Influence of a Patent Foramen Ovale and Biological Sex on Thermoregulatory and Cardiovascular Responses at Rest and during Exercise

by

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DISSERTATION ABSTRACT

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Doctor of Philosophy in Human Physiology

Influence of Patent Foramen Ovale and Biological Sex on Thermoregulatory and Responses at Rest and during Exercise

In healthy humans, core temperature (Tc) is maintained within narrow limits around ~37 °C. There is interindividual variability in resting Tc with most individuals between 36 and 37°C. Many factors contribute to the variability in the resting Tc including but not limited to, differences in basal metabolic rate, circadian rhythm differences [1], levels of inflammatory markers or endogenous pyrogens (i.e. tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, interferons) [2], and menstrual cycle phase [3]. Previous findings have also suggested that the presence of a patent foramen ovale in young, healthy men may be associated with elevations in resting esophageal temperature (Tesoph) that exist during thermal stressors (exercise, hot water immersion, cold water immersion). However, whether this difference in temperature exists in women with a PFO or whether this elevation in temperature is caused by differences in metabolic heat production are unknown. Additionally, while the elevations in temperature have been seen in a lab setting, whether the elevation in temperature influences self-paced 5k performance of thermoregulation during SCUBA diving is unknown.

In Chapter IV, we demonstrated that during 60 min of exercise at a controlled heat production (7 w/kg) that men without a PFO have higher Tc compared to men with a PFO. However, there was no difference in the change in Tc over the course of the hour, suggesting that although there may be variability in the baseline temperatures among individuals, the presence or absence or a PFO does not influence mechanisms of thermoregulation during exercise.

Additionally, no difference in baseline or exercise Tc or Tesoph existed between women with and without a PFO. In addition to Tc & Tesoph, we measured skin temperature, mean body temperature, heart rate and respiratory heat loss. There were no differences in any of these variables between PFO- and PFO+ groups.

In Chapter V, we showed that men with a PFO have a greater increase in Tc despite slower running times compared to men without a PFO. However, contrary to our hypothesis and unlike previous studies, men with a PFO did not have higher baseline Tc. The greater increase in Tc during the 5k may be due to differences in heat production, time for heat storage (longer running times), differences in body composition/body weight or variability in ambient conditions. There were no differences in Tc or 5k performance between PFO+ and PFO- women.

Finally, in Chapter VI, we demonstrated that the presence of a PFO had no effect on baseline or post-SCUBA diving core temperatures. We demonstrated that the most impactful predictors of the change in core temperature during a SCUBA dive were related to anthropometric characteristics such as body mass, body mass index, body surface area/mass ratio and wetsuit thickness.

This dissertation includes previously unpublished material.

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DEDICATION

To the mentors & scientists who believed in me and helped me believe in myself along the way.

Without your guidance, this would not have been possible.

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CHAPTER I

INTRODUCTION

This dissertation contains unpublished co-authored material. Chapters I, II, III, and VII do not contain co-authored material and are written entirely by me with editorial assistance solely from my dissertation committee. Chapters IV, V, and VI contain co-authored material. Co-authors are listed at the beginning of each applicable chapter and include Kaitlyn G. DiMarco, Aaron W. Betts, Tyler S. Kelly, Elizabeth M. Castillo, Dr. Christopher T. Minson, Joel E. Futral, Dr. Rachel N. Lord, Dr. Otto Barak, Dr. Justin Edward, Dr. Ivan Drvis, Dr. Igor Glavičić, Ivana Miloš, Dr. Željko Dujić, Dr. Nisha Charkoudian and Dr. Andrew T. Lovering.

Two of the most important physiological systems in the human body are the cardiovascular system and the respiratory system. Together, they make up the cardiopulmonary system, which includes the heart, lungs, and systemic and pulmonary circulations [4]. The primary function of the cardiopulmonary system is to ensure delivery of oxygenated blood and the removal of carbon dioxide, to and from the various organs of the body, respectively. In normal, healthy humans, as air is breathed into the lungs, oxygen diffuses from the alveoli into pulmonary microcirculation and carbon dioxide diffuses from the blood into the alveoli. This newly oxygenated blood travels from the pulmonary microcirculation, into the pulmonary vein, and back to the left side of the heart before being pumped into the aorta and traveling through the systemic circulation. While most of the blood entering the left side of the heart comes from the pulmonary circulation, blood may also return to the left atrium from the bronchial and thebesian circulations, and in some individuals, directly from the right atrium through a small hole called a patent foramen ovale.

