 ± 0.14
FVC (L)	5.36 ± 0.86	5.68 ± 0.66	5.53 ± 0.76
FEV_1 (L)	4.34 ± 0.72	4.62 ± 0.65	4.49 ± 0.68
$D_{L_{CO}}$ (ml•min^{-1}•$Torr^{-1}$)	41.1 ± 6.5	40.8 ± 9.7	41.0 ± 8.0

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

Table 5.3. Respiratory and metabolic measures during cold water immersion

	Pre-immersion			End of immersion		
	PFO– (n=13)	PFO+ (n = 14)	Overall (n = 27)	PFO– (n=13)	PFO+ (n = 14)	Overall (n = 27)
V_t (L)	0.9 ± 0.7	0.7 ± 0.2	0.8 ± 0.5	1.0 ± 0.5	1.0 ± 0.5	1.0 ± 0.5
RR (br/min)	15 ± 5	17 ± 5	16 ± 5	16 ± 5	14 ± 7	15 ± 6
V_E (L/min)	11.4 ± 4	10.1 ± 1.6	10.8 ± 3.2	14.2 ± 4.1	12.5 ± 3.3	13.4 ± 3.8
VO_2 (L/min)	0.35 ± 0.07	0.33 ± 0.06	0.34 ± 0.06	0.50 ± 0.14	0.45 ± 0.12	0.47 ± 0.13
VCO_2 (L/min)	0.31 ± 0.14	0.28 ± 0.05	0.29 ± 0.10	0.41 + 0.11	0.40 ± 0.11	0.41 ± 0.11
RER	0.86 ± 0.20	0.85 ± 0.09	0.85 ± 0.15	0.84 ± 0.14	0.91 ± 0.10	0.87 ± 0.13
$P_{ET}O_2$ (mmHg)	104 ± 7	103 ± 4	103 ± 6	106 ± 8	105 ± 6	106 ± 7
$P_{ET}CO_2$ (mmHg)	40 ± 3	42 ± 2	41 ± 3	40 ± 5	43 ± 5	41 ± 5
HR (bpm)	65 ± 11	70 ± 7	67 ± 9	58 ± 13*	67 ± 18	63 ± 16
SpO_2 (%)	98 ± 1	98 ± 1	98 ± 1	99 ± 0	98 ± 2	99 ± 1

Values are mean ± standard deviation. * indicates significant difference from pre-immersion ($p < .05$).

Table 5.4. Respiratory and metabolic measures during hot water immersion

	Pre-immersion			End of immersion		
	PFO– (n=13)	PFO+ (n = 14)	Overall (n = 27)	PFO– (n=13)	PFO+ (n = 14)	Overall (n = 27)
V_t (L)	0.8 ± 0.3	0.8 ± 0.2	0.8 ± 0.3	1.4 ± 0.7	1.1 ± 0.6	1.3 ± 0.7
RR (br/min)	16 ± 6	14 ± 6	15 ± 6	20 ± 8	20 ± 8	20 ± 8
V_E (L/min)	11.2 ± 2.0	9.9 ± 2.4	10.6 ± 2.3	24.1 ± 8.7	19.7 ± 8.0	22.0 ± 8.5
VO_2 (L/min)	0.33 ± 0.06	0.32 ± 0.07	0.33 ± 0.07	0.49 ± 0.08	0.48 ± 0.08	0.48 ± 0.08
VCO_2 (L/min)	0.28 ± 0.06	0.27 ± 0.07	0.27 ± 0.06	0.58 ± 0.16	0.52 ± 0.18	0.55 ± 0.17
RER	0.84 ± 0.07	0.83 ± 0.07	0.83 ± 0.07	1.19 ± 0.28	1.07 ± 0.25	1.13 ± 0.27
$P_{ET}O_2$ (mmHg)	104 ± 6	101 ± 4	103 ± 6	122 ± 16	114 ± 9	118 ± 14
$P_{ET}CO_2$ (mmHg) *	39 ± 4	42 ± 4	41 ± 4	32 ± 8*	37 ± 7	34 ± 8
HR (bpm)	63 ± 10	69 ± 10	66 ± 11	113 ± 10	115 ± 17	114 ± 13
SpO_2 (%)	99 ± 1	98 ± 1	98 ± 1	98 ± 2	97 ± 2	97 ± 2

Values are mean ± standard deviation. * indicates significant difference from PFO+ group ($p < .05$).
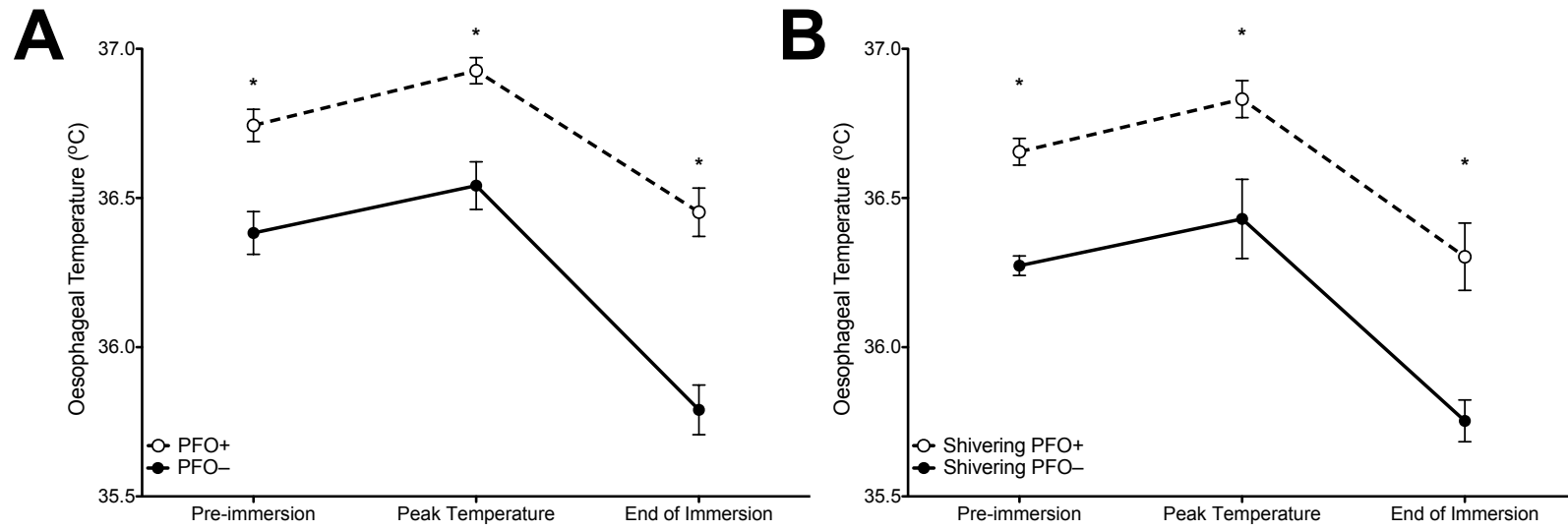
Figure 5.1. Effect of PFO on T$_{oesoph}$ during cold water immersion. Values are mean ± standard error. There was a main effect of PFO on T$_{oesoph}$ when looking at all subjects (Panel A), and when looking at subjects who began to shiver (6 PFO+, 3 PFO–, Panel B). Specific pairwise differences are indicated by * ($p < .05$).
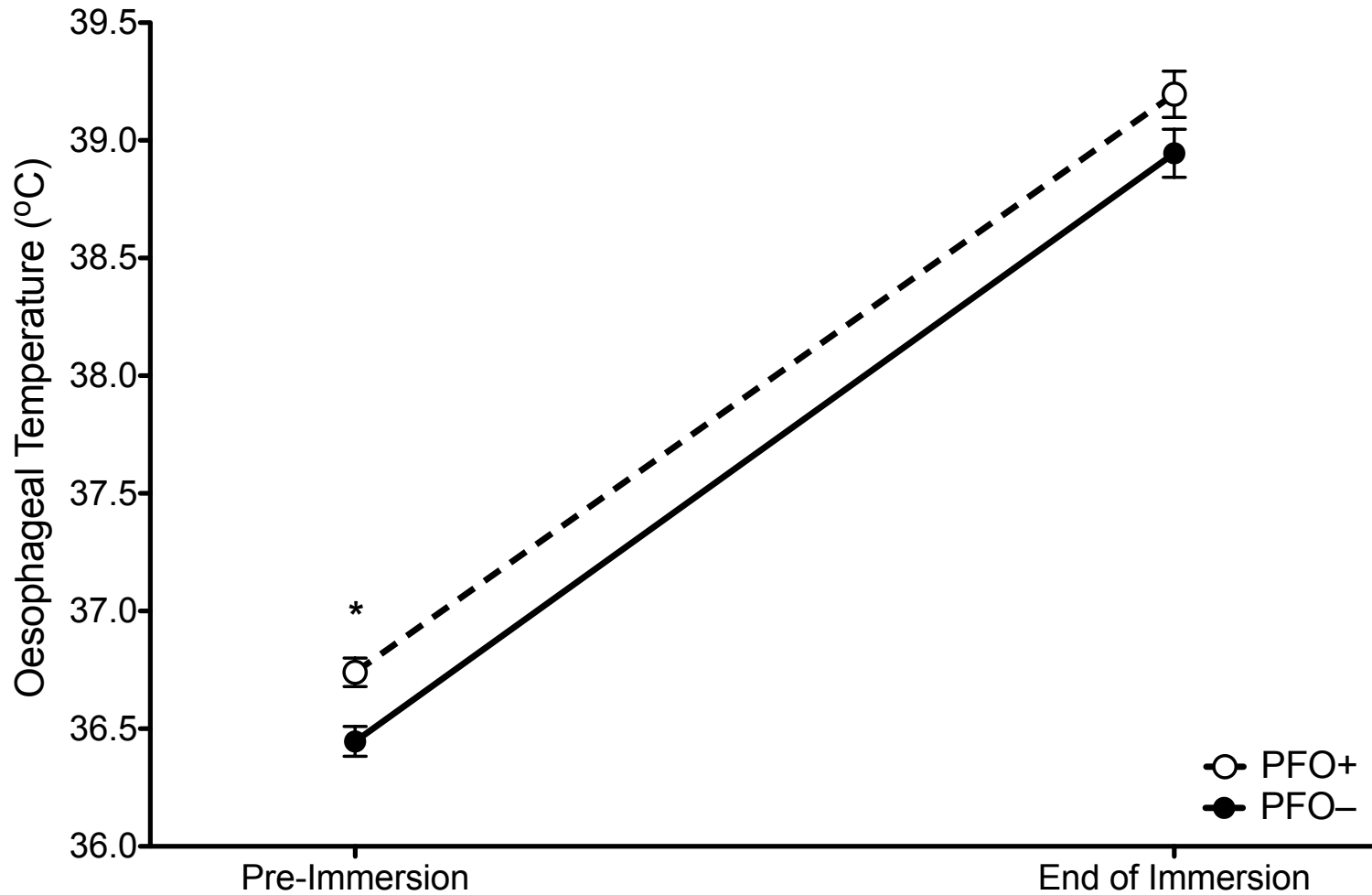
Figure 5.2. Effect of PFO on T$_{oesoph}$ during hot water immersion. Values are mean ± standard error. There was a main effect of PFO on T$_{oesoph}$ when looking at all subjects. Specific pairwise differences are indicated by * ($p < .05$).
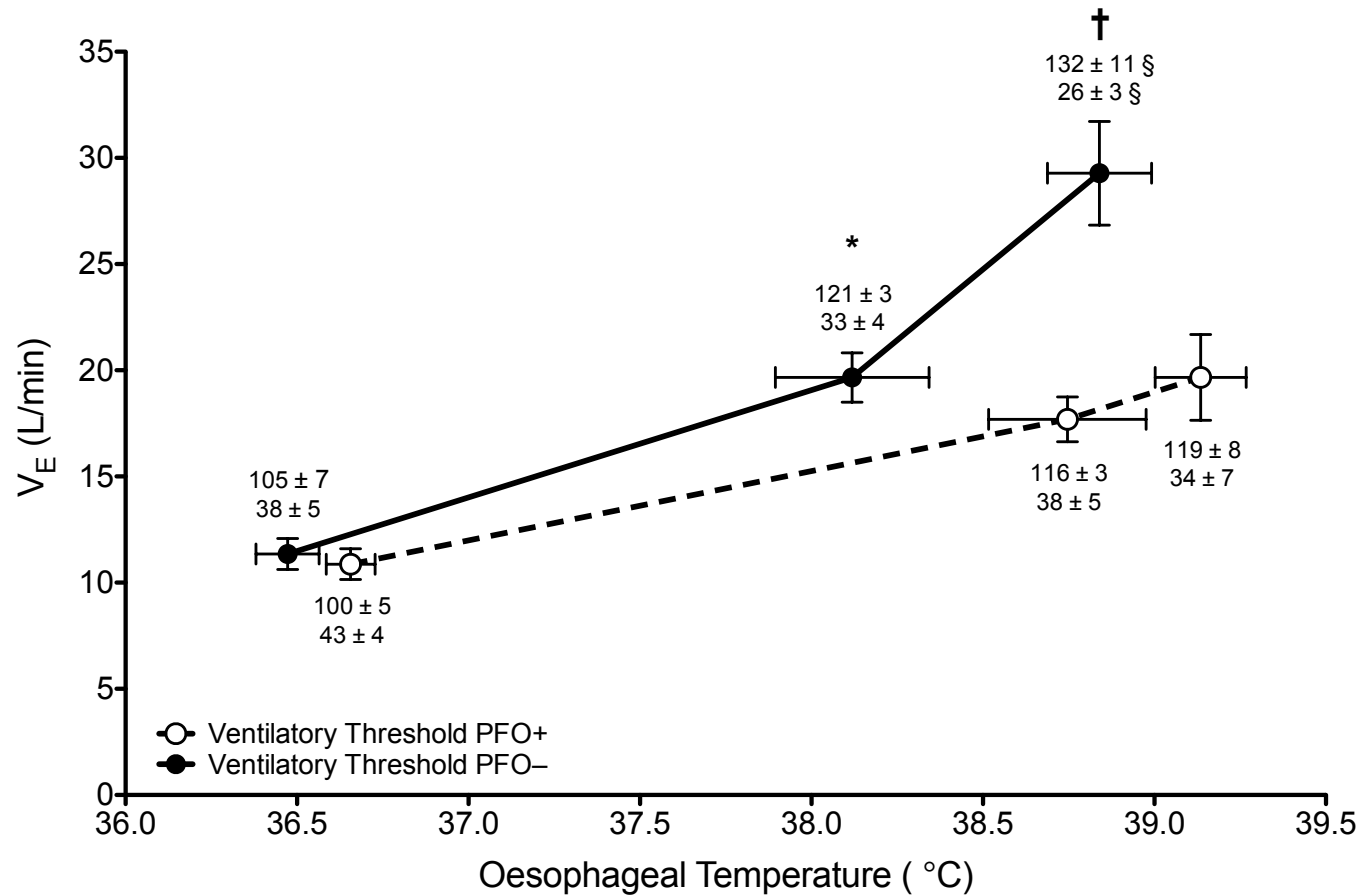
Figure 5.3. Effect of T$_{oesoph}$ on V$_E$ by group during hot water immersion for subjects who reached ventilatory threshold (n = 8 in each group). Points and bars on figure are mean ± standard error. Values on the top and bottom of each data point in text are mean ± standard deviation of P$_{ET}$O$_2$ and P$_{ET}$CO$_2$, respectively. * indicates a significant difference in T$_{oesoph}$ between groups, † indicates a significant difference in V$_E$ between groups, and § indicates significant difference between groups for P$_{ET}$O$_2$ or P$_{ET}$CO$_2$ ($p < .05$).

difference occurring prior to hot water immersion (p < 0.05, Figure 2A). Similarly, in the 16 subjects (8 PFO+) who reached a ventilatory T_{oesoph} threshold, there was no interaction effect; however, there was a main effect of PFO Group on T_{oesoph} with specific differences occurring at ventilatory threshold (PFO+: 38.7 ± 0.6°C, PFO– 38.1 ± 0.6 °C, Figure 3, p < 0.05).

Ventilatory Measures

During the cold tub and hot trial, there were no main effects of PFO Group on V_E, RR, V_t, or $P_{ET}O_2$. Similarly, there was no effect of PFO on $P_{ET}CO_2$ during cold water immersion (*p* > .05, **Tables 5.3**). However, there was a main effect of PFO Group on $P_{ET}CO_2$ during hot water immersion (*p* < .05, **Tables 5.4**)

Ventilatory Threshold During Hot Water Immersion

In the 8/13 PFO+ and 8/14 PFO– subjects who reached ventilatory threshold, there was a main effect of PFO Group on T_{oesoph} with specific differences occurring at the ventilatory threshold (PFO+: 38.7 ± 0.6°C, PFO–: 38.1 ± 0.6°C, **Figure 5.3,** *p* < .05).

Additionally, in the 8/13 PFO+ and 8/14 PFO– subjects who reached ventilatory threshold, there was a main effect of PFO on $P_{ET}O_2$ and $P_{ET}CO_2$, with specific differences occurring at the end of immersion for both measures (*p* < .05, **Figure 5.3,**). Thus, compared to PFO– subjects, PFO+ subjects had blunted ventilatory responses to heat with decreased V_E and increased $P_{ET}CO_2$. Consequently, it appears that in subjects who reached ventilatory threshold, that the ventilatory threshold occurred at a higher

T_{oesoph}, and with a blunted V_E response in PFO+ subjects, when compared to PFO–subjects.

Heart rate and Oxygen Saturation

During the cold tub trial, there was an interaction effect between PFO group and Time on HR ($p < .05$). While PFO+ subjects had a trend towards having a higher HR than PFO– subjects ($p = .07$, **Table 5.3**), there was not a main effect of PFO on SpO_2 ($p > .05$, **Table 5.3**). Moreover, in PFO– subjects only, HR was significantly lower at the end of immersion by ~7 bpm compared to prior to immersion (Prior – 65 ± 10 bpm, End – 58 ± 13 bpm, $p < .05$, **Table 5.3**).

During the hot tub trial, there was not an effect of PFO Group on HR ($p > .05$, **Table 5.4**), but there was an effect of PFO Group on SpO_2 ($p < .05$, **Table 5.4**). However, there were no specific pairwise differences between PFO and SpO_2.

Thus, it appears that during cold water immersion, compared to PFO– subjects, PFO+ subjects trend towards having a higher HR before and during immersion.

Metabolic Measures

During the cold tub and hot tub trials, there was no effect of PFO Group on VO_2, VCO_2, or RER (**Tables 5.3 and 5.4**, $p > .05$).

Respiratory Heat Loss

During cold and hot water immersion, there was no effect of PFO Group on CHL, EHL or RHL in a subset of 5 subjects (2 PFO+) (data not shown, $p > .05$). Similarly,

Table 5. Respiratory heat loss during cold and hot water immersion

		Cold water immersion			Hot water immersion		
		PFO+ (n=2)	PFO– (n = 3)	Overall (n = 5)	PFO+ (n=2)	PFO– (n = 3)	Overall (n = 5)
CHL	Pre-immersion	1.4 ± 0.8	1.7 ± 1.0	1.6 ± 0.8	1.4 ± 0.9	1.7 ± 0.4	1.6 ± 0.5
	End of immersion	1.7 ± 0.7	1.9 ± 1.2	1.8 ± 0.9	2.4 ± 0.6	4.0 ± 2.6	3.3 ± 2.0
EHL	Pre-immersion	2.8 ± 1.6	3.6 ± 2.1	3.3 ± 1.7	3.0 ± 1.8	3.5 ± 0.7	3.3 ± 1.1
	End of immersion	3.5 ± 1.5	3.8 ± 2.4	3.7 ± 1.8	5.1 ± 1.2	8.3 ± 5.3	7.0 ± 4.2
Total RHL	Pre-immersion	4.3 ± 2.4	5.3 ± 3.1	4.9 ± 2.6	4.4 ± 2.7	5.2 ± 1.1	4.9 ± 1.6
	End of immersion	5.3 ± 2.3	5.7 ± 3.5	5.5 ± 2.7	7.5 ± 1.8	12.3 ± 7.9	10.4 ± 6.2

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

when adding a correction factor to the expired air temperature for all subjects, there was no effect of PFO Group on CHL, EHL or RHL (see Appendix, $p > .05$).

Thermal Sensation

There were no differences in thermal sensation between PFO groups prior to water immersion in the cold tub (PFO+: 3.9 ± 0.3, PFO–: 3.8 ± 0.3, $p > .05$) or at the end of water immersion (PFO+: 1.5 ± 0.6, PFO–: 1.4 ± 0.8, $p > .05$). Similarly, there were no differences between groups in thermal sensation prior to water immersion in the hot tub (PFO+: 4.0 ± 0.1, PFO–: 4.0 ± 0.1, $p > .05$) or at the end of immersion (PFO+: 7.5 ± 0.5, PFO–: 6.9 ± 0.9, $p > .05$).

Whole Body Water Loss During Hot Water Immersion

There was a trend for PFO+ subjects to have a lower absolute amount of whole body water loss during hot water immersion (PFO+: 0.6 ± 0.2 kg, PFO–: 0.8 ± 0.3 kg, $p = .06$). However, there was a significant difference in the percentage of total body weight lost (PFO+: 0.7 ± 0.2 %, PFO–: $1.0 \pm 0.3\%$, $p < .05$). Similarly, there was a trend for PFO+ subjects to lose less water weight per hour than PFO– subjects (PFO+: 1.5 ± 0.6 kg/hour, PFO–: 1.9 ± 0.7 kg/hour, $p = .10$).

Immersion Duration and Rate of Temperature Change

There was no effect of PFO Group on the amount of time spent in the cold tub (PFO+: 49 ± 12 min PFO–: 51 ± 14 min, $p > .05$). However, there was a difference in the

rate of temperature change between groups during cold water immersion (PFO+: –0.014 ± 0.010°C/min, PFO–: –0.006 ± 0.008°C/min, $p < .05$). Nevertheless, there was not a difference in rate of temperature change when comparing the 6/13 PFO+ and 3/14 PFO– shivering subjects (PFO+: –0.020 ± 0.017°C/min, PFO–: –0.008 ± 0.009°C/min, $p > .05$).

When comparing all subjects there was no effect of PFO Group on the amount of time spent in the hot tub (PFO+: 24 ± 5 min PFO–: 25 ± 6 min, $p > .05$), or in the rate of temperature change between groups (PFO+: 0.11± 0.02°C°C/min, PFO–: 0.10 ± 0.02°C/min, $p > .05$).

Discussion

In this study we determined that during both cold water and hot water immersion, PFO+ subjects had a higher T_{oesoph} before immersion and maintained this higher T_{oesoph} throughout immersion. Furthermore, during hot water immersion, in the subjects that reached ventilatory threshold for thermal hyperpnoea (8/13 PFO+, 8/14 PFO–), PFO+ subjects reached ventilatory threshold at a higher T_{oesoph} and had blunted ventilatory responses when compared to PFO– subjects. These findings suggest that PFO+ subjects regulate and defend a higher T_{core} than PFO– subjects.

Presence of a PFO and Oesophageal Temperature During Cold Water Immersion

We recently showed that PFO+ subjects have a greater T_{oesoph} of ~0.4°C (Davis *et al.*, 2015). However, it was unknown if differences existed in the thermoregulatory responses to passive cooling. The present study aimed to determine if the presence of a PFO caused T_{oesoph} to change at a different rate during passive cooling. The current study

demonstrated that PFO+ subjects have a greater T_{oesoph} by ~0.4°C prior to cold water immersion, and this difference in T_{oesoph} is maintained throughout immersion. While there was a difference in the T_{oesoph} at which shivering occurred, the small number of subjects (PFO+: 6/13 vs. PFO–: 3/14) means this study is underpowered to definitively make that conclusion.

PFO+ and PFO– subjects maintained the same differences in T_{oesoph} of ~0.4°C prior to cold-water immersion and after peak T_{oesoph} was reached during cold-water immersion. However, in looking at the final measure made before subjects exited the tub, PFO+ subjects had a greater T_{oesoph} than PFO– subjects by ~0.7°C. These findings are likely explained in part by the difference in the T_{core} threshold at which sustained shivering occurs between the two groups and the variability that exists in this response. Only 9/27 subjects experienced 5-minutes of sustained shivering, which demonstrates variability in the shivering T_{core} threshold. Furthermore, 4 PFO– subjects had T_{oesoph} drop to 35.5°C during 60-minutes of cold water immersion, while none of the PFO+ subjects had their T_{oesoph} drop below 35.9°C. These findings support the notion that there are differences in thermoregulatory responses between PFO+ and PFO– groups. It is plausible that PFO+ subjects are "defending" a higher T_{oesoph} and thus begin shivering at a higher T_{oesoph} than PFO– subjects. However, we are unable to definitively make that conclusion. Additionally, there appears to be a difference between groups in heat retention. When comparing the 10 subjects (PFO+: 6/13 vs. PFO–: 4/14) that remained immersed for 60 minutes, the difference in T_{oesoph} was ~0.4°C through the first 40 minutes of immersion. However, after 60 minutes of immersion, the difference between groups had increased to 0.7°C. While the reasons for this response are unclear, this could

106

be due to differences in skin blood flow (Johnson *et al.*, 2014). If PFO+ subjects have reduced skin blood flow compared to PFO– subjects, this could limit the amount of convective heat loss that occurs, resulting in a greater T_{core}.

Presence of a PFO and Oesophageal Temperature During Hot Water Immersion

T_{oesoph} was also higher in PFO+ subjects compared to PFO– subjects during hot water immersion. Furthermore, when comparing the three main time points of interest which were pre-immersion, ventilatory threshold, and the end of immersion, there was an effect of PFO Group on T_{oesoph}. This finding was driven in part due to the ventilatory threshold occurring at a greater T_{oesoph} in PFO+ subjects (38.7 ± 0.6°C) compared to PFO– subjects (38.1 ± 0.6°C, Figures 2A and 4). When looking at all subjects, the T_{oesoph} where ventilatory threshold occurred is similar to the ~38.5°C reported by Cabanac and White (Cabanac & White, 1995), but lower than ~38.9°C that was given by Lucas et al.) (Lucas *et al.*, 2015). A possible explanation for the differences between those studies and the present study is that this study included an equal number of PFO+ and PFO– subjects, and the other studies did not determine if subjects had a PFO. As a result, it is plausible that there was an equal distribution of PFO+ and PFO– subjects in the study completed by Cabanac and White, as the average ventilatory threshold T_{oesoph} of all subjects in the present study is the same as their study. Similarly, the study completed by Lucas et al. might have had a higher prevalence of PFO+ subjects, which could explain the higher T_{oesoph} at which ventilatory threshold they reported.

Despite the differences in T_{oesoph} where ventilatory threshold occurred, the differences between initial T_{oesoph} and the final T_{oesoph} between the two groups of ~0.4°C

was similar, which is in line with findings from our previous work (Davis *et al.*, 2015). In that study during thermoneutral conditions and while breathing ambient air, PFO+ subjects had a greater T_{oesoph} by ~0.4°C prior to and after a 10-minute exercise bout.

Presence of a PFO and Ventilation During Cold Water Immersion

It has been previously been shown that PFO+ subjects had a greater V_E during a VO_{2MAX} test (Davis *et al.*, 2015). However, in this study there were no differences in V_E during the cold tub trial. These findings are unsurprising as V_E was not different in the previous study until the start of exercise. Furthermore, it has been shown that with the exception of an initial hyperventilation during the first minute, V_E does not significantly increase above rest until shivering occurs (Hayward & Eckerson, 1984). Thus, we would expect V_E to remain fairly constant until sustained shivering occurred. When looking at subjects who did shiver, there were no differences in V_E between PFO+ and PFO– subjects ($p > .05$, data not shown).

Presence of a PFO and Ventilation During Hot Water Immersion

Similar to cold tub immersion, there was no main effect of PFO on V_E during hot water immersion when looking at all subjects. However, when looking at the subjects who reached ventilatory threshold, PFO+ subjects had a lower V_E at the end of immersion than PFO– subjects by ~10 L/min (**Figures 5.2B and 5.5**). While the ventilatory responses to hyperthermia have been shown to be variable (Cabanac & White, 1995; Lucas *et al.*, 2015), some of this variability appears to be linked to the presence of a PFO. If thermal hyperpnoea occurs in an effort to lower T_{core}, these findings suggest

that PFO+ subjects defend a higher T_{core} than PFO– subjects. This argument is further strengthened when taken in combination with PFO+ subjects beginning to shiver at a higher T_{oesoph} than PFO– subjects. Additionally, these findings support the idea that PFO+ subjects not only defend a higher T_{core} than PFO– subjects, which is evidenced by a blunted ventilatory response to increased T_{core}. Results from the present study include a blunted ventilatory response to heat stress, while previous research has shown a blunted ventilatory response after 16 d of exposure to high altitude (Elliott *et al.*, 2015). While the reason for these blunted ventilatory responses are unknown, it appears the presence of a PFO plays an important role. One possible explanation for this blunted ventilatory response to heat might be that PFO+ subjects operate at a higher T_{core} and thus they have a shifted response to an increased T_{core}.

Presence of a PFO and Respiratory Heat Loss During Cold Water Immersion

In a subset of subjects, during cold-water immersion there were no differences in convective, evaporative or total respiratory heat loss. RHL is affected by V_E, inspired/expired air temperature and humidity Since this study was designed to prevent differences in T_{insp}, and Hum_{insp}, and we have previously shown no difference in resting V_E between PFO+ and PFO– subjects, the only way we would have seen differences in RHL would require differences in T_{exp} and/or Hum_{exp}. The fact there weren't any differences in T_{exp} and Hum_{exp} resulted in there being no differences in RHL prior to, and during cold-water immersion.

Presence of a PFO and Respiratory Heat Loss During Hot Water Immersion

We have previously suggested that PFO+ subjects have less respiratory heat loss than PFO– subjects (Davis *et al.*, 2015). These conclusions were based on the observation that during exercise PFO– subjects did not increase T_{oesoph} to the same degree breathing cold air that was ~0°C as they did breathing ambient air that was ~20°C. However, findings from both parts of the present study suggest there is no difference in RHL between PFO+ and PFO– subjects An alternative explanation to the findings from the previous study is that inhalation of cold air has been shown to induce peripheral vasoconstriction (Muller *et al.*, 2014). Consequently, PFO+ subjects might have had a more robust peripheral vasoconstrictor response in the skin than PFO– subjects during cold air inhalation, which could have attenuated the amount of heat lost through evaporation of sweat, and therefore the changes in T_{oesoph}.

Although there were no significant differences in RHL during hot water immersion, PFO+ subjects who showed a trend towards having a lower RHL prior to and at the end of hot water immersion. If there were in fact differences in RHL between PFO+ and PFO– subjects, it is possible that the experimental design prevented us from seeing these differences, as the T_{exp} that we measured during this study was ~4°C lower than expected (Hanson, 1974). Reasons for this low T_{exp} are likely explained by the temperature and humidity probes being placed in the expiratory port of the Hans Rudolph valve. Thus, the expired air had cooled off significantly from the time it left the mouth. Consequently, using 5 subjects (2 PFO+), we developed a regression model to predict the T_{exp} at the mouth based on the T_{exp} measured in the expiratory port. This resulted in T_{exp} increasing by ~5°C. However, the differences between the two groups in RHL were still non-significant. While PFO+ trended towards lower RHL than PFO– subjects, this

finding is likely due to the differences in V_E. If the average V_E for PFO– subjects was

substituted for the actual V_E measured in PFO+ subjects, the difference in RHL was

negligible. Thus, it appears that the differences in T_{oesoph} are likely due in large part to

other factors such as differences in skin blood flow or circulating factors such as

pyrogens that are known to increase T_{core} (Dinarello, 1999).

Presence of a PFO and Heart Rate During Cold Water Immersion

Although there were no differences in HR, PFO+ subjects had a HR that was ~9-

13 bpm greater in PFO– subjects at all time points. While not significant, these findings

are similar to those of Davis et al., who showed differences in HR prior to exercise

between PFO+ and PFO– subjects (Davis *et al.*, 2015). It has been well established that

increased T_{core} augments HR (Cabanac & White, 1995; Minson *et al.*, 1998). Since PFO+

subjects have a higher T_{oesoph} (Lovering *et al.*, 2011; Davis *et al.*, 2015), it is unsurprising

that PFO+ subjects trend towards a higher HR. Another possible contributing factor is

that PFO+ subjects likely have a lower partial pressure of oxygen and higher partial

pressure of carbon dioxide in the arteries than PFO– subjects, as blood flowing through a

PFO does not undergo gas exchange. Ultimately this would result in decreased oxygen

content. Assuming equal cardiac output between the two groups, PFO+ subjects would

have decreased oxygen delivery. However, this decreased oxygen content in PFO+

subjects could be overcome by increasing cardiac output via increased HR, which would

increase oxygen delivery.

Presence of a PFO and Heart Rate During Hot Water Immersion

Similar to the cold-water immersion, there were no differences in HR between PFO+ and PFO– subjects during hot water immersion. However, just as with the cold-water immersion, PFO+ subjects showed a trend toward an ~6 bpm higher HR prior to immersion. The reasons for this trend are the same as those during the cold-water immersion, which is that PFO+ subjects have a higher T_{oesoph}, and potentially lower arterial partial pressure of oxygen and higher arterial partial pressure of carbon dioxide. Furthermore, the increase in HR during hot water immersion can be attributed to the increase in T_{oesoph} (Cabanac & White, 1995; Minson *et al.*, 1998) and the increased oxygen uptake that occurs with immersion (Mekjavić & Bligh, 1989). However, unlike the end of cold-water immersion, at the end of hot-water immersion, PFO+ and PFO– subjects approximately had the same HR of ~113 bpm. It is possible that smaller absolute change in HR is due to the blunted ventilatory responses demonstrated by PFO+ subjects. Since PFO+ subjects have a lower V_E at the end of hot water immersion, their respiratory muscles would not need as much oxygen as PFO– subjects, resulting in a smaller absolute increase in HR during hot water immersion. Conversely, PFO– subjects would need to increase oxygen delivery by a greater degree, via augmented cardiac output, to provide more oxygen to respiratory muscles that augmented V_E.

Work done by Trinity et al. has demonstrated that during exercise in the heat, stroke volume is not limited by increased skin blood flow. Rather, stroke volume is attenuated by the reduced filling time which occurs with increased HR (Trinity *et al.*, 2010). However, it is plausible that during hot water immersion, skin blood flow is maximized, which could ultimately decrease stroke volume. This notion is supported by findings from Bonde-Petersen et al., who showed that during immersion in 44°C water,

skin blood flow, cardiac output, heart rate and stroke volume increased 110%, 44%, 32% and 9% respectively (Bonde-Petersen *et al.*, 1992). It is possible that the dramatic increase in skin blood flow caused a significant decrease in stroke volume. Consequently, if PFO– subjects have greater skin blood flow than PFO+ subjects, it is possible they would also have a smaller stroke volume than PFO+ subjects. Thus, PFO– subjects would only be able to maintain cardiac output by increasing HR to a greater magnitude than PFO+ subjects. While this notion is plausible, it is presently unknown if the work of breathing and/or stroke volume is different between these two groups prior to or during hot water immersion.

Presence of a PFO and Water Loss During Hot Water Immersion

Unlike cold-water immersion, during hot water immersion there was a significant amount of water loss before and after water immersion. Although, there were no significant differences in the absolute amount of water loss between the two groups, PFO– subjects lost a greater percentage of total body weight during hot water immersion. While the measurements do not allow us to determine the core or mean body temperature for the onset of sweating, these data suggest that PFO+ subjects 1) begin sweating at a higher T_{oesoph} and 2) have a lower sweat rate than PFO– subjects. Previous work has shown that sweating begins when T_{core} increases by ~0.1-0.2°C (Kenny *et al.*, 1997; 2003), which would mean subjects in both groups would have begun to sweat within the first 5 minutes of water immersion. However, this threshold might also be affected by skin temperature and rate of skin temperature change, which might have caused sweating to occur earlier (Nadel *et al.*, 1971). Additionally, if PFO+ subjects have blunted

sweating rates, this would support the notion that they have blunted thermoregulatory responses to increased T_{oesoph}. Furthermore, it is possible that water loss would also be increased towards the end of the hot water immersion when ventilation was greatest, as more insensible water loss occurs with augmented ventilation (Cox, 1987). Consequently, future studies need to be completed to determine if there are differences in the T_{oesoph} at which sweating begins, or in sweat rate.

Presence of a PFO and Shivering During Cold Water Immersion

In this study 9/27 subjects experienced sustained shivering as measured by a 25% increase in metabolic rate for 5 minutes. Out of these 9 subjects, the 6 PFO+ subjects began shivering at a higher T_{oesoph} than the 3 PFO– subjects. If all subjects remained in the cold tub until they began to shiver the average T_{oesoph} at which shivering occurred would be lower. Consequently, the actual T_{oesoph} at which shivering occurred in ~20°C water is still unknown. Despite this, both groups showed similar initial responses by increasing T_{oesoph} by ~0.2°C during the first 10-15 minutes. However, after the peak T_{oesoph} was attained, PFO+ subjects decreased T_{oesoph} by ~0.5°C, while PFO– subjects decreased T_{oesoph} by ~0.8°C. This suggests that PFO+ subjects do not lose heat at the same rate as PFO– subjects, and further supports the idea that PFO+ subjects defend a higher T_{oesoph} and/or are able to maintain T_{oesoph} better during a cold challenge. However, shivering is not only influenced by T_{core}, but by skin temperature as well (Lopez *et al.*, 1994; Cheng *et al.*, 1995). Consequently, conclusions made from the present study can only be applied to immersion in 20°C water.

Limitations

While PFO+ subjects showed a trend towards a higher HR than PFO– subjects prior to immersion, this study was not designed to detect differences in resting HR. There is likely a sympathetic response just prior to immersion, which would elevate HR. Future studies directed at answering this question should be completed in an environment conducive to achieving a true resting HR.

Additionally, people with a greater amount of subcutaneous fat have more insulation, which could prevent them from decreasing T_{core} at the same rate as people with less fat (Daniels & Baker, 1961). Although we did not measure actual body composition, the fact that we did not have any differences between groups in height, weight and body surface area (**Table 5.2**) suggest body composition was similar. However, future studies should determine body composition to ensure that the amount of subcutaneous fat is not a confounding factor.

Finally, we measured sweat rate by taking the difference of weight prior to and after water immersion. This will overestimate actual sweat rate due to insensible water loss that occurs due to ventilation. Additionally, the measurements made in this study did not allow determination of the T_{core} threshold for the onset of sweating, and consequently it still remains to be determined if the T_{core} at which sweating occurs is affected by the presence of a PFO. Therefore, future studies should be designed in a manner that allows for this T_{core} to be determined.