The foramen ovale is a normal part of the fetal circulation [5]. In utero, the foramen ovale allows blood to travel directly from the right side of the heart to the left side of the heart, bypassing the pulmonary circulation. This right to left blood flow is facilitated by the elevation in right atrial pressure that results from the increased pulmonary vascular resistance caused by hypoxic pulmonary vasoconstriction. The minimal blood flow through the pulmonary circulation does not compromise gas exchange in the fetus as gas exchange occurs with the placenta, and not in the lungs.

At birth when the baby starts breathing ambient air, pulmonary vascular resistance decreases, and subsequently left atrial pressure exceeds right atrial pressure. This elevation in left atrial pressure causes the septum primum to come into contact with the septum secundum and through the process of fibrosis and the conversion of endothelial cells to mesenchymal cells, the foramen ovale closes [6]. While the foramen ovale closes within the first two years of life in most of the population [5], in ~25-40% of the population, it fails to close and is then termed a patent foramen ovale, or PFO [7].

Historically, little research has been done examining the influence of the PFO on human physiology. More recently, however, research has shown that the PFO may influence various physiological processes and responses including pulmonary gas exchange efficiency [8], the prevalence of Acute Mountain Sickness in those rapidly ascending to high altitude [9,10], ventilatory responses to hypoxia, hypercapnia, and thermal stressors [9,11], and baseline core temperature [11,12]. In terms of core temperature and thermoregulation, previous studies have reported that men with a PFO have elevated core temperatures (measured via esophageal temperature probe) at rest, during exercise and during passive heating and cooling [11,12] compared to men without a PFO. While the exact mechanisms responsible for this elevation in core

temperature are unclear, authors hypothesized that one potential mechanism may be that there are differences in respiratory heat loss. The blood that travels through the PFO bypasses the lungs and therefore does not participate in respiratory system cooling and could decrease heat dissipation in those with a PFO.

With rising global temperatures and climate change increasing heat strain in the general population every year [13], more research has been focused on understanding the factors that affect an individual's ability to thermoregulate and mitigate heat stress. Since previous research has been shown that men with a PFO have higher core temperatures compared to men without a PFO, understanding whether the presence of a PFO can influence human health and performance during active heat stress is of the utmost importance.

The first objective of this dissertation, addressed in Chapter IV, was to determine if men and women with a PFO have a higher core temperature compared to men and women without a PFO during 60 min of exercise at a controlled heat production. The second objective, addressed in Chapter V, was to determine if the presence of a PFO in men and women was associated with altered the thermoregulatory and cardiovascular responses and performance during a self-paced 5k outdoor time trial. The third and final objective of this dissertation, addressed in Chapter VI, was to determine if the presence of a PFO was associated with altered the core temperature responses in SCUBA divers during two dive profiles.

Historical Perspective

The start of thermoregulation research began in the late 1700s with the development of the mercury thermometer [14]. In 1871, due to the publication of *Manual of Medical Thermometry* by C.A. Wunderlich [15], thermometry became more widely used in clinical practice, which paved the way for the inclusion of temperature measurements in research studies. The use of

thermocouples to measure thermal topography and partitional calorimetry for the calculation of metabolic rate began in the early 1900s [16], and both are now widely used in thermoregulation research. The development of these initial tools allowed early scientists to make great strides in understanding the regulation of body temperature in health and disease.

The first to discover that body temperature was a regulated variable were Currie [17] and Liebermeister [18]. From there, using brain lesioning studies, scientists were able to determine that the hypothalamus played an important role in the neural control of thermoregulatory responses and was the key brain center responsible for the regulation of body temperature. It was later determined that while the mechanism for how the "set point" or operating range for temperature (defense temperature) is regulated is still unknown, body and core temperature are in fact regulated around an ideal set point within in the brain [14]. Over time, thermoregulation research progressed from understanding that body temperature is a regulated variable, to showing the responses of warm sensitive and cold sensitive neurons in the brain, which affect the responses of the smooth muscle in the vasculature and heat production, to later understanding the key neurotransmitters involved in these responses. Fast forward to the modern scientific era, and while there is still much to be known and understood about the physiological responses that allow humans to regulate their body temperature within a narrow range, we now have a much better understanding about how humans respond to various thermal stressors, whether that be exercise, heat or cold exposure, or fever.