Summary

This study has again demonstrated that PFO+ subjects have a greater resting T_{oesoph} of ~0.4°C than PFO− subjects. Additionally, we have shown that compared to PFO− subjects, PFO+ subjects 1) begin to shiver at a higher T_{oesoph} during cold water immersion and 2) have a higher T_{oesoph} prior to and during cold water and hot water immersion. Additionally, in subjects who reached ventilatory threshold during hot water immersion, PFO+ subjects attained the ventilatory threshold at a greater T_{oesoph} and have a blunted ventilatory response. These findings are important because they support the notion that PFO+ subjects might be more susceptible to heat related illnesses as they might attain a critical T_{core} sooner than PFO− subjects when all other things are held constant. Although the rate of change of T_{oesoph} was the same in PFO+ and PFO− subjects, the difference in resting T_{oesoph} between these two groups could be important as it relates to both fatigue and potential cellular damage. PFO+ subjects are hotter than PFO− subjects and are therefore closer to a T_{core} at which fatigue and/or cellular damage could occur. Consequently, it is plausible that PFO+ subjects are more susceptible to heat related illnesses than PFO− subjects.

Similarly, PFO− subjects might be more disposed to experiencing cold related illnesses. Based on these findings, research examining exercise performance in hot and cold environments may want to consider the effect of a PFO on T_{core} and performance. Finally, this study further supports the idea that some of the normal biological variability found in T_{core}, V_E and HR might be explained by the presence/absence of a PFO.

Acknowledgments

CHAPTER VI

THE EFFECT OF A PATENT FORAMEN OVALE ON ACUTE VENTILATORY

RESPONSES TO HYPOXIA AND HYPERCAPNIA

This chapter will be submitted for publication to the *Journal of Physiology* and Lindsey Boulet, B.S. Alyssa M. Hardin, Alex Chang, Dr. Glen Foster and Dr. Andrew T. Lovering are co-authors. I performed the experimental work, led the project, and the writing is entirely my own; Lindsey Boulet, Alyssa Hardin, Alex Chang assisted with data collection and data analysis; Dr. Glen Foster and Dr. Andrew T. Lovering helped develop the protocol, and provided guidance and editorial assistance. All co-authors will formally approve this manuscript prior to submission.

Introduction

The existence of a patent foramen ovale (PFO) has been known for nearly 2,000 years (Christie, 1930; Patten, 1931; Aslam *et al.*, 2006). Individuals with a PFO (PFO+) have a small tunnel between the right and left atria, which can allow varying degrees of cardiac output (Q_c) to bypass the respiratory system. Autopsy studies (Hagen *et al.*, 1984; Kerut *et al.*, 2001) and studies using saline contrast echocardiography in living humans (Woods *et al.*, 2010; Marriott *et al.*, 2013; Elliott *et al.*, 2013) have shown that a PFO is present in a substantial proportion of the population with a prevalence of ~25-40%. Despite its high prevalence, little work has explored potential effects of a PFO on physiology in healthy humans.

Previous work by our lab has shown there is no difference in resting ventilation in PFO+ subjects after rapid ascent to 5260 m from sea-level (Elliott *et al.*, 2015). However,

after 16 days of exposure to 5260 m, PFO+ subjects showed blunted ventilation compared to PFO– subjects, despite PFO+ subjects having a lower arterial partial pressure of oxygen (PaO_2), higher arterial partial pressure of carbon dioxide ($PaCO_2$), and lower arterial oxygen saturation (SaO_2). However, there were a disproportionate number of females in the PFO+ group, and sex hormones can affect ventilatory responses (Schoene *et al.*, 1986; Slatkovska *et al.*, 2006). Consequently, it is possible these findings were driven by differences in sex hormones and not the presence of a PFO. Additionally, we have shown that in subjects who reached ventilatory threshold for thermal hyperpnoea during passive heating, compared to PFO– subjects, PFO+ subjects had blunted ventilatory responses (in submission).

Taken together these studies suggest that PFO+ subjects have blunted ventilatory responses compared to PFO– subjects. Presently it is unknown if PFO+ subjects exhibit blunted ventilatory responses during acute exposure to isocapnic hypoxia, as well as hyperoxic hypercapnia and normoxic hypercapnia, when compared to PFO– controls. Therefore, the purpose of this study was fourfold. We wanted to determine if the presence of a PFO affects ventilatory responses during acute exposure to 1) poikilocapnic hypoxia, 2) isocapnic hypoxia, 3) hyperoxic hypercapnia and 4) normoxic hypercapnia. These conditions were chosen to isolate differences between central and peripheral chemoreceptor sensitivity, as well as the cumulative effect of both. Based on findings from Elliott et al. (Elliott *et al.*, 2015) and Davis et al. (in submission), it was hypothesized that compared to PFO– subjects, PFO+ subjects would have blunted ventilatory responses to all four conditions.

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*.

Participants

A total of 62 subjects were recruited for participation in this study. Researchers described orally, and in writing, the nature of the study to all subjects, who subsequently provided their written consent. A total of 31 subjects (16 female) qualified and completed the entire study. These 31 subjects included 15 PFO+ subjects (8 female) and 16 PFO− subjects (8 female). Of the remaining 31 subjects who did not complete the study, 5 were excluded for having poor pulmonary function (forced vital capacity < 85% of predicted), 11 withdrew before completing the entire protocol for reasons not associated with the study (e.g. time commitment, moved out of town, etc.), we were unable to place an intravenous catheter in 3 subjects to screen for PFO, 7 had late appearing contrast (i.e. contrast appearing after more than 3 cardiac cycles with greater than 4 bubbles appearing in the left ventricle), 5 subjects were excluded because their group was already filled (i.e. we already had 8 PFO− males). In total we had a PFO prevalence of 46% which is greater than what has been previously reported (Hagen *et al.*, 1984; Kerut *et al.*, 2001; Woods *et al.*, 2010; Marriott *et al.*, 2013; Elliott *et al.*, 2013), because some subjects were invited to participate that had been previously identified as being PFO+ or PFO−, six of which (4 PFO+) had participated in a study looking at the effect of a PFO on T_{core} (Davis *et al.,*

2015). Ultimately, 31 (15 PFO+) healthy, non-smoking volunteers, age 25 ± 8 yr., without history of cardiopulmonary disease were recruited and, after written informed consent was given, agreed to proceed with the study.

Ultrasound Screening

Ultrasound screening has been previously described in detail (Lovering & Goodman, 2012). Initial agitated saline contrast studies were performed with subjects breathing room air and reclined at 45° in the left lateral decubitus position where a clear apical, four-chamber view was obtained. Care was taken to optimally visualize all four chambers, interatrial septum and delineate myocardial and valvular structures by individually adjusting the receiver gain settings. Each saline contrast injection was created by manually agitating 3 ml of sterile saline with 1 ml of room air for 15 s between two 10 ml syringes connected in parallel to two 3-way stopcocks. The saline–air microbubble suspension was then immediately injected in a constant, forceful manner into a peripheral antecubital vein via an IV catheter (20–22 G). This mixture of saline and air provides excellent right-sided contrast. Following opacification of the right atrium and ventricle, the subsequent 20 cardiac cycles were recorded at >30 frames/s for further analysis.

The appearance of ≥1 microbubble in the left atrium or ventricle in any frame during the subsequent 20 cardiac cycles served as the criterion that subjects were either positive for an intracardiac right-to-left shunt (i.e. PFO) or demonstrated the transpulmonary passage of contrast (Freeman & Woods, 2008; Woods *et al.*, 2010; Marriott *et al.*, 2013; Elliott *et al.*, 2013). Saline contrast injections were performed

during normal breathing, as well as immediately following the release of a Valsalva

manoeuvre in order to transiently elevate right atrial pressure and create conditions

optimal for the detection of an intracardiac right-to-left shunt. Effective Valsalva

manoeuvres were confirmed by a transient leftward shift of the interatrial septum.

Valsalva manoeuvres do not increase left heart contrast in the absence of a PFO. An

intracardiac right-to-left shunt was suspected if microbubbles appeared in the left heart

≤ 3 cardiac cycles following right heart opacification. Subsequently, these subjects were

classified as PFO+, while all others without left sided contrast were categorized as PFO−.

Using this approach we have shown that we have the sensitivity to accurately detect PFO

in the general healthy population (Elliott *et al.*, 2013).

Pulmonary Function and Lung Diffusion Capacity

Prior to the pulmonary function testing, using an electronic scale (Ohaus

Corporation, ES200L, Pinebrook, NJ) researchers obtained the subject's weight while

wearing shorts as well as the subject's standing height. Baseline pulmonary function

testing included measures of forced vital capacity (FVC), forced expiratory volume in 1 s

(FEV1), and forced midexpiratory flows (FEF25−75). Measurements were made with a

computerized spirometry system (Ultima PFX, MedGraphics, St. Paul, MN) according to

American Thoracic Society/European Respiratory Society (ATS/ERS) standards

(Macintyre *et al.*, 2005). Lung volumes and capacities were determined using whole-

body plethysmography (Wanger *et al.*, 2005). Lung diffusion capacity for carbon

monoxide (DL_{CO}) was determined by the single-breath, breath-hold method (Knudson *et*

al., 1987) using the Jones and Meade method for timing and alveolar sample collection

(MedGraphics Ultima PFX, Breeze v.6.3.006) . Predicted values for DL_{CO} were

calculated as previously described (Gutierrez *et al.*, 2004).

Protocols

Subjects completed four different protocols, 1) hyperoxic hypercapnia, 2)

normoxic hypercapnia, 3) isocapnic hypoxia and 4) poikilocapnic hypoxia. Each trial was

separated by a minimum of 40 minutes. The hypercapnic trials were completed prior to

the hypoxic trials. This was done in an effort to prevent the carry over effect of

sympoathoexcitation which can occur following hypoxic exposure (Morgan *et al.*, 1995).

Furthermore, the order of the hypercapnic and hypoxic trials were each randomized.

Respiratory Measurements

Acquisition of respiratory parameters occurred at 200 Hz using an analog-to-

digital converter (Powerlab/16SP ML 880; ADInstruments, Colorado Springs, CO)

interfaced with a personal computer. Commercially available software was used to

analyze ventilatory and cardiovascular variables (LabChart V7.1, ADInstruments,

Colorado Springs, CO). Subjects breathed through a mouthpiece with a noseclip, a

bacteriological filter, and a two-way nonrebreathing valve (7900 series, Hans Rudolph,

Shawnee, KS) during all four trials. Airflow resistance for the breathing apparatus was

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. Respired

gas pressures were sampled at the mouth, dried with nafion tubing, and analyzed for end

tidal oxygen ($P_{ET}O_2$) and end tidal carbon dioxide ($P_{ET}CO_2$) (ML206; ADInstruments,

Colorado Springs, CO). Gas analyzers were calibrated with gases of known

concentration. Measured PO_2 and PCO_2 were time corrected for gas analyzer sample delay, and the values corresponding to the end of expiration (i.e., when respiratory flow crossed zero in the positive to negative direction) were identified as the $P_{ET}O_2$ and $P_{ET}CO_2$. The fraction of inspired oxygen (F_IO_2) and carbon dioxide (F_ICO_2) delivered to the subject for each breath were determined by the dynamic end tidal forcing (DEF) system (see below) and recorded in a text file for subsequent analysis. Respiratory flow was measured near the mouth using a pneumotachograph (HR 800L, Hans Rudolph, Shawnee, KS), and a differential pressure amplifier (ML141, ADInstruments, Colorado Springs, CO), which was calibrated with a 3-liter syringe. Total apparatus dead space was 250 ml.

End-Tidal Forcing

A portable DEF system controlled $P_{ET}O_2$ and $P_{ET}CO_2$. This system used independent gas solenoid valves for O_2, CO_2, and N_2, which controlled the volume of each gas being delivered to the inspiratory reservoir through a mixing and humidification chamber. $P_{ET}O_2$, $P_{ET}CO_2$, tidal volume (V_t), breathing frequency (*f*), and minute ventilation (V_E) were determined for each breath using specifically designed software (Labview 13.0, National Instruments, Austin, TX). Using feedback regarding $P_{ET}O_2$, $P_{ET}CO_2$, inspired V_t, and expired V_t, the DEF system adjusted the inspirate to bring end-tidal gas to the desired target values. Feed-forward control of the inspirate was based on estimates of baseline metabolic O_2 consumption and CO_2 production, and employed the alveolar gas equation to determine the required F_IO_2 and F_ICO_2 and fraction of inspired nitrogen. This system has been used previously to control end-tidal gases during

124

physiological stressors (Bain *et al.*, 2013; Tremblay *et al.*, 2014; Tymko *et al.*, 2015; 2016). End-tidal steady state was defined as the timepoint when $P_{ET}O_2$ and $P_{ET}CO_2$ values remained within 1 mmHg of the desired target for 3 consecutive breaths.

Hyperoxic Hypercapnia

Baseline values of $P_{ET}CO_2$ values were determined after 5-minute of breathing room air without aid of the DEF system. The DEF system was then utilized, such that the F_IO_2 and F_ICO_2 were adjusted to maintain $P_{ET}O_2$ and $P_{ET}CO_2$ at 250 mm Hg and baseline values, respectively. Once steady-state was attained, subjects continued to breathe that mix of gas for 90 seconds. $P_{ET}CO_2$ was then increased in a stepwise fashion +3, +6 and +9 mm Hg from baseline. Each step in $P_{ET}CO_2$ lasted for 90 seconds after a steady state in end-tidal gases was achieved. HCVR was determined by creating regression lines for individual subjects of $P_{ET}CO_2$ based on the change from baseline $P_{ET}CO_2$ (0, +3, +6, +9 mmHg) and associated V_E. Significance was determined by comparing the average group slopes. Although the use of a hyperoxic condition does not eliminate all afferent input from the peripheral chemoreceptor, it will reduce peripheral chemoreceptor input below that found in normoxic conditions. Thus, the use of this protocol allowed us to estimate central chemoreceptor sensitivity, while minimizing peripheral chemoreceptor input. For all subjects used in the analysis, the Pearson correlation (r) was suitably linear, with $r > 0.7$.

Normoxic Hypercapnia

125

Baseline values of $P_{ET}O_2$ and $P_{ET}CO_2$ values were determined after 5-minute of breathing room air without aid of the DEF system. The DEF system was then utilized, such that the F_IO_2 and F_ICO_2 were adjusted so that $P_{ET}O_2$ and $P_{ET}CO_2$ were clamped at baseline values for both gases. Once steady-state was attained, subjects continued breathed that mix of gas for 90 seconds. $P_{ET}CO_2$ was then increased in a stepwise fashion +3, +6 and +9 mm Hg from baseline. Each step in $P_{ET}CO_2$ lasted for 90 seconds after attainment of steady state. HCVR was determined as above. For all subjects used in the analysis, the Pearson correlation (r) was suitably linear, with $r > 0.7$.

Isocapnic Hypoxia

After 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 and the time at which V_E was greatest after 5 minutes of hypoxic exposure. The hypoxic ventilatory response (HVR) was calculated using two time points – the 60-second average prior to administration of the hypoxic mixture, and the 60-second average during hypoxic administration where V_E was the greatest. HVR was defined as the change in V_E divided by the change in SpO_2.

Poikilocapnic Hypoxia

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 (as described above),

126

subjects remained in this hypoxic condition for 20 minutes. Time points of interest were baseline and the time at which V_E was greatest after 5 minutes of hypoxic exposure. HVR was calculated as above.

Statistical Analyses

Data were analysed 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 analysed using a two-way mixed ANOVA (PFO x time point) with α = .05. When determining the HCVR, individual regression lines were calculated based on the +3, +6 and +9 time points. An average regression line was calculated for each group, and an independent *t*-test with α = .05 was run on the average slope.

To calculate HVR, we used an average of the final 15 seconds prior to hypoxic exposure as our baseline. The hypoxic time point was defined as the 15 second average with the greatest V_E after a minimum of 5-minutes of hypoxic exposure. HVR was calculated as the difference in V_E between baseline and hypoxic exposure divided by the difference in SpO_2 between baseline and hypoxic exposure. For all other variables a two-way mixed ANOVA (PFO x time point) with α = .05 was utilized.

Results

Anthropometrics and Lung Function

Anthropometric, pulmonary function, and DL_{CO} data for PFO+ and PFO− subjects are presented in **Table 6.1**. There were no significant differences between PFO+

and PFO– groups ($p > .05$). However, there were differences between males and females in height, weight, FVC, FEV_1, and DL_{CO} **(Table 6.1)**

Hypercapnic Ventilatory Response

Figures 6.1 and 6.2 illustrate the group averages during the hyperoxic hypercapnia (HH) and normoxic hypercapnia (NH) trials, respectively. During the HH trial there were 4 PFO+ (2 male) and 1 PFO– (1 female) subjects that were excluded. Similarly, during the NH trial there were 3 PFO+ (1 male) and 3 PFO– (1 male) subjects that were excluded. During both trials, PFO+ subjects had a significantly lower HCVR than PFO– subjects ($p < .05$). However, there were no differences in HCVR within each group between HH and NH trials (PFO+: 1.24 ± 0.15 L min^{-1} mmHg^{-1} vs. 1.23 ± 0.13 L min^{-1} mmHg^{-1}, $p > .05$; PFO–: 1.80 ± 0.18 L min^{-1} mmHg^{-1} vs. 1.77 ± 0.23 L min^{-1} mmHg^{-1}, $p > .05$).

Hypoxic Ventilatory Response

There was no between group differences (i.e. PFO+ vs. PFO–) in the HVR during the isocapnic hypoxia (IH) trial (**Figure 6.3**) or poikilocapnic hypoxia (PH) trial (**Figure 6.4**). However, there was a difference between IH and PH for both groups (PFO+: 1.30 ± 0.55 L min^{-1} %SpO$_2^{-1}$, vs. 0.66 ± 0.68 L min^{-1} %SpO$_2^{-1}$, $p < .05$; PFO–: 1.26 ± 0.54 L min^{-1} %SpO$_2^{-1}$ vs. 0.61 ± 0.35 L min^{-1} %SpO$_2^{-1}$, $p < .05$), as expected.

Cardiorespiratory Measures

Table 6.1. Anthropometric, and pulmonary function data

	PFO+			PFO–			Overall		
	Females $n = 8$	Males $n = 7$	Overall $n = 15$	Females $n = 8$	Males $n = 8$	Overall $n = 16$	Females $n = 16$	Males $n = 15$	Overall $n = 31$
Age (years)	21 ± 1	$28 \pm 6^*$	24 ± 5	25 ± 10	28 ± 9	27 ± 9	23 ± 7	28 ± 7	25 ± 7
Height (cm)	163 ± 8	$180 \pm 9^*$	170 ± 11	165 ± 6	$180 \pm 6^*$	172 ± 10	164 ± 7	$180 \pm 7^*$	171 ± 10
Weight (kg)	59 ± 8	$82 \pm 10^*$	69 ± 14	59 ± 5	$82 \pm 15^*$	71 ± 16	59 ± 6	$82 \pm 12^*$	70 ± 15
BSA (m^2)	1.6 ± 0.1	$2.0 \pm 0.2^*$	1.8 ± 0.2	1.6 ± 0.1	2.0 ± 0.2	1.8 ± 0.2	1.6 ± 0.1	$2.0 \pm 0.2^*$	1.8 ± 0.2
FVC (L)	3.7 ± 0.4	$5.5 \pm 1.1^*$	4.5 ± 1.3	3.9 ± 0.5	$5.4 \pm 0.5^*$	4.6 ± 0.9	3.8 ± 0.5	$5.4 \pm 0.8^*$	4.7 ± 1.1
FEV$_1$ (L)	3.4 ± 0.3	$4.7 \pm 0.4^*$	4.1 ± 0.8	3.3 ± 0.4	$4.6 \pm 0.3^*$	4.0 ± 0.7	3.4 ± 0.3	$4.6 \pm 0.4^*$	4.0 ± 0.8
DL$_{CO}$ (ml•min^{-1}•Torr^{-1})	27.0 ± 4.4	$45.1 \pm 7.9^*$	36.0 ± 11.2	32.1 ± 4.3	$39.9 \pm 6.7^*$	36.3 ± 6.9	29.5 ± 5.0	$42.3 \pm 7.5^*$	36.1 ± 9.1
DL$_{CO}$/V$_A$(ml•min^{-1}•Torr^{-1}•L^{-1})	6.0 ± 0.6	6.1 ± 0.4	6.0 ± 0.5	6.2 ± 0.5	5.7 ± 0.8	5.9 ± 0.7	6.1 ± 0.5	5.9 ± 0.6	6.0 ± 0.6

Values are mean $\pm$ standard deviation. * indicates difference from females, $p < .05$.

Table 6.2. Ventilatory and cardiovascular measures during hyperoxic and normoxic hypercapnia

		Rest		+3		+6		+9	
		PFO–	PFO+	PFO–	PFO+	PFO–	PFO+	PFO–	PFO+
Hyperoxic Hypercapnia	V_E (L•min^{-1})	16.1 ± 4.8	17.5 ± 4.2	20.0 ± 5.1	19.9 ± 4	25.5 ± 5.3	25.1 ± 5.6	31.9 ± 7.3	28.2 ± 4.0
	V_t (L)	1.5 ± 0.6	1.4 ± 0.5	1.6 ± 0.5	1.6 ± 0.5	1.8 ± 0.7	1.9 ± 0.5	2.0 ± 0.7	2.0 ± 0.6
	RR (breaths•min^{-1})	12.5 ± 4.7	13.2 ± 3.8	13.8 ± 4.9	13.5 ± 3.5	15.3 ± 5.0	14.1 ± 3.5	16.9 ± 5.6	14.8 ± 3.1
	$P_{ET}O_2$ (mmHg)	237.9 ± 29.2	239.9 ± 24	245.5 ± 9.0	234.3 ± 35.2	246.1 ± 3.7	245.2 ± 4.8	245.3 ± 4.1	242 ± 15.5
	$P_{ET}CO_2$ (mmHg)	41.8 ± 3.1	41.7 ± 2.7	45.3 ± 3.2	45.5 ± 3.2	47.9 ± 2.9	48.4 ± 2.6	50.9 ± 2.9	51.2 ± 2.8
	HR (bpm)	66.4 ± 14.6	65.2 ± 8.0	67.2 ± 14.1	66.1 ± 7.9	69.3 ± 14.8	69.6 ± 9.3	70.9 ± 15	69.1 ± 7.8
	SpO_2 (%)	98.4 ± 1.3	98.3 ± 1.3	98.4 ± 1.9	98.2 ± 1.1	98.5 ± 1.4	98.3 ± 1.1	98.4 ± 1.2	97.9 ± 1.4
Normoxic Hypercapnia	V_E (L•min^{-1})	14.6 ± 3.9	16.0 ± 5.1	18.9 ± 5.3	19.0 ± 3.2	24.3 ± 6.7	22.5 ± 3.9	29.8 ± 9.5	27.1 ± 5.2
	V_t (L)	1.4 ± 0.5	1.4 ± 0.6	1.6 ± 0.6	1.6 ± 0.5	1.9 ± 0.8	1.8 ± 0.6	2.1 ± 0.8	2.0 ± 0.7
	RR (breaths•min^{-1})	12.1 ± 4.3	12.6 ± 3.3	12.9 ± 4.8	13.0 ± 3.4	14.5 ± 5.2	13.2 ± 3.1	15.3 ± 5.6	14.1 ± 2.8
	$P_{ET}O_2$ (mmHg)	100.6 ± 6.3	99.6 ± 6.9	10.0 ± 4.0	98.6 ± 6.8	100.3 ± 2.7	99.0 ± 5.9	100.1 ± 3.6	98.6 ± 6.3
	$P_{ET}CO_2$ (mmHg)	41.1 ± 3.1	41.9 ± 2.6	44.0 ± 2.8	45.2 ± 2.7	47.2 ± 2.6	48.1 ± 2.1	49.9 ± 2.8	51.0 ± 1.9
	HR (bpm)	67.9 ± 14.8	65.6 ± 8.7	68.9 ± 14.0	68.2 ± 8.5	71.7 ± 18.3	69.1 ± 9.3	72.7 ± 15.6	70.8 ± 8.7
	SpO_2 (%)	96.6 ± 1.9	96.2 ± 1.6	96.5 ± 2.0	96.3 ± 2.0	96.6 ± 1.5	96.6 ± 1.4	97.2 ± 1.4	97.1 ± 1.3

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

Table 6.3. Ventilatory and cardiovascular 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•min^{-1})	12.7 ± 5	11.6 ± 4.7	35.9 ± 11.5	34.4 ± 11.6	12.5 ± 3.6	11.9 ± 4.1	23.3 ± 10.3	22.4 ± 9.4
V_t (L)	1.2 ± 0.5	1.0 ± 0.4	2.2 ± 0.9	2.1 ± 0.8	1.1 ± 0.4	1.0 ± 0.5	1.9 ± 0.9	1.6 ± 0.9
RR (breaths•min^{-1})	11.8 ± 4.5	12.5 ± 2.7	17.7 ± 6.6	17.4 ± 4.4	12.4 ± 4.1	12.8 ± 3.1	13.4 ± 4.7	15.0 ± 4.3
$P_{ET}O_2$ (mmHg)	99.3 ± 4.7	99.4 ± 4.6	43.3 ± 4.5	44.1 ± 4.4	102.0 ± 5.9	101.9 ± 4.8	51.3 ± 5.4	51.5 ± 7.6
$P_{ET}CO_2$ (mmHg)	37.8 ± 3.7	38.2 ± 2.5	40.6 ± 2.9	39.4 ± 3.8	36.0 ± 5.6	38.3 ± 2.9	29.7 ± 9.1	28.5 ± 10.2
HR (bpm)	62.7 ± 13.4	60.7 ± 8.3	80.4 ± 15.5	82.9 ± 9.3	60.2 ± 12.7	63.5 ± 9	73.0 ± 16.4	76.9 ± 11.5
SpO_2 (%)	97.3 ± 1.7	97.4 ± 1.6	78.6 ± 4.2	78.9 ± 4.6	97.8 ± 1.3	97.3 ± 1.2	79.6 ± 5.4	78.9 ± 4.8

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

Figure 6.1 Effect of PFO on HCVR during acute exposure to hyperoxic hypercapnia. Dashed line and open circles represent average $P_{ET}CO_2$ and V_E and the associated regression line for PFO+ subjects. Solid line and filled circles represent average change from resting $P_{ET}CO_2$ values plotted against V_E and the associated regression line for PFO+ subjects. There was a main effect of PFO on HCVR during exposure to hyperoxic hypercapnia. * indicates significant difference in slope from PFO+, $p < .05$.
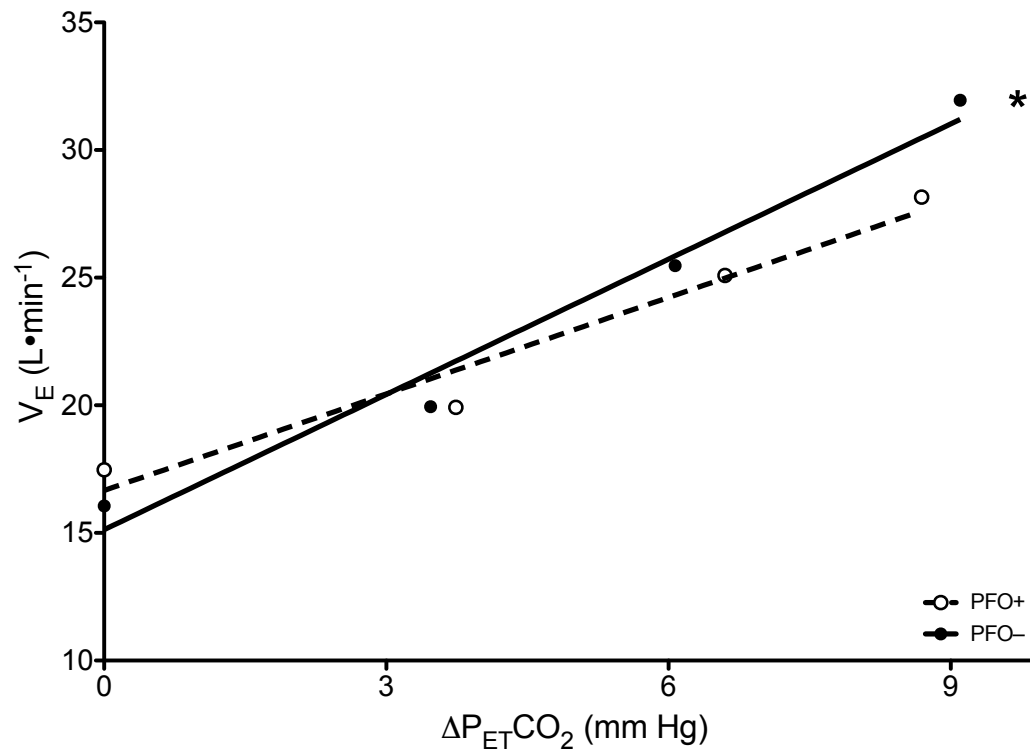
Figure 6.2. Effect of PFO on HCVR during acute exposure to normoxic hypercapnia. Dashed line and open circles represent average $P_{ET}CO_2$ and V_E and the associated regression line for PFO+ subjects. Solid line and filled circles represent average change from resting $P_{ET}CO_2$ values plotted against V_E and the associated regression line for PFO+ subjects. There was a main effect of PFO on HCVR during exposure to normoxic hypercapnia. * indicates significant difference in slope from PFO+, $p < .05$.
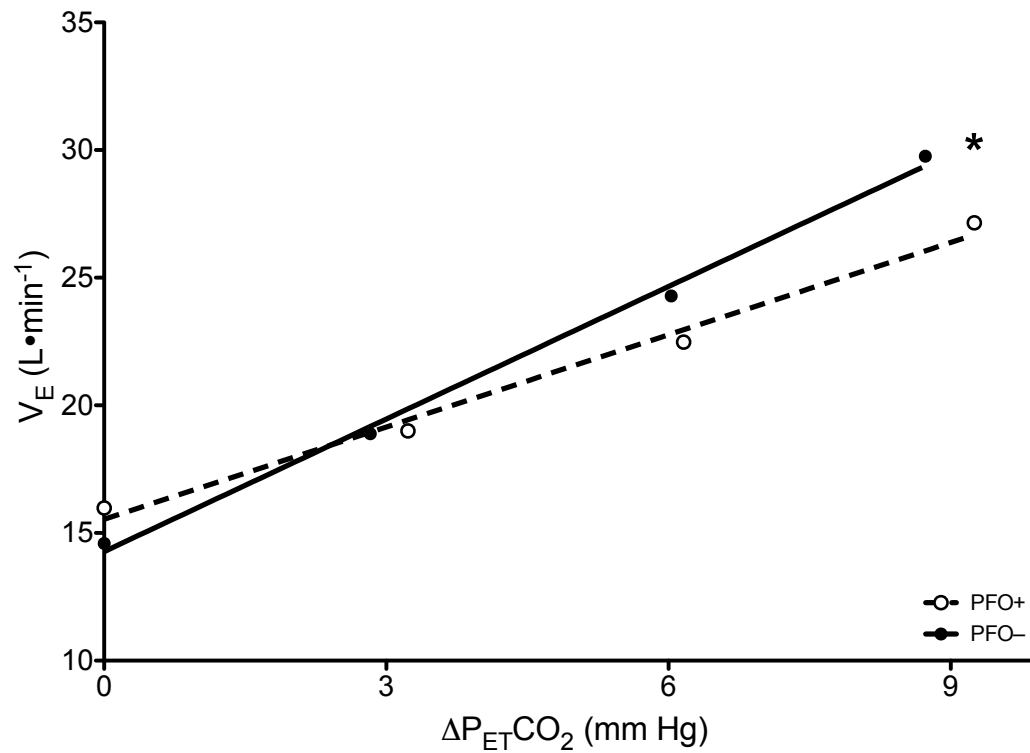
Figure 6.3. Effect of PFO on HVR during acute exposure to isocapnic hypoxia. Thick dashed and solid lines represent group averages for PFO+ and PFO− subjects respectively. Values are mean ± standard deviation. There was no effect of PFO on HVR during exposure to isocapnic hypoxia ($p > .05$).
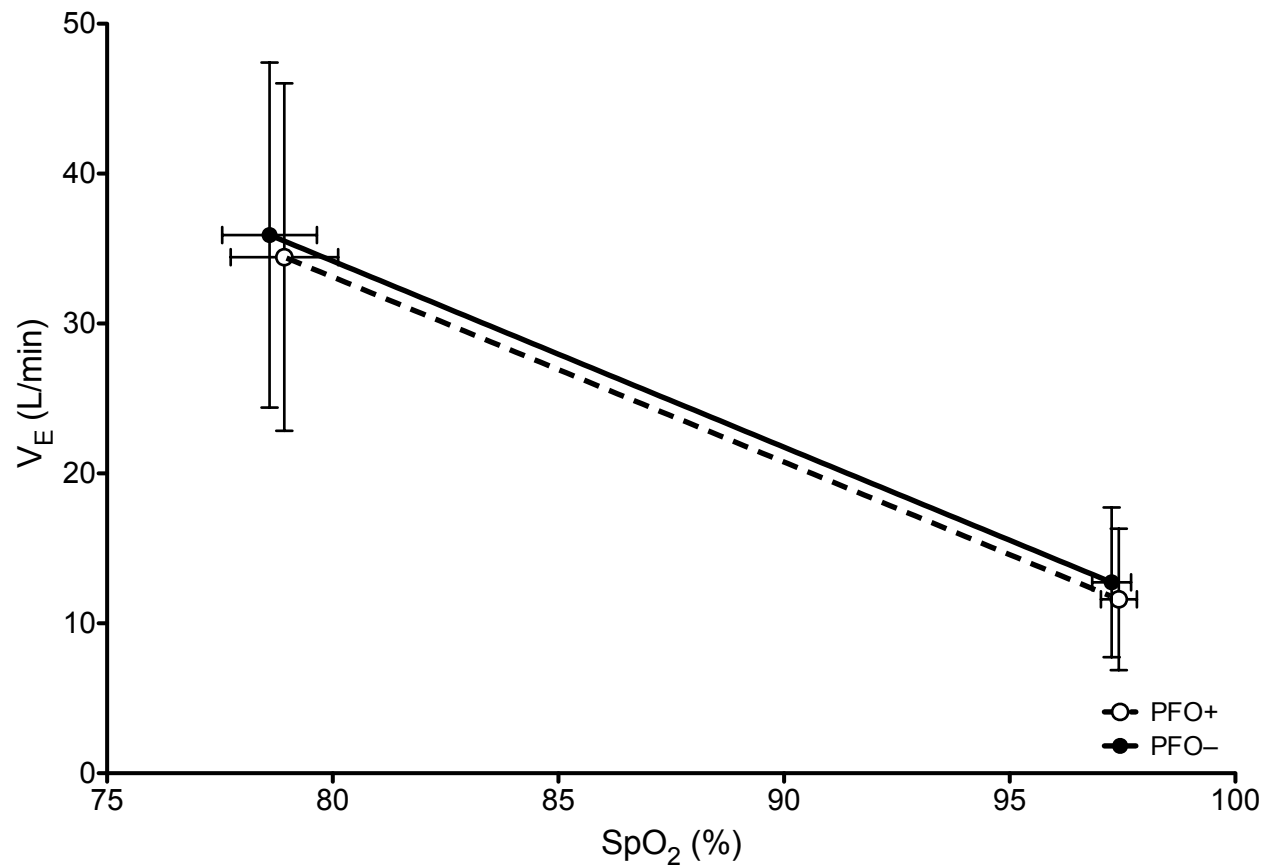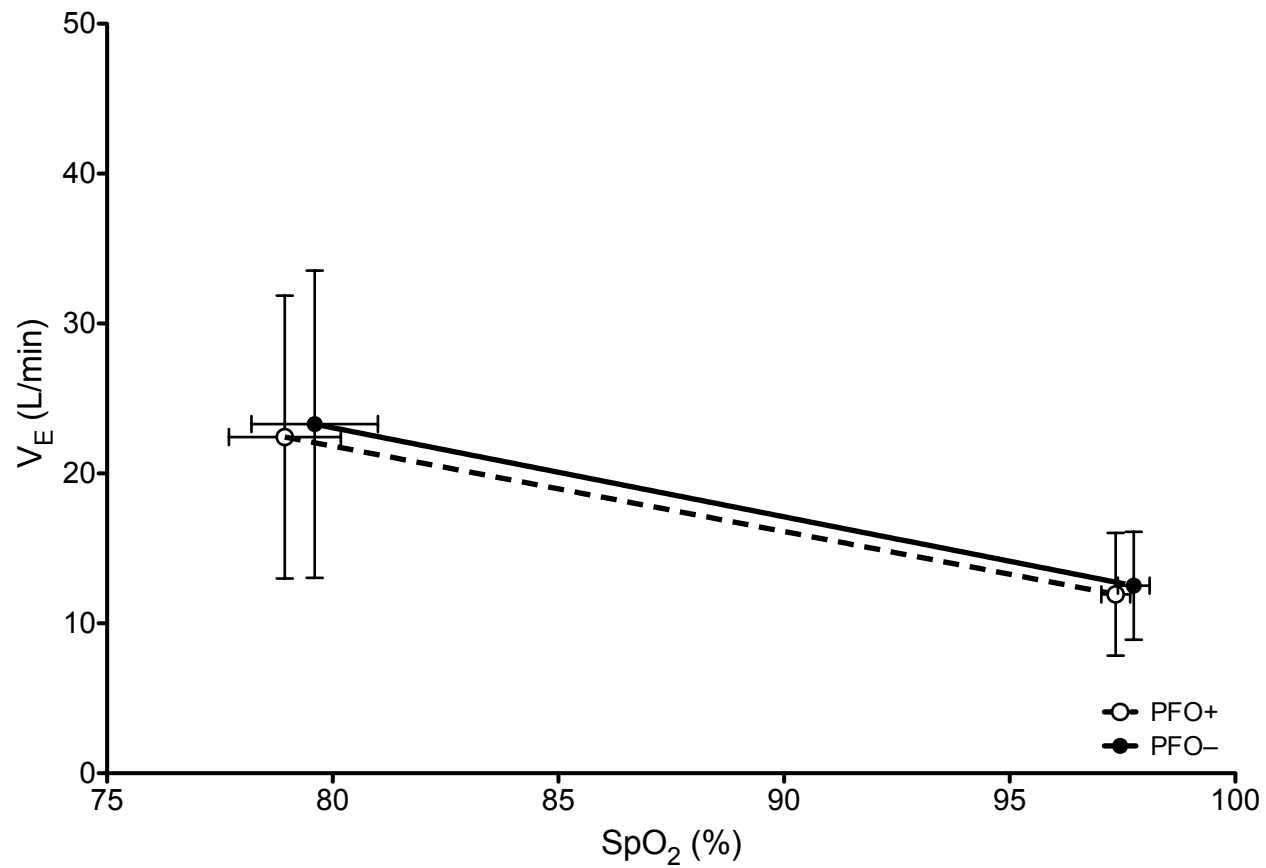
Figure 6.4. Effect of PFO on HVR during acute exposure to poikilocapnic hypoxia. Thick dashed and solid lines represent group averages for PFO+ and PFO– subjects respectively. Values are mean ± standard deviation. There was no effect of PFO on HVR during exposure to poikilocapnic hypoxia ($p > .05$).

There was no effect of PFO on V_E, V_t, RR, $P_{ET}O_2$ or $P_{ET}CO_2$ during the HH or

NH trials ($p > .05$, **Table 6.2**). Similarly, there was no effect of PFO on V_E, V_t, RR,

$P_{ET}O_2$ or $P_{ET}CO_2$ during the IH or PH trials ($p > .05$, Table 3). There was no effect of

PFO on HR or SpO_2 during any of the trials ($p > .05$, **Tables 6.2 and 6.3**).