More recently research has shown that an additional factor that may influence this set point for thermoregulation in humans is the presence or absence of a patent foramen ovale. The foramen ovale is a normal part of the fetal cardiopulmonary circulation and is a small hole between the atria of the heart. Generally, the foramen ovale closes in the first few years of life,

however, in ~25-40% of the population, the foramen ovale fails to close and is then termed a patent foramen ovale.

The existence of a PFO was first described by Claudius Galen in 200 AD:

"There is a kind of orifice or fenestra 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. …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 28 animal, you would say there had never been a time when it was open; and, on the other hand, in a foetus, before or immediately after birth when this membrane is attached so to speak, only by it's root, the rest of it hanging free in the vascular cavity, you would hardly believe in it's ever becoming agglutinated." – (Opera Omnia, vol. IV, p.243; translation by Dalton, 1884, p.69.) [19].

The prevalence of a PFO in the population has been studied since early 1900s. In 1938, Patten published a paper summarizing the prevalence of a PFO in nine autopsy studies between 1837 and 1934 [20]. Out of 4083 autopsy patients, 864, or ~21% were identified as having a PFO. Among the 9 individual studies, the prevalence of the PFO ranged from 15% to 43%. More recent studies using both autopsy [7,21] and saline contrast echocardiography [22–24] have also reported these findings that 25-40% of the population has a PFO. Interestingly, the incidence of PFO decreased 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 [7]. In addition to the finding that prevalence of the PFO decreased with age, the size of the PFO was found to increase with age. During the first decade

of life, the average of a PFO was ~3.4 mm and increases to ~5.8 mm during the 10th decade of life.

While the presence of a PFO has been noted since the early observations of Galen, little research has been done examining the influence of this intracardiac shunt on normal physiological processes. In 2011, work done by Lovering et al. [8] demonstrated that subjects with a PFO (PFO+) have a worse pulmonary gas exchange efficiency (measured by alveolar-to-arterial oxygen difference; $AaDO_2$) at rest. Another intriguing finding in this study was that at maximal exercise, subjects with a PFO had a higher esophageal temperature (measured via esophageal probe) by ~0.4 °C compared to subjects without a PFO. While this was an interesting finding, authors hypothesized that the elevation in esophageal temperature in PFO+ subjects was a result of the lack of controls for cofounding variables that would affect temperature such as hydration status, time of day, menstrual cycle phase in female participants, etc. However, follow up work showed that men with a PFO did in fact have higher esophageal temperatures during short duration exercise, as well as during passive heating and cooling all well controlling for these variables [11,12]. Whether or not these findings apply to PFO+ women has yet to be explored.

The purpose of providing this historical perspective is to show that while both thermoregulation research and the knowledge of the PFO have been around since the early 1800s, little work has been done examining the potential influence of this shunt of the thermoregulatory and cardiovascular responses to various forms of thermal stress. While it may be that the presence of a PFO contributes to some of the normal biological variability we see in resting core temperatures, additional work needs to be done to determine whether a PFO influences other aspects of temperature regulation.

Background and Significance

Thermoregulation

In normal humans, core temperature (Tc) is maintained within narrow limits around ~37 °C. There is interindividual variability in resting Tc among a group of individuals, with most between 36 and 37°C, and factors such as differences in basal metabolic rate, circadian rhythm differences [1], levels of inflammatory markers or endogenous pyrogens (i.e. tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, interferons) [2], and menstrual cycle phase [3] all contribute to this variability in resting Tc seen among individuals [25].