Effect of Biological Sex

With the exception of V_t during the IH trial as well as V_E and V_t during the PH

trial, there was no effect of biological sex on any measures for any of the trials ($p > .05$,

See Appendix). Despite the differences in V_E and V_t, there were no differences in IH

HVR (Males = 1.4 ± 0.6, Females = 1.2 ± 0.5, $p > .05$) or PH (Males = 0.8 ± 0.7, Females

= 0.5 ± 0.3, $p > .05$). Similarly, there was no effect of biological sex on HVR during the

IH or PH trials (see Appendix)

Discussion

In this study we determined that compared to PFO− subjects, PFO+ subjects have

blunted ventilatory responses to acute normoxic and hyperoxic hypercapnia. However,

there were no differences in ventilatory responses between PFO+ and PFO− during acute

hypoxic exposure. These findings suggest that PFO+ subjects have a blunted central

chemoreflex when compared to PFO− subjects.

Presence of a PFO and Hypercapnic Ventilatory Responses

The hypercapnic ventilatory response has been well characterized (Sahn *et al.*,

1977; Katayama *et al.*, 1998; Ainslie & Poulin, 2004). During wakefulness, small

increases in carbon dioxide tension cause significant increases in V_E. This increase in V_E is due to an augmentation of both V_t and frequency. However, there is variability within this response, as some people are characterized as having a blunted ventilatory response to carbon dioxide. We have recently shown in subjects who reached a T_{core} threshold for thermal hyperpnoea during passive heating, PFO+ subjects have blunted ventilatory responses when compared to PFO– subjects (in submission). Furthermore, we have shown that during 16 days of exposure to 5260 m, PFO+ subjects had blunted ventilatory acclimatization compared to PFO– subjects (Elliott *et al.*, 2015). However, it was unknown if PFO+ subjects had blunted ventilatory responses during exposure to acute hypercapnia. The present study demonstrated that PFO+ subjects have a blunted ventilatory response to acute hypercapnia.

Prior to exposure to hypercapnia during hyperoxic and normoxic conditions, there were no differences in resting V_E or $P_{ET}CO_2$ between PFO+ and PFO– subjects. During hyperoxic and normoxic hypercapnia, PFO+ subjects had blunted ventilatory responses (**Figures 6.1 and 6.2**). Since this blunted response only occurred during the hypercapnic trials, it follows this is possibly due to a central chemoreflex component. While the mechanism behind this blunted response is unknown, it appears that PFO+ subjects have decreased sensitivity to CO_2. Although we did not detect any differences in resting $P_{ET}CO_2$ between PFO groups, other studies have demonstrated PFO+ subjects trended towards a higher $P_{ET}CO_2$ (Davis *et al.*, 2015) or $PaCO_2$ (Lovering *et al.*, 2011; Elliott *et al.*, 2015) than PFO– subjects. If PFO+ subjects have a higher $PaCO_2$ of 2-3 mmHg than PFO– subjects, as suggested by data from Elliott et al. and Lovering et al. (Lovering *et al.,* 2011; Elliott *et al.,* 2015), this could result in PFO+ subjects increasing their V_E at a

higher $PaCO_2$ compared to PFO– subjects. It is also plausible if PFO+ subjects have a greater $PaCO_2$ than PFO– subjects, that they have become acclimatized to a $PaCO_2$ and have a blunted response for a given increase in $PaCO_2$.

While findings from this study support the notion that PFO+ subjects have a blunted central chemoreflex, they also support the idea that the peripheral chemoreceptor is not acutely affected by the presence of a PFO, as there were no differences between HCVR during the HH and NH trials within groups. However, the peripheral chemoreceptor response during both of these trials would be minimal in healthy normoxic humans, since its firing rate does not significantly increase until PaO_2 drops below 60 mm Hg (Prabhakar & Semenza, 2015). Nevertheless, denervation studies of the carotid body have shown a slight decrease in V_E at rest in normoxia (Smith *et al.*, 1986; Blain *et al.*, 2010). Accordingly, we did see slight, but nonsignificant, differences between normoxic and hyperoxic HCVRs in both PFO+ and PFO– groups.

Presence of a PFO and Hypoxic Ventilatory Responses

It has been well established that PaO_2 is monitored by the peripheral chemoreceptor (Prabhakar & Peng, 2004; Prabhakar, 2006; Smith *et al.*, 2010; Prabhakar & Semenza, 2015). Under normal resting conditions at sea-level, PaO_2 is 100 mm Hg. The firing rate of the peripheral chemoreceptor does not significantly increase until PaO_2 drops below ~60 mm Hg (Prabhakar & Semenza, 2015). It is at this point that small decreases in PaO_2 result in large reductions in SaO_2. In an effort to prevent large decreases in SaO_2, peripheral chemoreceptor firing rate increases when PaO_2 decreases below ~60 mmHg. Consequently, this increased V_E augments PaO_2 which then leads to a

reduction in the firing rate of the peripheral chemoreceptor, while simultaneously increasing SaO_2. Thus, the $P_{ET}O_2$ utilized for the hypoxia trials in this study was 45 mm Hg, which would increase the likelihood that significant differences in V_E, if they exist, would occur.

During IH exposure, V_E is regulated by afferent input from the peripheral and central chemoreceptors. Completing an IH trial allowed for PaO_2 to be reduced, which would increase input from the peripheral chemoreceptor, while at the same time afferent input from the central chemoreceptor also increases. Similarly, during PH exposure, afferent input from the peripheral chemoreceptors will exceed input that occurs during normal, resting conditions; however, afferent input from the central chemoreceptor will be reduced due to the increase in V_E reducing $PaCO_2$.

While there were differences in HCVR, we did not see any differences in HVR during the IH trial. The average HVR that we saw for both groups during IH (PFO+: 1.30 $\pm$ 0.55 L min^{-1} SpO_2^{-1}, PFO–: 1.26 $\pm$ 0.54 L min^{-1} SpO_2^{-1}) was similar to previous research completed in healthy males (Kolb *et al.*, 2004), which suggest there is no difference in afferent peripheral input between PFO+ and PFO– groups. Although there were differences in HCVR during both hypercapnic trials, there were no differences in V_E or $P_{ET}CO_2$ prior to exposure to acute hypercapnia, with resting afferent central chemoreceptor input. Thus, the findings from the hypoxic and hypercapnic trials support each other, as well as the notion that the blunted ventilatory responses in PFO+ subjects is likely centrally mediated.

Elliott et al. showed that upon initial ascent to 5260 m there was no effect of PFO on HVR (Elliott *et al.*, 2015). However, in that study there were unequal proportions of

139

males and females in each group (PFO+: 63% female, PFO–: 20% female). It is possible that the lack of differences seen in that study were caused by this disproportionate number of females in the PFO+ group. However, the present study was designed so that there were approximately the same proportion of males and females in each group (PFO+: 50% female, PFO–: 53% female). Consequently, the present study is better designed to determine if PFO has an effect on HVR during acute PH exposure than the one completed by Elliott et al.

Similar to IH, the ventilatory drive to breathe during exposure to PH is regulated by afferent input from the peripheral chemoreceptor. However, at the same time that peripheral afferent input is increasing, central afferent input is decreasing due to decreased $PaCO_2$. This is evident by $P_{ET}CO_2$ after PH compared to IH (PFO+: 39.4 ± 3.8 mm Hg vs. 28.5 ± 10.2; PFO– 40.6 ± 3.0 mm Hg vs. 29.7 ± 9.1 mm Hg). Not surprisingly, the HVR for both groups was significantly lower during the PH trial than the IH trial (PFO+: 0.66 ± 0.68 L min^{-1} SpO$_2$$^{-1}$, PFO–: 0.61 ± 0.35 L min^{-1} SpO$_2$$^{-1}$). The fact that there were no differences between groups during PH supports work done by Elliott et al., which suggested that the presence of a PFO does not affect ventilatory responses during initial exposure to 5260 m. In that study SaO_2 and $PaCO_2$ values decreased by ~20% upon initial exposure to 5260 m. Furthermore, V_E increased by ~7 L/min, which resulted in an HVR of ~0.4. During the PH trial of this study, V_E increased by ~10 L/min above baseline values during hypoxic exposure, while SpO_2 decreased by ~18%. This resulted in an HVR that was slightly higher than what was seen by Elliott et al. These differences are likely explained by the variability that occurs with this measure. Based on these findings, it is plausible that the blunted ventilatory responses during acute

exposure to hypercapnia have a central component to them, and not a peripheral component.

Limitations

It has been shown that female sex hormones can affect ventilatory responses. Specifically, ventilation is greatest during the luteal phase when these hormones are their highest concentrations (Schoene *et al.*, 1981; Slatkovska *et al.*, 2006). Consequently, it is possible that differences in the menstrual cycle might account for some of our findings. However, findings reported by Macnutt et al. stated that menstrual cycle phase had no effect of HVR (Macnutt *et al.*, 2012).

Additionally, it is possible the DEF system induced anxiety in some subjects, as resting values for V_E were higher than expected. This could have induced a minor hyperventilation in subjects, which would have artificially lowered $P_{ET}CO_2$. This did not appear to occur during the hypercapnic trials, as baseline values were ~40 mmHg for both groups. However, it is possible that a minor hyperventilation occurred during the hypoxic trials as resting $P_{ET}CO_2$ values were ~37 mmHg. Future studies might want to allow subjects to have a longer rest period to increase familiarity and decrease anxiety.

Furthermore, this study used end-tidal gases as estimates of arterial gases. Tymko et al. have recently shown that arterial blood gas values are overestimated by end-tidal gas values (Tymko *et al.*, 2015). Since $P_{ET}O_2$ and $P_{ET}CO_2$ are calculated based on expired oxygen and carbon dioxide values, there is no way to account for the blood going through the PFO which would decrease PaO_2 while increasing $PaCO_2$. Consequently, $P_{ET}O_2$ and $P_{ET}CO_2$ are likely overestimating and underestimating PaO_2 and $PaCO_2$

respectively (Tymko *et al.*, 2016). Thus, it is possible that there were differences in PaO_2 and $PaCO_2$ between these two groups that we were unable to detect.

Finally, PaO_2 and $PaCO_2$ in PFO+ subjects are likely affected by the amount of blood flowing through a PFO. As the blood flow through a PFO increases, it is plausible that PaO_2 would decrease while $PaCO_2$ would increase. However, we did not measure blood flow through a PFO during this study.

Summary

This study has demonstrated that PFO+ subjects have blunted ventilatory responses to hypercapnia, but not hypoxia. Specifically, we have shown that PFO+ subjects have a blunted ventilatory response during acute exposure to hyperoxic and normoxic hypercapnia. Additionally, we have shown that there is no difference in ventilatory responses between PFO+ and PFO– subjects during acute exposure to isocapnic or poikilocapnic hypoxia. While the reason that PFO+ subjects have blunted ventilatory responses have yet to be fully elucidated, it appears that there is a centrally mediated component, as this study showed differences in HCVR, but not HVR between PFO+ and PFO– subjects.

Acknowledgments

CHAPTER VII

CONCLUSIONS

Main Findings

Most research looking at the effect of a PFO on physiological processes has focused on clinical populations (i.e. stroke, transient ischemic attack, etc.), and not on physiologic processes in a healthy population. Prior to this dissertation, two studies in healthy humans had shown that gas exchange and ventilatory acclimatization were affected by the presence of a PFO (Lovering *et al.*, 2011; Elliott *et al.*, 2015). In the study completed by Lovering et al., the primary finding was that gas exchange efficiency, as measured by the $AaDO_2$, was different between PFO+ and PFO– subjects at rest during normoxic and hypoxic conditions. An unexpected finding was that PFO+ subjects have a higher T_{esoph} than PFO– subjects. However, proper controls for a thermoregulatory study were not implemented, and therefore this study was not designed to find differences in T_{esoph}. Similarly, the work completed by Elliott et al. had an unexpected finding that PFO+ subjects had blunted ventilatory acclimatization after 16 days of exposure to 5260 m.

The main finding of this dissertation was that the presence of a PFO significantly affects physiological responses in otherwise healthy humans, which supports the work completed by Lovering et al. and Elliott et al. Specifically, we have shown in Chapters IV and V that PFO+ subjects have a T_{esoph} that is ~0.4 °C higher than PFO– subjects. These studies were designed with appropriate controls in place to measure T_{esoph} including subjects coming in fasted, and all trials completed at same time of day. Thus we are confident in concluding that PFO+ subjects have a greater T_{esoph} than PFO– subjects. We

144

initially hypothesized that these differences in T_{esoph} were partially due to decreased respiratory heat loss (RHL). However, findings from Chapter V suggest that RHL does not play a significant role in the differences in T_{esoph}. While the reasons for these differences have not been elucidated, other plausible explanations include PFO+ subjects having a greater concentration of pyrogens, or decreased skin blood flow, which could lead to them having a higher T_{esoph} than PFO− subjects.

In addition to showing that PFO+ subjects have a T_{esoph} that is ~0.4 °C higher than PFO− subjects, the study in Chapter V also showed that compared to PFO− subjects, PFO+ subjects do not cool off as quickly during cold water immersion. However, during hot water immersion, PFO+ and PFO− subjects increase T_{esoph} at the same rate. Taken together, results from Chapters IV and V suggest that PFO+ and PFO− subjects operate at, and defend, a different T_{esoph}.

The main findings from Chapters V and VI demonstrate that PFO+ subjects have altered ventilatory responses to heat and acute hypercapnic exposure, respectively. In subjects that experience hyperthermia-induced hyperventilation during Chapter V, PFO+ subjects increased their ventilation at a higher T_{esoph}, but not to the same magnitude as PFO− subjects. If thermal tachypnea occurs in an effort to dissipate heat, it is plausible that PFO+ subjects prefer to be warmer and that PFO− subjects prefer to be cooler, which would provide the rationale for the differences in thermoregulation between these two groups. Furthermore, the blunted ventilatory responses to heat are likely due to an altered hypothalamic response.

Similarly, during acute exposure to hypercapnic environments, PFO+ subjects had blunted ventilatory responses, which suggests that PFO+ subjects have a reduced

sensitivity to carbon dioxide. Since blood flowing across the PFO will increase the overall $PaCO_2$ of blood exiting the left side of the heart and entering the systemic circulation, it is possible that PFO+ subjects who have a greater resting $PaCO_2$ while breathing room air are less sensitive to increases in carbon dioxide since their resting $PaCO_2$ could potentially be higher than PFO– subjects (Lovering *et al.*, 2011; Elliott *et al.*, 2015).

While there were differences in HCVR, we saw no differences in ventilatory responses during acute exposure to isocapnic or poikilocapnic hypoxia. This was not surprising since findings from Elliott et. al showed no differences in HVR between PFO+ and PFO– subjects upon initial exposure to 5260 m. Thus, it might be possible that the differences, or lack thereof, in acute ventilatory responses to hypercapnia or hypoxia are mediated by central chemoreceptors, while the blunted ventilatory acclimatization to altitude is primarily mediated by the peripheral chemoreceptor. However, these conclusions are speculative at this time.

Summary and Future Directions

Existence of a PFO and its high prevalence is not novel. However, despite this fact, little research has examined its effect on physiological processes in otherwise healthy humans. It has now been confirmed that PFO+ subjects have a higher T_{esoph} than PFO– subjects. However, the mechanism behind this finding has not been completely elucidated. It is plausible that these differences can be explained in part by respiratory heat loss. However, findings from this dissertation have not shown differences in RHL. Thus, it is possible that this difference in T_{esoph} is also affected by an unknown substance

that is metabolized by the lungs. For example, if there exists a pyrogen that increases T_{core} which is cleared out in the lungs, it follows that PFO+ subjects would have a higher T_{core}, since they would metabolize less of this pyrogen. Similarly, PFO+ subjects could simply have a higher blood concentration of a pyrogen. Another possibility for this difference in T_{esoph} is that there are differences in heat loss mechanisms between PFO+ and PFO– subjects such as skin blood flow. If PFO+ subjects have reduced skin blood flow, this would attenuate the amount of heat loss they experience, which would result in a higher T_{core}. Therefore, future studies need to be designed in a way to accurately measure respiratory heat loss, pyrogen concentrations and skin blood flow.

Interestingly, findings from Chapters IV and V also suggest that PFO+ subjects have a greater resting HR than PFO– subjects in thermoneutral environments, as well as during cold water immersion. Potential explanations for this finding include increased T_{esoph} or decreased oxygen content. Conversely, the findings from Chapter VI did not indicate there were any differences in HR between the two groups. This could be explained by the studies completed in Chapters V and VI only had male subjects, while the studies completed in Chapter VI had male and female subjects.

Therefore, the work completed in this dissertation has not provided definitive evidence that the presence of a PFO has any effect on HR. Consequently, future studies could include those where a true, resting HR is obtained. Findings from this type of study could provide strong evidence as to whether or not the presence of a PFO affects HR. Similarly, during the first two studies contained in this dissertation, HR was measured with a pulse oximeter, which is less accurate than an EKG. Consequently, studies aiming to determine if the presence of a PFO increases HR, should consider using an EKG.

Additionally, none of the thermoregulation studies completed for this dissertation involved females. Therefore, it is possible that the differences we are seeing only occur in males. Although previous research has shown that T_{core} only varies by ~0.3°C during the menstrual cycle (Pivarnik *et al.*, 1992; Fukuoka *et al.*, 2002; Kuwahara, 2005), it is possible the presence of a PFO can affect the difference in T_{core} between the luteal and follicular phase. Consequently, similar studies done in Chapters IV and V could be completed in women while accounting for menstrual cycle.

Finally, Chapters IV and V of this dissertation have demonstrated that T_{core} as measured by T_{esoph} was greater in PFO+ subjects. While T_{esoph} is considered to be the gold standard measure of T_{core}, because it provides an estimate of left ventricle temperature, there are other measures of T_{core} that can, and should be, utilized. For example, rectal temperature, a commonly used analogue of core temperature, was not used during any studies of this dissertation. Similarly, as mentioned earlier, research comprising this dissertation did not measure skin temperature. Skin temperature in conjunction with T_{core} can be used to determine whole body temperature. Utilizing all these measures can help determine if the effects of a PFO are localized to T_{esoph}, or if they are more systemic.

The presence of a PFO definitely plays a role in physiological processes in healthy humans, as has been demonstrated within Chapters IV-VI. However, the work completed within this dissertation has only answered a couple of questions. Accordingly, the potential for future research looking at the effects of a PFO is exciting, as there remain many unanswered questions.

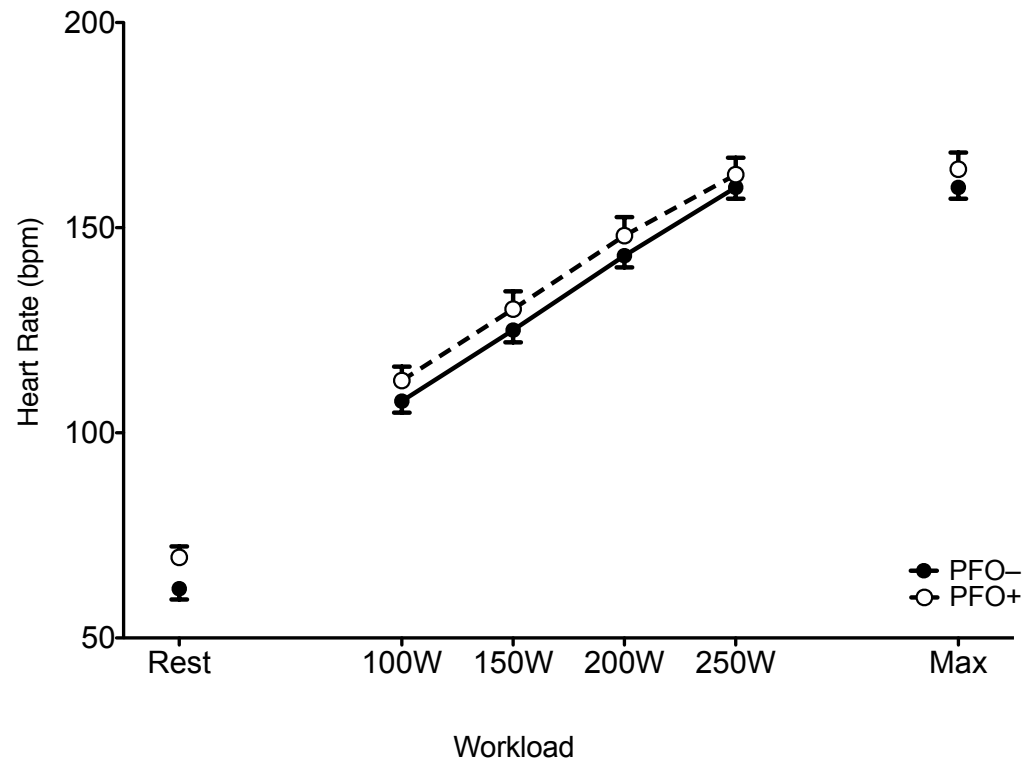**HEART RATE DURING VO$_{2MAX}$ TEST**

Figure 7.1. Effect of PFO on HR during a VO$_{2MAX}$ test. Dashed and solid lines represent group averages for PFO+ and PFO– subjects respectively. Values are mean ± standard deviation. There was no effect of PFO on HR prior to and during a VO$_{2MAX}$ test ($p >$ .05).
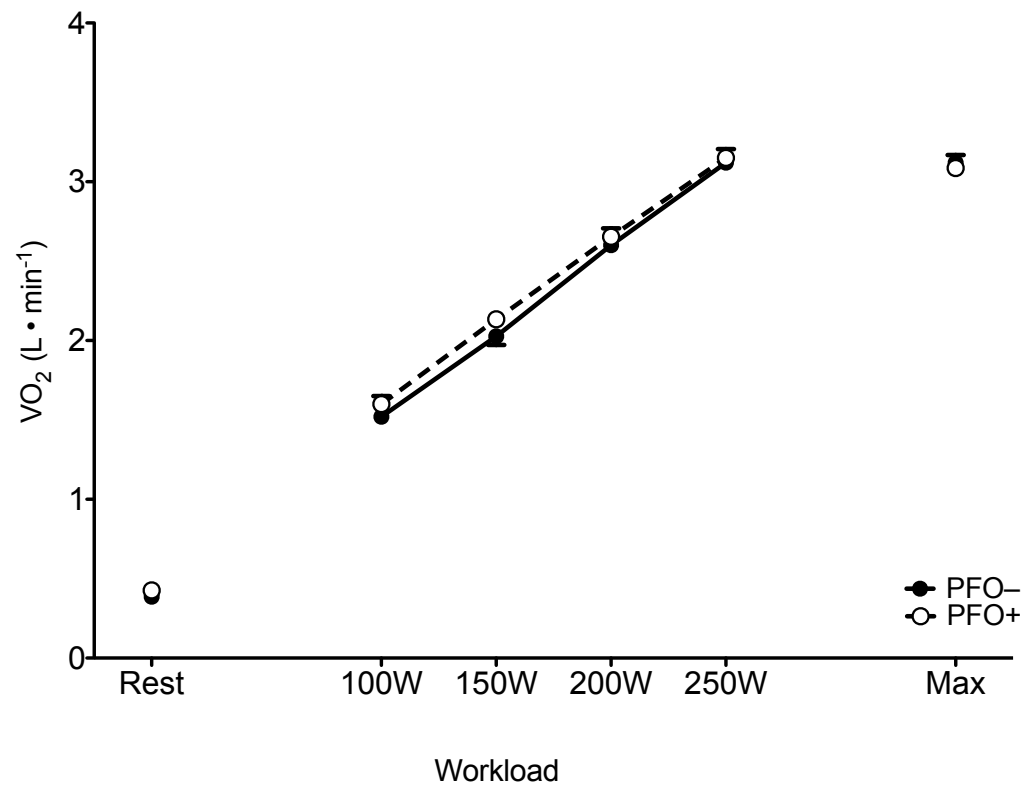
VO$_2$ DURING VO$_{2MAX}$ TEST

Figure 7.2. Effect of PFO on VO$_2$ during a VO$_{2MAX}$ test. Dashed and solid lines represent group averages for PFO+ and PFO– subjects respectively. Values are mean ± standard deviation. There was no effect of PFO on VO$_2$ prior to and during a VO$_{2MAX}$ test ($p > .05$).
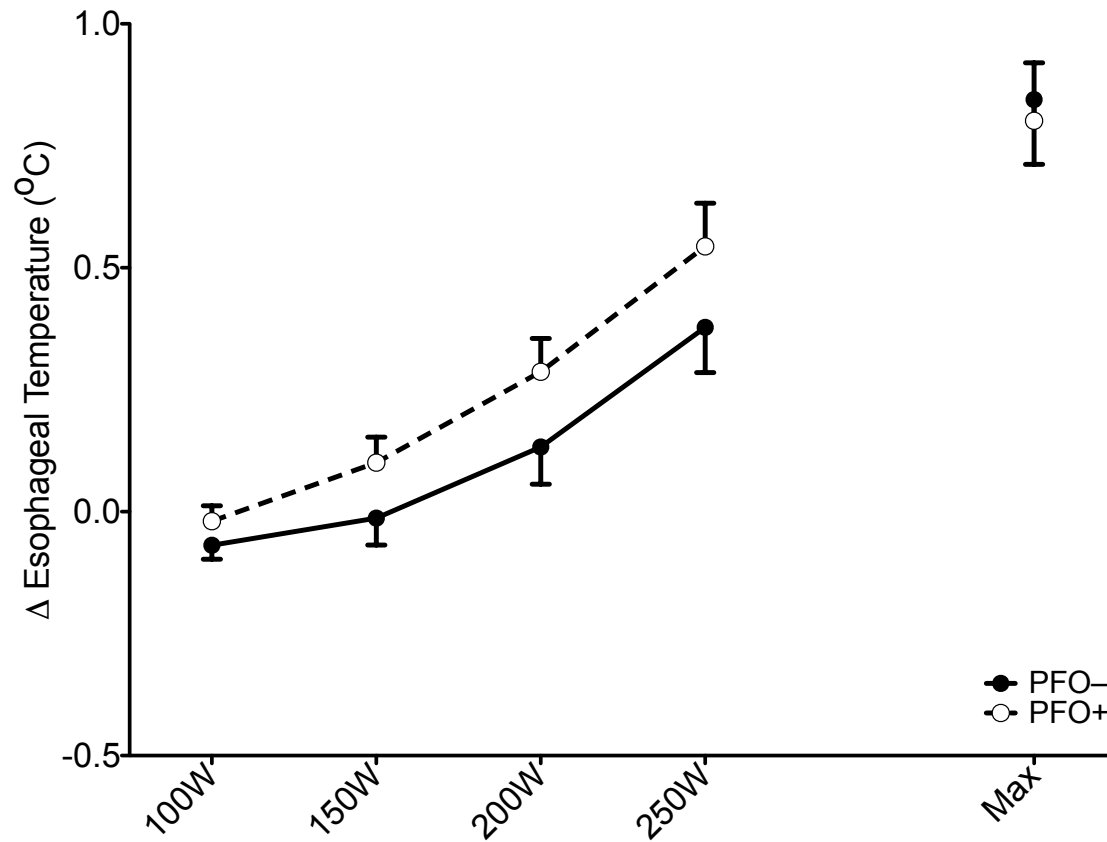
Figure 7.3. Effect of PFO on change in T$_{oesoph}$ during a VO$_{2MAX}$ test. Dashed and solid lines represent group averages for PFO+ and PFO– subjects respectively. Values are mean ± standard deviation. There was no effect of PFO on change in T$_{oesoph}$ during a VO$_{2MAX}$ test ($p > .05$).

HEAT OF BLOOD CROSSING PFO

Blood entering the right atrium has a temperature of 37.5°C;

Blood crossing the PFO also has a temperature of 37.5°C (T_{PFO});

Blood cooled by respiratory system heat loss which enters the left atrium via the pulmonary vein has a temperature of 36.5°C (T_{resp}),

Total cardiac output at rest is 5 L•min^{-1} (Q_{TOT});

Blood flow across the PFO is 250 ml•min^{-1} (Q_{PFO}),

Specific heat of blood is 3.49 kJ•L^{-1}•°C,

Effect of Q_{PFO} on T_{core} can then be calculated using the following equation:

$$\text{Specific heat of } Q_{TOT} = (3.49 \text{ kJ} \bullet Q_{PFO}^{-1} \bullet T_{PFO}^{-1}) + (3.49 \text{ kJ} \cdot (Q_{TOT}\text{-}Q_{PFO})^{-1} \bullet T_{resp}^{-1})$$

$$(3.49 \text{ kJ} \bullet L^{-1} \bullet °C \bullet 0.25 \text{ L} \bullet 37.5 \text{ °C}) + (3.49 \text{ kJ} \bullet L^{-1} \bullet °C \bullet (5.00 - 0.25) \text{ L} \bullet 36.5°C)$$

$$(32.72 \text{ kJ} \bullet L^{-1} \bullet °C) + (605.08 \text{ kJ} \bullet L^{-1} \bullet °C)$$

$$637.80 \text{ kJ} \bullet L^{-1} \bullet °C$$

Such that 637.80 kJ•L^{-1}•°C with a Q_{TOT} of 5 L results in a T_{core} of 36.6°C, which would be an increase of ~0.1°C.
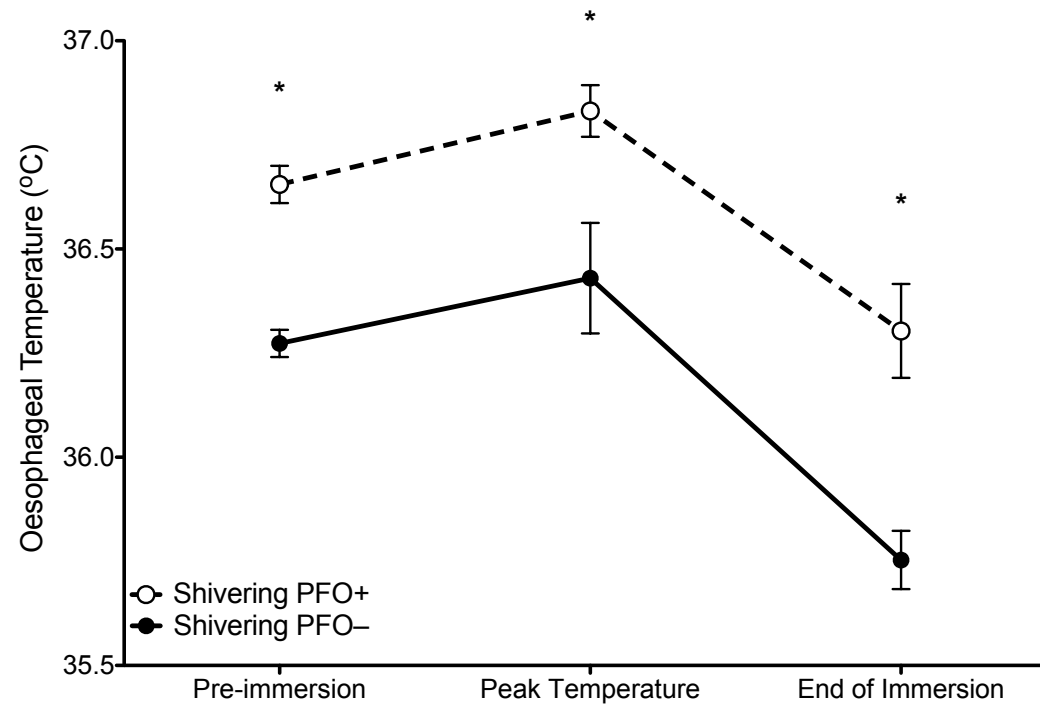
Figure 7.4. Effect of PFO on T$_{oesoph}$ in subjects who shivered during cold water immersion. Dashed and solid lines represent group averages for PFO+ and PFO– subjects respectively. Values are mean ± standard deviation. There was no effect of PFO on T$_{oesoph}$ in subjects who shivered prior to and during cold water immersion ($p > .05$).

Figure 7.5. Effect of PFO on respiratory heat loss during cold water immersion. Dashed and solid lines represent group averages for PFO+ and PFO– subjects respectively. Values are mean ± standard deviation. There was no effect of PFO on convective heat loss (Panel A), evaporative heat loss (Panel B) or total respiratory heat loss (Panel C) during cold water immersion ($p > .05$).
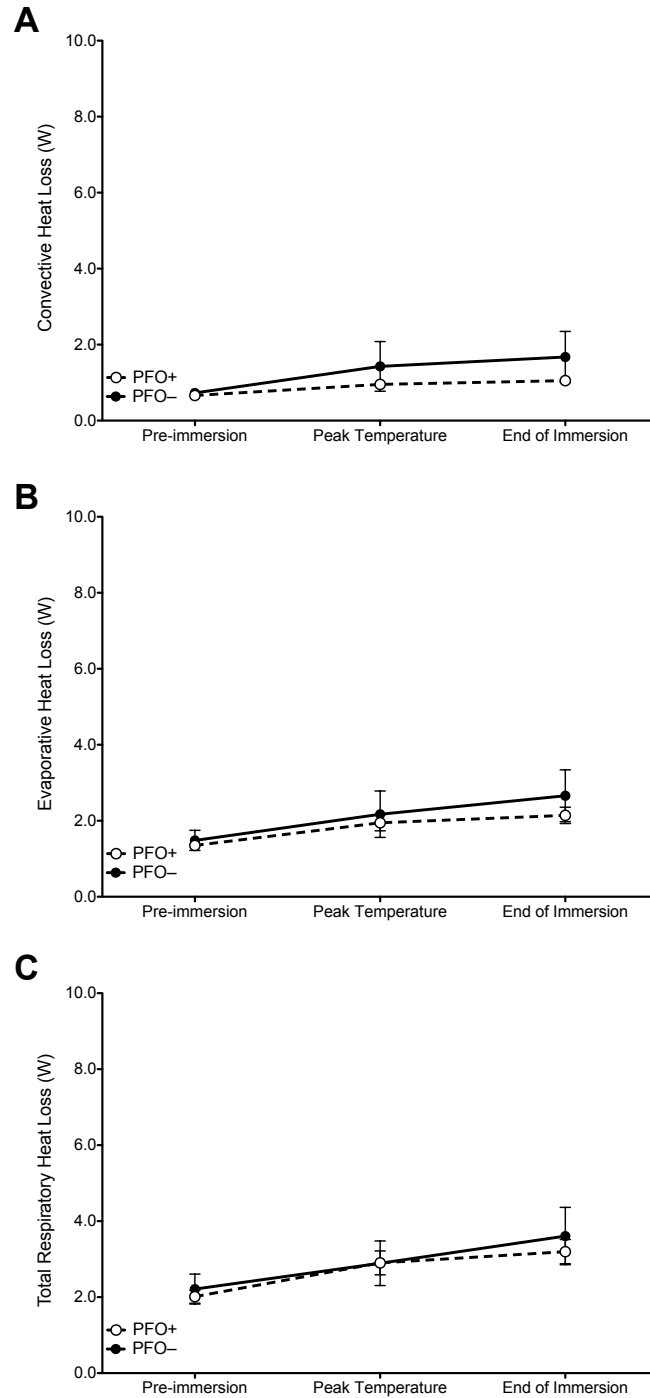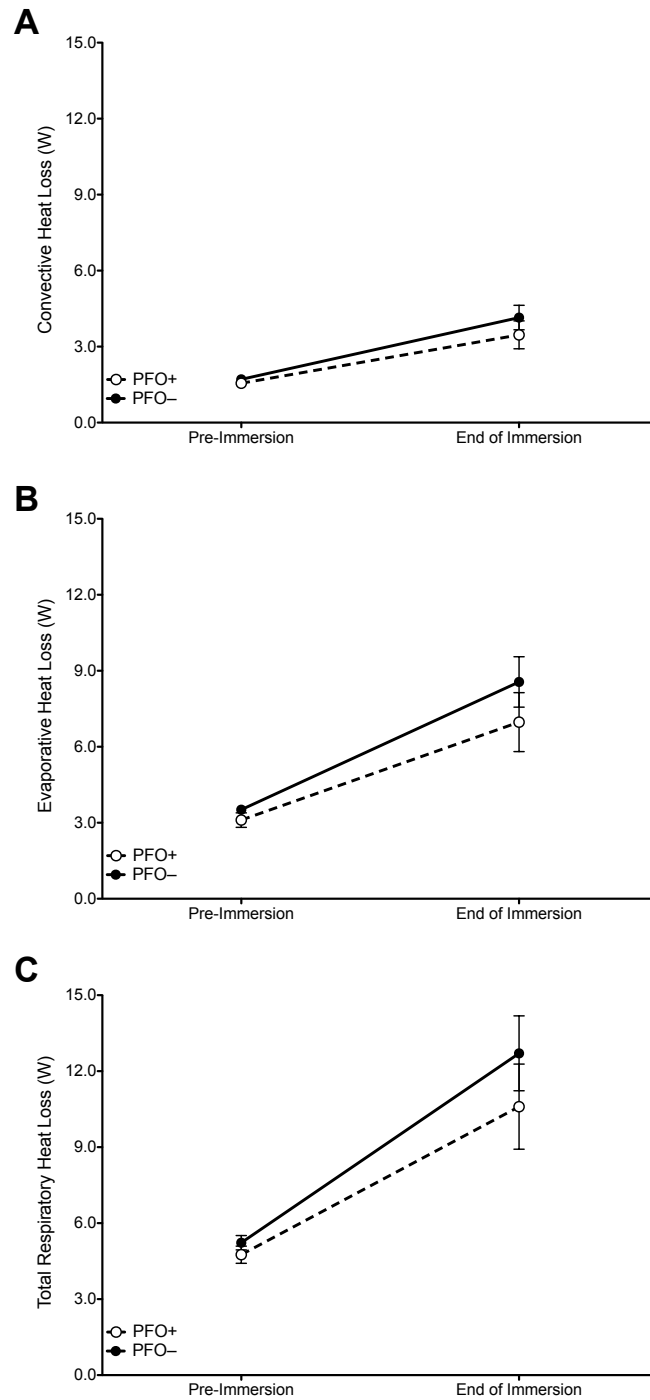
Figure 7.6. Effect of PFO on respiratory heat loss during hot water immersion. Dashed and solid lines represent group averages for PFO+ and PFO– subjects respectively. Values are mean ± standard deviation. There was no effect of PFO on convective heat loss (Panel A), evaporative heat loss (Panel B) or total respiratory heat loss (Panel C) during hot water immersion $(p > .05)$.