In response to thermal stressors, whether hot or cold exposure, several physiological adjustments can be made to attempt to maintain core temperature. The appropriate responses are coordinated in the pre-optic anterior hypothalamus in the brain. In the case of heat stress, to dissipate more heat, there will be an increase in cutaneous vasodilation as well as the onset/increase in sweating. If the goal is to produce or conserve heat, as would be the case during cold exposure, the cutaneous vasculature will vasoconstrict and heat production will increase via shivering and non-shivering thermogenesis.

Sex Differences with Thermoregulation and the Menstrual Cycle

In women, the thermoregulatory responses to thermal stressors vary over the course of the menstrual cycle due to the cyclical changes in the sex hormone concentrations of progesterone and estradiol [26]. In the luteal phase of the menstrual cycle, core temperature is 0.3-0.7 °C higher compared to the follicular phase [27]. Similar shifts in the regulation of Tc have been seen in the low hormone (placebo) vs high hormone phase of birth control/oral contraceptives [28]. The elevation in progesterone during the luteal phase (or high hormone phase) affects both the set points for thermoregulation as well as core temperature for the onset of heat dissipation and

conservation mechanisms [3,29]. While there are these known physiological differences in women over the course of the menstrual cycle with regards to temperature regulation, little thermoregulation research has included women. In fact, women only make up 30% of the study participants in thermoregulation research up to 2019 [30], therefore, there are several knowledge gaps when it comes to women and thermoregulation.

Patent Foramen Ovale

The development of the heart and lungs starts during embryonic development in utero. The heart, which starts out as a single cavity eventually develops into an organ with four separate chambers, known as the two atria and two ventricles. In addition to the development of the chamber's walls, valves also develop between the chambers and major blood vessels to assist with unidirectional blood flow, allowing the blood to circulate through both the systemic and pulmonary circulations. While blood flow through the pulmonary circulation is crucial for gas exchange after birth, during fetal development, pulmonary blood flow is minimal. Due to the elevated pulmonary vascular resistance resulting from hypoxic pulmonary vasoconstriction in the fetal lungs, blood cannot flow easily through the pulmonary vasculature.

The lack of pulmonary blood flow is not an issue in utero as gas exchange is occurring with the placenta, and not at the level of the lungs. However, due to the presence of a small hole between the two smaller and upper chambers of the heart (also known as the atria) and elevated right atrial pressure resulting from the increased pulmonary resistance, blood travels from the right side of the heart to the left side and continues throughout the fetal systemic circulation. This hole is known as the foramen ovale.

At birth when the baby starts breathing ambient air, pulmonary vascular resistance decreases, and left atrial pressure exceeds right atrial pressure. This elevation in left atrial

pressure causes the septum primum to come into contact with the septum secundum and through the process of fibrosis and the conversion of endothelial cells to mesenchymal cells, the foramen ovale closes [6]. While in most of the population, the foramen ovale closes within the first two years of life [5], as previously mentioned, in ~25-40% of the population, it fails to close and is then termed a patent foramen ovale, or PFO [7,23,24].

Patent Foramen Ovale and Thermoregulation

As previously stated, it has been shown that men with a PFO have a higher core temperature when measured via esophageal probe compared to men without a PFO at rest, during short duration exercise, and during passive heating and cooling [8,11,12]. While these higher esophageal temperatures have been noted, the mechanisms responsible for the shift in temperature are unknown. Higher esophageal temperatures are not the only difference that has been shown in PFO+ men compared to PFO- men with regards to thermal responses. Other differences include blunted thermal hyperpnea and a higher core temperature for the onset of shivering. It is well established that during heating as core temperature increases, ventilation increases due to the influence of temperature on the carotid body [31]. During the passive heating study [11], PFO+ men did not increase their ventilation until they reached an esophageal temperature that was 0.7 °C higher than PFO- men. In fact, in the subset of subjects who reached a ventilatory threshold, PFO+ men (8/13) reached their ventilatory threshold at a higher core temperature vs PFO- men (10/14), but once the threshold was reached, they had a blunted ventilatory response, evidence by a lower VE (by ~10 L/min) and a higher end tidal CO_2 (35 ± 6 mmHg vs 28 ± 4 mmHg, respectively). During passive cooling, or cold water immersion, PFO+ men shivered at a higher core temperature compared to PFO- men and were able to maintain higher core temperatures regardless of it they shivered or not. [11]

Statement of the Problem

A patent foramen ovale is present in ~25-40% of the general population (Marriott et al., 2013; Elliott et al., 2013). While previous research has shown that men with a PFO have higher core temperatures at rest, during exercise, and during passive heating and cooling, the mechanisms to explain these differences are unclear. Additionally, whether these findings apply to both men and women with a PFO has yet to be determined. Finally, although these differences in core temperature have been measured, it is unknown if this elevation in temperature has an impact on health and performance during real world situations (i.e., outdoor 5k run and SCUBA diving).

Purpose & Hypotheses

The primary purpose of this dissertation was three-fold: to determine if the presence of a PFO affects 1) core temperature at rest and during exercise at a controlled heat production in both men and women; 2) the core temperature responses and time trial performance in men and women during self-paced exercise; and 3) the core temperature responses during SCUBA diving during two dive profiles.

Aim #1: Determine the influence of 60 min of cycling exercise at a standardized heat production on thermoregulatory and cardiovascular responses in men and women with and without a patent foramen ovale.

The purpose of this aim was to determine whether or not the previously seen differences in core temperature [11,12], as measured by esophageal probe, between PFO+ and PFO- men are the result of differences in heat production in those who participated in the studies, rather than differences in thermoregulatory mechanisms or the set point for temperature regulation. It has been shown

that when comparing different groups of individuals, if heat production is not controlled for then it can lead to differences in core temperature responses [32]. Additionally, while these differences in esophageal temperature have been measured between PFO+ and PFO- men, women have yet to be included in these studies, therefore, whether similar differences in core temperature exist in PFO+ women has not been determined.

Aim #2: Determine the association of the presence of a patent foramen ovale and biological sex assigned at birth on core temperature and heart rate responses during a self-paced, outdoor 5k time trial run.

It is well established that during self-paced exercise, physiological variables such as core temperature and heart rate affect the perception of effort for a given task and therefore can affect performance. As core temperature and heart rate increase, it increases the perceived difficulty of the task, which could lead to an individual to slow down (in the case of running) and compromise performance. Since it has been shown that men with a PFO have higher core temperatures at rest and during exercise [12], it is unknown if this higher core temperature would affect self-paced exercise performance in an outdoor, real world scenario. Thus, the second aim of this dissertation was to determine whether the presence of a PFO affects core temperature responses and performance during self-paced outdoor 5k run in men and women.

Aim #3: Determine the influence of wetsuit type and non-shivering thermogenesis on core temperatures responses during two dive profiles in SCUBA divers in those with and without a PFO.

A previous study showed that men with a PFO are better able to maintain core temperatures when exposed to cold environments, specifically during cold water immersion (CWI). During CWI, PFO+ men had higher core temperatures throughout the protocol, regardless of whether they shivered or not. Additionally, men with a PFO shivered at higher core temperatures compared to men without a PFO. These data suggest that men with a PFO may be better able to handle cold environments. SCUBA divers are a group of individuals who are often exposed cold environments and to water temperatures that are far below the temperature that would be considered thermoneutral for human beings (~34-35°C) [33], which can be detrimental to physical and cognitive performance and even life threatening [34]. During cold water immersion, various mechanisms including shivering, non-shivering thermogenesis and behavioral thermoregulation (i.e. choice of suit) all affect the core temperature changes. What has yet to be studied is if SCUBA divers with a PFO are better able to maintain core temperatures during diving. In this study we will look at both the influence wetsuit thickness and the contribution of non-shivering thermogenesis (i.e., UCPs and FGF21) to the maintenance of core temperature. The purpose of the third dissertation aim is to determine the influence of wetsuit type and non-shivering thermogenesis on core temperatures responses during two dive profiles in SCUBA divers in those with and without a PFO.

Chapter IV will be submitted to the *Journal of Physiology* and, Aaron W. Betts, Elizabeth M. Castillo, Kaitlyn G. DiMarco, Christopher T. Minson, Nisha Charkoudian, and Andrew T. Lovering are co-authors. I performed the experimental work, led the project, and the writing is entirely my own; Aaron W. Betts, Elizabeth Castillo, and Kaitlyn G. DiMarco assisted with data collection; and Dr. Andrew T. Lovering and Dr Nisha Charkoudian helped develop the protocol, and provided guidance and editorial assistance.