EFFECT OF BIOLOGICAL SEX DURING ACUTE EXPOSURE TO HYPERCAPNIA AND HYPOXIA

Table 7.1. Effect of biological sex on ventilatory and cardiovascular measures during acute exposure to hyperoxic and normoxic hypercapnia

		Rest		+3		+6		+9	
		Male	Female	Male	Female	Male	Female	Male	Female
Hyperoxic Hypercapnia	V_E (L•min^{-1})	14.1 ± 5.3	10.4 ± 2.6	18.3 ± 5.0	15.6 ± 3.3	21.5 ± 4.9	18.7 ± 3.3	27.9 ± 5.8	22.8 ± 3.4
	V_t (L)	1.3 ± 0.6	0.9 ± 0.3	1.6 ± 0.6	1.3 ± 0.4	1.8 ± 0.6	1.4 ± 0.3	2.1 ± 0.7	1.6 ± 0.3
	RR (breaths•min^{-1})	12.2 ± 4.7	12.0 ± 3.7	12.3 ± 4.5	13.4 ± 4.1	13.2 ± 4.6	14.1 ± 4.0	14.5 ± 4.8	14.8 ± 4.1
	$P_{ET}O_2$ (mmHg)	98.5 ± 8.4	96.6 ± 6.1	246.3 ± 13.7	246.2 ± 16.5	244.1 ± 6.8	244.4 ± 9.6	245.7 ± 3.7	245.4 ± 4.8
	$P_{ET}CO_2$ (mmHg)	40.5 ± 4.3	39.2 ± 2.7	42.0 ± 2.8	41.4 ± 3.0	45.9 ± 3.5	44.7 ± 2.8	48.3 ± 2.9	47.7 ± 2.5
	HR (bpm)	63.2 ± 12.5	66.2 ± 7.2	64.2 ± 14.3	67.9 ± 8.3	64.9 ± 13.5	69.2 ± 8.3	67.5 ± 15	72.2 ± 8.3
	SpO_2 (%)	96.7 ± 3.7	97.2 ± 2.2	98.1 ± 1.2	98.6 ± 1.5	98.4 ± 1.1	98.6 ± 1.2	98.2 ± 1.4	98.6 ± 1.1
Normoxic Hypercapnia	V_E (L•min^{-1})	14.4 ± 4.1	12.0 ± 5.7	17.0 ± 5.5	13.5 ± 2.4	20.8 ± 4.3	17.1 ± 3.7	25.2 ± 6.2	21.6 ± 4
	V_t (L)	1.3 ± 0.4	1.0 ± 0.4	1.6 ± 0.7	1.2 ± 0.3	1.8 ± 0.7	1.4 ± 0.4	2.1 ± 0.8	1.6 ± 0.4
	RR (breaths•min^{-1})	12.4 ± 5.4	12.1 ± 3.6	12.1 ± 4.4	12.6 ± 3.3	12.4 ± 3.7	13.5 ± 4.5	13.3 ± 4.5	14.4 ± 4.1
	$P_{ET}O_2$ (mmHg)	102.2 ± 13.7	99.5 ± 5.5	99.1 ± 7.5	101.0 ± 5.3	99.1 ± 6.7	100.5 ± 4.3	99.4 ± 5.6	100.0 ± 3.4
	$P_{ET}CO_2$ (mmHg)	38.8 ± 3.4	39.9 ± 2.6	42.1 ± 3.1	40.9 ± 2.4	45.1 ± 2.8	44.0 ± 2.7	48.1 ± 2.1	47.1 ± 2.7
	HR (bpm)	63.0 ± 13.1	68.4 ± 12	63.2 ± 10.1	70.3 ± 13.0	64.9 ± 9.5	72.1 ± 12.2	65.8 ± 10.5	74.9 ± 16.5
	SpO_2 (%)	97.4 ± 1.4	98.0 ± 1.2	96.1 ± 1.9	96.7 ± 1.6	96.5 ± 1.8	96.3 ± 2.2	96.3 ± 1.5	96.9 ± 1.3

Values are mean ± standard deviation. No significant differences between men and women.

Table 7.2. Effect of biological sex on ventilatory and cardiovascular measures during isocapnic and poikilocapnic hypoxia

| | Isocapnic Hypoxia | | | | Poikilocapnic Hypoxia | | | |
| | Rest | | Max V_E | | Rest | | Max V_E | |
	Males	Females	Males	Females	Males	Females	Males	Females
V_E (L•min^{-1})	14.5 ± 4.9	10.0 ± 3.3	37.4 ± 13.6	32.9 ± 8.0	13.9 ± 4.2	10.6 ± 2.5*	27.2 ± 11.5	18.5 ± 4.7*
V_t (L)	1.3 ± 0.6	0.9 ± 0.3*	2.6 ± 1.0	1.7 ± 0.2*	1.3 ± 0.6	0.9 ± 0.2*	2.3 ± 1.0	1.3 ± 0.3*
RR (breaths•min^{-1})	12.7 ± 3.4	11.8 ± 4.1	15.6 ± 5.2	19.7 ± 5.1	12.3 ± 3.6	12.9 ± 3.7	12.9 ± 3.7	15.6 ± 4.9
$P_{ET}O_2$ (mmHg)	98.4 ± 4.3	100.5 ± 4.6	45.3 ± 3.0	42.1 ± 5.0	102.8 ± 6.2	101.2 ± 4.3	64.4 ± 40.3	48.9 ± 5.5
$P_{ET}CO_2$ (mmHg)	38.6 ± 2.1	37.2 ± 3.9	39.8 ± 3.9	40.2 ± 2.8	35.6 ± 5.5	38.7 ± 2.7	26.5 ± 10.9	31.7 ± 7.2
HR (bpm)	61.0 ± 13.5	64.3 ± 8.8	78.3 ± 13.6	86.4 ± 10.7	60 ± 12.6	67.1 ± 16.7	70.3 ± 14.0	79.2 ± 13.3
SpO_2 (%)	96.6 ± 1.6	98.2 ± 1.3	78.8 ± 3.7	78.3 ± 5.1	97.2 ± 1.3	97.9 ± 1.2	79.2 ± 5.3	79.3 ± 5.0

Values are mean ± standard deviation. No significant differences between biological sex.
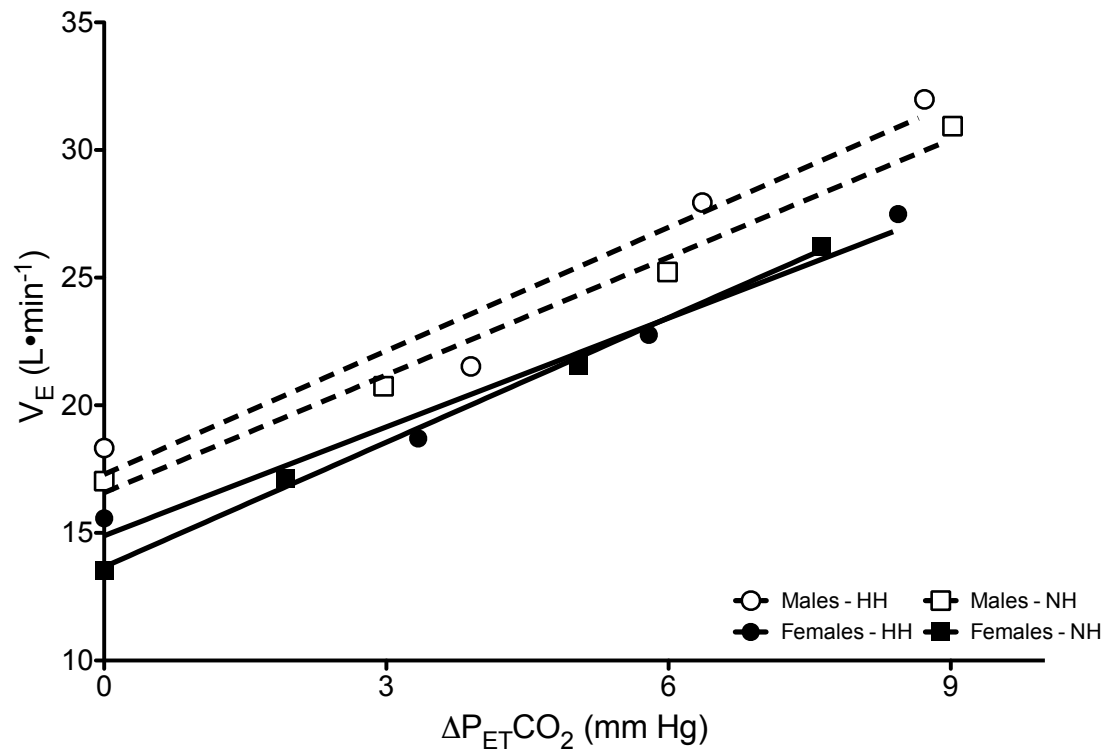
Figure 7.7. Effect of biological sex on HCVR. Filled symbols and solid lines represent average change from resting PETCO2 values plotted against V_E and the associated regression line for female subjects. Open symbols and dashed lines represent average change from resting PETCO2 values plotted against V_E and the associated regression line for male subjects. Circles represent females and squares represent males. There was no effect of biological sex on HCVR, $p > .05$.
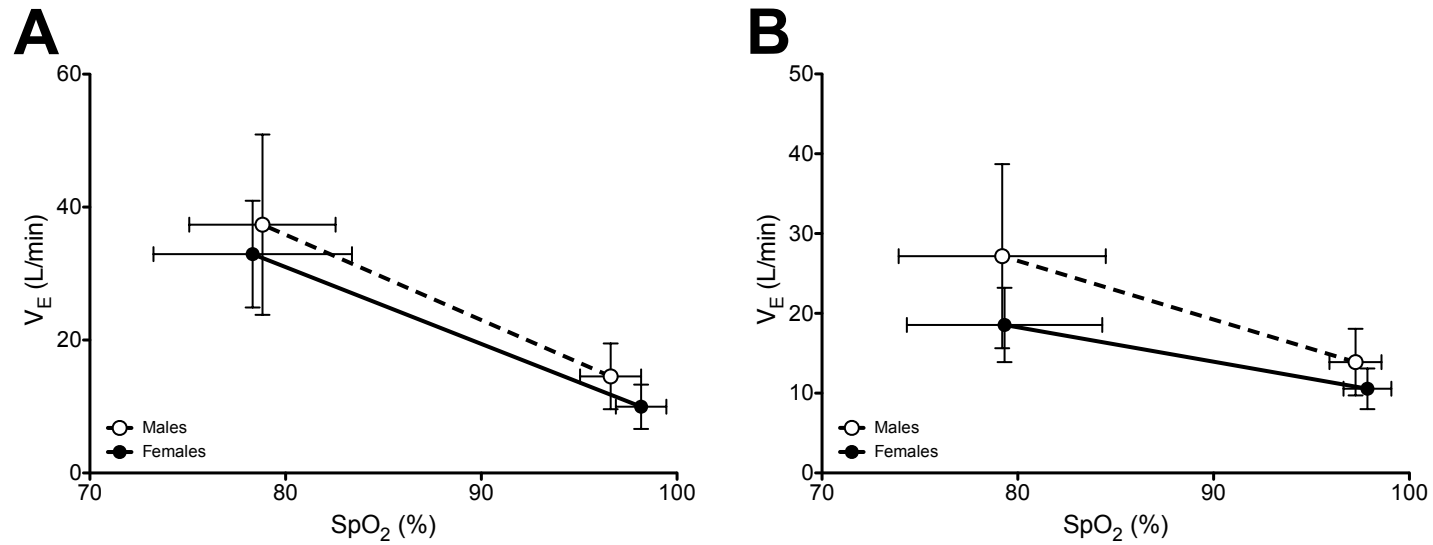
Figure 7.8. Effect of biological sex on HVR. Panel A shows HVR during isocapnic hypoxia and Panel B shows HVR during poikilocapnic hypoxia. Thick dashed and solid lines represent group averages for male and female subjects respectively. Values are mean ± standard deviation. There was no effect of biological sex on HVR during exposure to isocapnic or poikilocapnic hypoxia ($p > .05$).

REFERENCES CITED

Afonso S, Rowe GG, Castillo CA & Crumpton CW (1962). Intravascular and intracardiac blood temperatures in man. *J Appl Physiol* **17,** 706–708.

Ainslie PN & Poulin MJ (2004). Ventilatory, cerebrovascular, and cardiovascular interactions in acute hypoxia: regulation by carbon dioxide. *J Appl Physiol* **97,** 149–159.

Altareki N, Drust B, Atkinson G, Cable T & Gregson W (2009). Effects of environmental heat stress (35 degrees C) with simulated air movement on the thermoregulatory responses during a 4-km cycling time trial. *Int J Sports Med* **30,** 9–15.

Anon (1987). *Glossary of terms for thermal physiology. Second edition. Revised by The Commission for Thermal Physiology of the International Union of Physiological Sciences (IUPS Thermal Commission).*

Aschoff J (1983). Circadian control of body temperature. *Journal of Thermal Biology* **8,** 143–147.

Aslam F, Shirani J & Haque AA (2006). Patent foramen ovale: assessment, clinical significance and therapeutic options. *South Med J* **99,** 1367–1372.

Bain AR, Smith KJ, Lewis NC, Foster GE, Wildfong KW, Willie CK, Hartley GL, Cheung SS & Ainslie PN (2013). Regional changes in brain blood flow during severe passive hyperthermia: effects of PaCO2 and extracranial blood flow. *J Appl Physiol* **115,** 653–659.

Blain GM, Smith CA, Henderson KS & Dempsey JA (2009). Contribution of the carotid body chemoreceptors to eupneic ventilation in the intact, unanesthetized dog. *J Appl Physiol* **106,** 1564–1573.

Blain GM, Smith CA, Henderson KS & Dempsey JA (2010). Peripheral chemoreceptors determine the respiratory sensitivity of central chemoreceptors to CO(2). *J Physiol* **588,** 2455–2471.

Bligh J (2006). A theoretical consideration of the means whereby the mammalian core temperature is defended at a null zone. *J Appl Physiol* **100,** 1332–1337.

Bligh J & Johnson KG (1973). Glossary of terms for thermal physiology. *J Appl Physiol* **35,** 941–961.

Bonde-Petersen F, Schultz-Pedersen L & Dragsted N (1992). Peripheral and central blood flow in man during cold, thermoneutral, and hot water immersion. *Aviat Space Environ Med* **63,** 346–350.

Borg GA (1973). Perceived exertion: a note on "history" and methods. *Med Sci Sports* **5,** 90–93.

Boulant JA (2005). Neuronal basis of Hammel's model for set-point thermoregulation. *J Appl Physiol* **100,** 1347–1354.

Burch GE (1945). Rate of water and heat loss from the respiratory tract of normal subjects in a subtropical climate. *Arch Intern Med (Chic)* **76,** 315–327.

Cabanac M (2006). Adjustable set point: to honor Harold T. Hammel. *J Appl Physiol* **100,** 1338–1346.

Cabanac M & White MD (1995). Core temperature thresholds for hyperpnea during passive hyperthermia in humans. *Eur J Appl Physiol Occup Physiol* **71,** 71–76.

Cheng C, Matsukawa T, Sessler DI, Ozaki M, Kurz A, Merrifield B, Lin H & Olofsson P (1995). Increasing mean skin temperature linearly reduces the core-temperature thresholds for vasoconstriction and shivering in humans. *Anesthesiology* **82,** 1160–1168.

Christie A (1930). Normal closing time of the foramen ovale and the ductus arteriosus: an anatomic and statistical study. *Archives of Pediatrics & Adolescent Medicine* **40,** 323.

Chu AL, Jay O & White MD (2007). The effects of hyperthermia and hypoxia on ventilation during low-intensity steady-state exercise. *Am J Physiol Regul Integr Comp Physiol* **292,** R195–R203.

Cooper KE & Veale WL (1986). Effects of temperature on breathing. *Compr Physiol*.

Coris E, Ramirez A & Van Durme D (2004). Heat illness in athletes: the dangerous combination of heat, humidity and exercise. *Sports Med* **34,** 9–16.

Cox P (1987). Insensible water loss and its assessment in adult patients: a review. *Acta Anaesthesiol Scand* **31,** 771–776.

Cunningham DJ & O'Riordan JL (1957). The effect of a rise in the temperature of the body on the respiratory response to carbon dioxide at rest. *Q J Exp Physiol Cogn Med Sci* **42,** 329–345.

Curras MC, Kelso SR & Boulant JA (1991). Intracellular analysis of inherent and synaptic activity in hypothalamic thermosensitive neurones in the rat. *J Physiol* **440,** 257–271.

Curtis AN, Walsh ML & White MD (2007). Influence of passive hyperthermia on human ventilation during rest and isocapnic hypoxia. *Appl Physiol Nutr Metab* **32,** 721–732.

Dalton JC (1884). Doctrines of the Circulation.

Daniels F & Baker P (1961). Relationship between body fat and shivering in air at 15 C. *J Appl Physiol* **16,** 421–425.

Davis JT, Ng C-YA, Hill SD, Padgett RC & Lovering AT (2015). Higher oesophageal temperature at rest and during exercise in humans with patent foramen ovale. *J Physiol* **593,** 4615–4630.

Dempsey JA, Forster HV & DoPico GA (1974). Ventilatory acclimatization to moderate hypoxemia in man. The role of spinal fluid (H+). *J Clin Invest* **53,** 1091–1100.

Devuyst G, Piechowski-Józwiak B, Karapanayiotides T, Fitting J-W, Kémeny V, Hirt L, Urbano LA, Arnold P, van Melle G, Despland P-A & Bogousslavsky J (2004). Controlled contrast transcranial Doppler and arterial blood gas analysis to quantify shunt through patent foramen ovale. *Stroke* **35,** 859–863.

Dinarello CA (1999). Cytokines as endogenous pyrogens. *J Infect Dis* **179 Suppl 2,** S294–S304.

Doufas AG, Lin C-M, Suleman M-I, Liem EB, Lenhardt R, Morioka N, Akça O, Shah YM, Bjorksten AR & Sessler DI (2003). Dexmedetomidine and meperidine additively reduce the shivering threshold in humans. *Stroke* **34,** 1218–1223.

Duffin J (2007). Measuring the ventilatory response to hypoxia. *J Physiol* **584,** 285–293.

Elliott JE, Laurie SS, Kern JP, Beasley KM, Goodman RD, Kayser B, Subudhi AW, Roach RC & Lovering AT (2015). AltitudeOmics: impaired pulmonary gas exchange efficiency and blunted ventilatory acclimatization in humans with patent foramen ovale after 16 days at 5,260 m. *J Appl Physiol* **118,** 1100–1112.

Elliott JE, Nigam SM, Laurie SS, Beasley KM, Goodman RD, Hawn JA, Gladstone IM, Chesnutt MS & Lovering AT (2013). Prevalence of left heart contrast in healthy, young, asymptomatic humans at rest breathing room air. *Respir Physiol Neurobiol* **188,** 71–78.

Emmanouilides GC, Moss AJ, Duffie ER Jr. & Adams FH (1964). Pulmonary arterial pressure changes in human newborn infants from birth to 3 days of age. *J Pediatr* **65,** 327–333.

Fenster BE, Curran-Everett D, Freeman AM, Weinberger HD, Kern Buckner J & Carroll JD (2013*a*). Saline Contrast Echocardiography for the Detection of Patent Foramen Ovale in Hypoxia: A Validation Study Using Intracardiac Echocardiography. *Echocardiography* **31,** 420–427.

Fenster BE, Nguyen BH, Buckner JK, Freeman AM & Carroll JD (2013*b*). Effectiveness of percutaneous closure of patent foramen ovale for hypoxemia. *Am J Cardiol* **112,** 1258–1262.

Foster GE, Ainslie PN, Stembridge M, Day TA, Bakker A, Lucas SJE, Lewis NCS, Macleod DB & Lovering AT (2014). Resting pulmonary haemodynamics and shunting: a comparison of sea-level inhabitants to high altitude Sherpas. *J Physiol* **592,** 1397–1409.

Fox SM & Haskell WL (1970). The exercise stress test: needs for standardization. *Cardiology: Current Topics and Progress*149–154.

Fox SM, Naughton JP & Haskell WL (1971). Physical activity and the prevention of coronary heart disease. *Annals of Clinical Research* **3,** 404–432.

Freeman JA & Woods TD (2008). Use of saline contrast echo timing to distinguish intracardiac and extracardiac shunts: failure of the 3- to 5-beat rule. *Echocardiography* **25,** 1127–1130.

Fuhlbrigge AL, Weiss ST, Kuntz KM, Paltiel ADfor the CAMP Research Group (2006). Forced Expiratory Volume in 1 Second Percentage Improves the Classification of Severity Among Children With Asthma. *PEDIATRICS* **118,** e347–e355.

Fujii N, Honda Y, Hayashi K, Soya H, Kondo N & Nishiyasu T (2008). Comparison of hyperthermic hyperpnea elicited during rest and submaximal, moderate-intensity exercise. *J Appl Physiol* **104,** 998–1005.

Fukuoka Y, Kaneko Y, Takita C, Hirakawa M, Kagawa H & Nakamura Y (2002). The effects of exercise intensity on thermoregulatory responses to exercise in women. *Physiology & Behavior* **76,** 567–574.

Galloway S & Maughan R (1997). Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Med Sport Sci* **29,** 1240–1249.

Gao Y & Raj JU (2010). Regulation of the Pulmonary Circulation in the Fetus and Newborn. *Physiol Rev* **90,** 1291–1335.

Gaudio R Jr & Abramson N (1968). Heat-induced hyperventilation. *J Appl Physiol* **25,** 742–746.

Geibel A, Zehender M & Kasper W (2007). Importance of a Patent Foramen Ovale. 79–89.

Geladas N & Banister EW (1988). Effect of cold air inhalation on core temperature in exercising subjects under heat stress. *J Appl Physiol* **64,** 2381–2387.

Gonzalez-Alonso J (2007). Hyperthermia impairs brain, heart and muscle function in exercising humans. *Sports Med* **37,** 371–373.

Gonzalez-Alonso J, Teller C, Andersen S, Jensen F, Hyldig T & Nielsen B (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J Appl Physiol* **86,** 1032–1039.

Griffin JD & Boulant JA (1995). Temperature effects on membrane potential and input resistance in rat hypothalamic neurones. *J Physiol* **488 (Pt 2),** 407–418.

Gutierrez C, Ghezzo RH, Abboud RT, Cosio MG, Dill JR, Martin RR, McCarthy DS, Morse JLC & Zamel N (2004). Reference values of pulmonary function tests for Canadian Caucasians. *Can Respir J* **11,** 414–424.

Hagen PT, Scholz DG & Edwards WD (1984). Incidence and size of patent foramen ovale during the first 10 decades of life: an autopsy study of 965 normal hearts. *Mayo Clin Proc* **59,** 17–20.

Haldane JS (1905). The Influence of High Air Temperatures No. I. *Journal of Hygiene* **5,** 494–513.

Hanson R de G (1974). Respiratory heat loss at increased core temperature. *J Appl Physiol* **37,** 103–107.

Hayward JS & Eckerson JD (1984). Physiological responses and survival time prediction for humans in ice-water. *Aviat Space Environ Med* **55,** 206–211.

Heindl S, Struck J, Wellhöner P, Sayk F & Dodt C (2004). Effect of facial cooling and cold air inhalation on sympathetic nerve activity in men. *Respir Physiol Neurobiol* **142,** 69–80.

Hills BA (1973). Chemical facilitation of thermal conduction in physiological systems. *Science* **182,** 823–825.

Hopkins SR, Belzberg AS, Wiggs BR & McKenzie DC (1996). Pulmonary transit time and diffusion limitation during heavy exercise in athletes. *Respir Physiol* **103,** 67–73.

Johnson JM, Minson CT & Kellogg DL (2014). Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation. *Compr Physiol* **4,** 33–89.

Katayama K, Sato Y, Ishida K, Mori S & Miyamura M (1998). The effects of intermittent exposure to hypoxia during endurance exercise training on the ventilatory responses to hypoxia and hypercapnia in humans. *Eur J Appl Physiol Occup Physiol* **78,** 189–194.

Kelman GR & Nunn JF (1966). Nomograms for correction of blood Po2, Pco2, pH, and base excess for time and temperature. *J Appl Physiol* **21,** 1484–1490.

Kenefick R, Ely B, Cheuvront S, Palombo L, Goodman D & Sawka M (2009). Prior heat stress: effect on subsequent 15-min time trial performance in the heat. *Med Sport Sci* **41,** 1311–1316.

Kenny GP & Jay O (2011). *Thermometry, Calorimetry, and Mean Body Temperature during Heat Stress*. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Kenny GP, Chen AA, Johnston CE, Thoden JS & Giesbrecht GG (1997). Intense exercise increases the post-exercise threshold for sweating. *Eur J Appl Physiol Occup Physiol* **76,** 116–121.

Kenny GP, Périard J, Journeay WS, Sigal RJ & Reardon FD (2003). Effect of exercise intensity on the postexercise sweating threshold. *J Appl Physiol* **95,** 2355–2360.

Kernohan JC & Roughton FJ (1968). Thermal studies of the rates of the reactions of carbon dioxide in concentrated haemoglobin solutions and in red blood cells. A. The reactions catalysed by carbonic anhydrase. B. The carbamino reactions of oxygenated and deoxygenated haemoglobin. *J Physiol* **197,** 345–361.

Kerut EK, Bourgeois B, Serio J & Nanda NC (2013). Intrapulmonary Shunts and Its Implications for Assessment in Stroke and Divers with Type II Decompression Illness. *Echocardiography* **31,** 3–4.

Kerut EK, Norfleet WT, Plotnick GD & Giles TD (2001). Patent foramen ovale: a review of associated conditions and the impact of physiological size. *J Am Coll Cardiol* **38,** 613–623.

Knudson RJ, Kaltenborn WT, Knudson DE & Burrows B (1987). The single-breath carbon monoxide diffusing capacity. Reference equations derived from a healthy nonsmoking population and effects of hematocrit. *Am Rev Respir Dis* **135,** 805–811.

Kolb JC, Ainslie PN, Ide K & Poulin MJ (2004). Protocol to measure acute cerebrovascular and ventilatory responses to isocapnic hypoxia in humans. *Respir Physiol Neurobiol* **141,** 191–199.

Kutty S, Sengupta PP & Khandheria BK (2012). Patent foramen ovale: the known and the to be known. *J Am Coll Cardiol* **59,** 1665–1671.

Kuwahara T (2005). Effects of menstrual cycle and physical training on heat loss responses during dynamic exercise at moderate intensity in a temperate environment. *AJP: Regulatory, Integrative and Comparative Physiology* **288,** R1347–R1353.

Levitzky M (2013). *Pulmonary Physiology 8/E*. McGraw Hill Professional.

Little M & Rummel J (1971). Circadian variations in thermal and metabolic responses to heat exposure. *J Appl Physiol* **31,** 556–561.

Lopez M, Sessler DI, Walter K, Emerick T & Ozaki M (1994). Rate and gender dependence of the sweating, vasoconstriction, and shivering thresholds in humans. *Anesthesiology* **80,** 780–788.

Lovering AT & Goodman RD (2012). Detection of intracardiac and intrapulmonary shunts at rest and during exercise using saline contrast echocardiography ed. Ainslie PN. *Applied Aspects of Ultrasonography in Humans* 159–174.

Lovering AT, Stickland M, Amann M, O'Brien M, Hokanson J & Eldridge M (2011). Effect of a patent foramen ovale on pulmonary gas exchange efficiency at rest and during exercise. *J Appl Physiol* **110,** 1354–1361.

Lucas RAI, Pearson J, Schlader ZJ & Crandall CG (2015). Cardiopulmonary and arterial baroreceptor unloading during passive hyperthermia does not contribute to hyperthermia-induced hyperventilation. *Experimental Physiology* **100,** 1309–1318.

Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CPM, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, Gustafsson P, Hankinson J, Jensen R, McKay R, Miller MR, Navajas D, Pedersen OF, Pellegrino R & Wanger J (2005). Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* **26,** 720–735.

Macnutt MJ, De Souza MJ, Tomczak SE, Homer JL & Sheel AW (2012). Resting and exercise ventilatory chemosensitivity across the menstrual cycle. *J Appl Physiol* **112,** 737–747.

Mariak Z, White MD, Lewko J, Lyson T & Piekarski P (1999). Direct cooling of the human brain by heat loss from the upper respiratory tract. *J Appl Physiol* **87,** 1609–1613.

Marriott K, Manins V, Forshaw A, Wright J & Pascoe R (2013). Detection of right-to-left atrial communication using agitated saline contrast imaging: experience with 1162 patients and recommendations for echocardiography. *J Am Soc Echocardiogr* **26,** 96–102.

Maughan R, Watson P & Shirreffs S (2007). Heat and cold : what does the environment do to the marathon runner? *Sports Med* **37,** 396–399.

McFadden ER, Pichurko BM, Bowman HF, Ingenito E, Burns S, Dowling N & Solway J (1985). Thermal mapping of the airways in humans. *J Appl Physiol* **58,** 564–570.

Meissner I, Khandheria BK, Heit JA, Petty GW, Sheps SG, Schwartz GL, Whisnant JP, Wiebers DO, Covalt JL, Petterson TM, Christianson TJH & Agmon Y (2006). Patent Foramen Ovale: Innocent or Guilty?: Evidence From a Prospective Population-Based Study. *JAC* **47,** 440–445.

Mekjavić IB & Bligh J (1989). The increased oxygen uptake upon immersion. The raised external pressure could be a causative factor. *Eur J Appl Physiol Occup Physiol* **58,** 556–562.

Mekjavić IB & Rempel ME (1990). Determination of esophageal probe insertion length based on standing and sitting height. *J Appl Physiol* **69,** 376–379.

Mekjavić IB, Sundberg CJ & Linnarsson D (1991). Core temperature "null zone." *J Appl Physiol* **71,** 1289–1295.

Minson CT, Wladkowski SL, Cardell AF, Pawelczyk JA & Kenney WL (1998). Age alters the cardiovascular response to direct passive heating. *J Appl Physiol* **84,** 1323–1332.

Montain S & Coyle E (1992). Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *J Appl Physiol* **73,** 1340–1350.

Morgan BJ, Crabtree DC, Palta M & Skatrud JB (1995). Combined hypoxia and hypercapnia evokes long-lasting sympathetic activation in humans. *J Appl Physiol* **79,** 205–213.

Muller MD, Gao Z, Drew RC, Herr MD, Leuenberger UA & Sinoway LI (2011). Effect of cold air inhalation and isometric exercise on coronary blood flow and myocardial function in humans. *J Appl Physiol* **111,** 1694–1702.

Muller MD, Gao Z, McQuillan PM, Leuenberger UA & Sinoway LI (2014). Coronary responses to cold air inhalation following afferent and efferent blockade. *AJP: Heart and Circulatory Physiology* **307,** H228–H235.

Murphy PJ (2005). The fetal circulation. *Continuing Education in Anaesthesia, Critical Care & Pain* **5,** 107–112.

Nadel ER, Bullard RW & Stolwijk JA (1971). Importance of skin temperature in the regulation of sweating. *J Appl Physiol* **31,** 80–87.

Nattie E (1999). CO2, brainstem chemoreceptors and breathing. *Progress in Neurobiology* **59,** 299–331.

Nielsen B, Hales J, Strange S, Christensen N, Warberg J & Saltin B (1993). Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *J Physiol* **460,** 467–485.

Nielsen B, Strange S, Christensen NJ, Warberg J & Saltin B (1997). Acute and adaptive responses in humans to exercise in a warm, humid environment. *Pflugers Arch* **434,** 49–56.

Nielsen M & Smith H (1952). Studies on the regulation of respiration in acute hypoxia; with a appendix on respiratory control during prolonged hypoxia. *Acta Physiol Scand* **24,** 293–313.

Noakes T, Peltonen J & Rusko H (2001). Evidence that a central governor regulates exercise performance during acute hypoxia and hyperoxia. *J Exp Biol* **204,** 3225–3234.

Patten BM (1931). The closure of the foramen ovale. *American Journal of Anatomy* **48,** 19–44.

Patten BM (1938). Developmental defects at the foramen ovale. *Am J Pathol* **14,** 135–162.139.

Pivarnik JM, Marichal CJ, Spillman T & Morrow JR (1992). Menstrual cycle phase affects temperature regulation during endurance exercise. *J Appl Physiol* **72,** 543–548.

Powell FL (2007). The influence of chronic hypoxia upon chemoreception. *Respir Physiol Neurobiol* **157,** 154–161.

Prabhakar NR (2006). O2 sensing at the mammalian carotid body: why multiple O2 sensors and multiple transmitters? *Experimental Physiology* **91,** 17–23.

Prabhakar NR & Peng Y-J (2004). Peripheral chemoreceptors in health and disease. *J Appl Physiol* **96,** 359–366.

Prabhakar NR & Semenza GL (2015). Oxygen Sensing and Homeostasis. *Physiology* **30,** 340–348.

Querido JS, Ainslie PN, Foster GE, Henderson WR, Halliwill JR, Ayas NT & Sheel AW (2013). Dynamic cerebral autoregulation during and following acute hypoxia: role of carbon dioxide. *J Appl Physiol* **114,** 1183–1190.

Rasanen J, Wood DC, Debbs RH, Cohen J, Weiner S & Huhta JC (1998). Reactivity of the human fetal pulmonary circulation to maternal hyperoxygenation increases during the second half of pregnancy: a randomized study. *Circulation* **97,** 257–262.

Robbins PA, Conway J, Cunningham DA, Khamnei S & Paterson DJ (1990). A comparison of indirect methods for continuous estimation of arterial PCO2 in men. *J Appl Physiol* **68,** 1727–1731.

Roughton FJ (1935). Thermochemistry of the oxygen-haemoglobin reaction: Direct measurements of the heat of reaction under various conditions. *Biochem J* **29,** 2604–2621.

Roughton FJ, Adair GS, Barcroft J, Goldschmidt G, Herkel W, Hill RM, Keys AB & Ray GB (1936). The thermochemistry of the oxygen-haemoglobin reaction: Comparison of the heat as measured directly on purified haemoglobin with that calculated indirectly by the Van't Hoff Isochore. *Biochem J* **30,** 2117–2133.

Sahn SA, Zwillich CW, Dick N, McCullough RE, Lakshminarayan S & Weil JV (1977). Variability of ventilatory responses to hypoxia and hypercapnia. *J Appl Physiol Respir Environ Exerc Physiol* **43,** 1019–1025.

Satinoff E (1978). Neural organization and evolution of thermal regulation in mammals. *Science* **201,** 16–22.

Sawka MN, Leon LR, Montain SJ & Sonna LA (2011). *Integrated Physiological Mechanisms of Exercise Performance, Adaptation, and Maladaptation to Heat Stress*. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Saxton C (1981). Effects of severe heat stress on respiration and metabolic rate in resting man. *Aviat Space Environ Med* **52,** 281–286.

Schoene RB, Hackett PH, Henderson WR, Sage EH, Chow M, Roach RC, Mills WJ & Martin TR (1986). High-altitude pulmonary edema. Characteristics of lung lavage fluid. *JAMA* **256,** 63–69.

Schoene RB, Robertson HT, Pierson DJ & Peterson AP (1981). Respiratory drives and exercise in menstrual cycles of athletic and nonathletic women. *J Appl Physiol Respir Environ Exerc Physiol* **50,** 1300–1305.

Slatkovska L, Jensen D, Davies GAL & Wolfe LA (2006). Phasic menstrual cycle effects on the control of breathing in healthy women. *Respir Physiol Neurobiol* **154,** 379–388.

Smith CA, Bisgard GE, Nielsen AM, Daristotle L, Kressin NA, Forster HV & Dempsey JA (1986). Carotid bodies are required for ventilatory acclimatization to chronic hypoxia. *J Appl Physiol* **60,** 1003–1010.

Smith CA, Forster HV, Blain GM & Dempsey JA (2010). An interdependent model of central/peripheral chemoreception: Evidence and implications for ventilatory control. *Respir Physiol Neurobiol* **173,** 288–297.

Spurr GB, Prentice AM, Murgatroyd PR, Goldberg GR, Reina JC & Christman NT (1988). Energy expenditure from minute-by-minute heart-rate recording: comparison with indirect calorimetry. *Am J Clin Nutr* **48,** 552–559.

Strunk BL, Cheitlin MD, Stulbarg MS & Schiller NB (1987). Right-to-left interatrial shunting through a patent foramen ovale despite normal intracardiac pressures. *Am J Cardiol* **60,** 413–415.

Sun X-G, Hansen JE, Oudiz RJ & Wasserman K (2002). Gas exchange detection of exercise-induced right-to-left shunt in patients with primary pulmonary hypertension. *Circulation* **105,** 54–60.

Swanson GD & Bellville JW (1975). Step changes in end-tidal CO2: methods and implications. *J Appl Physiol* **39,** 377–385.

Tikuisis P, Jacobs I, Moroz D, Vallerand AL & Martineau L (2000). Comparison of thermoregulatory responses between men and women immersed in cold water. *J Appl Physiol* **89,** 1403–1411.

Tremblay JC, Lovering AT, Ainslie PN, Stembridge M, Burgess KR, Bakker A, Donnelly J, Lucas SJE, Lewis NCS, Dominelli PB, Henderson WR, Dominelli GS, Sheel AW & Foster GE (2014). Hypoxia, not pulmonary vascular pressure, induces blood flow through intrapulmonary arteriovenous anastomoses. *J Physiol* n/a–n/a.

Trinity JD, Pahnke MD, Lee JF & Coyle EF (2010). Interaction of hyperthermia and heart rate on stroke volume during prolonged exercise. *J Appl Physiol* **109,** 745–751.

Tymko MM, Ainslie PN, Macleod DB, Willie CK & Foster GE (2015). End tidal-to-arterial CO2 and O2 gas gradients at low- and high-altitude during dynamic end-tidal forcing. *AJP: Regulatory, Integrative and Comparative Physiology* **308,** R895–R906.

Tymko MM, Hoiland RL, Kuca T, Boulet LM, Tremblay JC, Pinske BK, Williams AM & Foster GE (2016). Measuring the human ventilatory and cerebral blood flow response to CO 2: a technical consideration for the end-tidal-to-arterial gas gradient. *J Appl Physiol* **120,** 282–296.

Vestbo J, Edwards LD, Scanlon PD, Yates JC, Agusti A, Bakke P, Calverley PMA, Celli B, Coxson HO, Crim C, Lomas DA, MacNee W, Miller BE, Silverman EK, Tal-Singer R, Wouters E & Rennard SI (2011). Changes in Forced Expiratory Volume in 1 Second over Time in COPD. *N Engl J Med* **365,** 1184–1192.

Wadhwa A, Sengupta P, Durrani J, Akça O, Lenhardt R, Sessler DI & Doufas AG (2005). Magnesium sulphate only slightly reduces the shivering threshold in humans. *Br J Anaesth* **94,** 756–762.

Wanger J et al. (2005). Standardisation of the measurement of lung volumes. *Eur Respir J* **26,** 511–522.

White MD (2006). Components and mechanisms of thermal hyperpnea. *J Appl Physiol* **101,** 655–663.

White MD & Cabanac M (1996). Exercise hyperpnea and hyperthermia in humans. *J Appl Physiol* **81,** 1249–1254.

Woods TD, Harmann L, Purath T, Ramamurthy S, Subramanian S, Jackson S & Tarima S (2010). Small- and moderate-size right-to-left shunts identified by saline contrast echocardiography are normal and unrelated to migraine headache. *Chest* **138,** 264–269.

Wyman J (1939). The heat of oxygenation of hemoglobin. *J Biol Chem* **127,** 581–599.

Young AJ (2011). Homeostatic responses to prolonged cold exposure: human cold acclimatization.

Young AJ, Sawka MN, Epstein Y, Decristofano B & Pandolf KB (1987). Cooling different body surfaces during upper and lower body exercise. *J Appl Physiol* **63,** 1218–1223.