Chapter V was submitted to *Medicine and Science in Sports & Exercise* and Aaron W. Betts, Kaitlyn G. DiMarco, Tyler S. Kelly, Nisha Charkoudian, and Andrew T. Lovering are co-authors. I performed the experimental work, led the project, and the writing is entirely my own; Aaron W. Betts, Tyler S. Kelly, and Kaitlyn G. DiMarco assisted with data collection; and Dr. Andrew T. Lovering and Dr Nisha Charkoudian helped develop the protocol, and provided guidance and editorial assistance.

Chapter VI was submitted to *Journal of Science and Medicine in Sport* and, Kaitlyn G. DiMarco, Joel E. Futral Rachel Lord, Justin Edwards, Otto Barak, Željko Dujić, Igor Glavičiç, Ivana Miloš, Ivan Drvis, and Andrew T. Lovering, are co-authors. I performed the experimental work, led the project, and the writing is entirely my own; Kaitlyn G. DiMarco, Andrew T. Lovering assisted with data collection; 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 outline the topics discussed in Chapters IV-VI. The first topic will be a general introduction to the main concepts of thermoregulation, including physiological responses to both hot and cold environments. Next, the factors that contribute to baseline and exercise core temperature and cardiovascular responses including the influence of sex hormones, circadian rhythm, inflammation, and heat production will be explained. Finally, this review of the literature will explain the previous research completed on how the presence of a patent foramen ovale may influence these thermoregulatory responses.

Principles of Thermoregulation

In healthy humans, core temperature (Tc) is maintained within narrow limits around ~37 °C. There is interindividual variability in resting Tc with most individuals between 36 and 37°C, and factors such as differences in basal metabolic rate, circadian rhythm differences [1], levels of inflammatory markers or endogenous pyrogens (i.e. tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, interferons) [2], and menstrual cycle phase [3] all contributing to the variability in resting Tc among these individuals [25].

In a given individual, the maintenance of Tc is achieved through the integration of sensory afferent information in the pre-optic anterior hypothalamus (PO/AH) [3]. Depending on the information received, the PO/AH will coordinate the efferent signals and organize a series of physiological responses. In the case of elevations in temperature, increases in skin blood flow (SKBF) and sweating will occur to allow for the dissipation of heat from the body to the external environment. On the other hand, if decreases in temperature are sensed by cold sensitive

neurons, cutaneous vasoconstriction attenuates SKBF to increase heat conservation and both shivering and non-shivering thermogenesis (NST) occur to increase heat production (Hprod) [3].

Heat Dissipation Mechanisms

During heat stress, cutaneous vasodilation and increases in SKBF occur as the result of both neural mechanisms and the local effect of increased temperature on the blood vessels in the skin. The cutaneous vasculature is under both sympathetic noradrenergic vasoconstrictor control as well as sympathetic noradrenergic active vasodilator control [35]. At rest, there is tonic vasoconstriction to the skin which causes SKBF to be ~250 ml/min. This low blood flow is due to norepinephrine binding to alpha 1 (α_1) and 2 (α_2) receptors in the skin. As Tc and skin temperature (Tsk) increase, the elevation in temperature is sensed by thermoreceptors in the skin, brain, and throughout the body and afferent information is sent back to the PO/AH, which will then activate mechanisms responsible for increasing SKBF. The initial increase in SKBF caused by passive vasodilation and is the result of a decrease in NE release from the sympathetic adrenergic nerves and a decrease in the α_2 receptors affinity for NE. This removes the tonic vasoconstrictor input to the smooth muscle cells in the vasculature of the skin, increasing SKBF. As Tc and Tsk continue to increase, active vasodilation is initiated. The exact mechanism that leads to an increase in SKBF via active vasodilation is unknown, however, it involves co-transmission with acetylcholine from sympathetic cholinergic nerves [27,36,37]. Additionally, local warming of a given area (independent of neural input) leads to increased nitric oxide production, which is another mechanism that facilitates increases in SKBF [38].

During both exercise and passive heat stress, increased skin blood flow and evaporation of sweat are the primary mechanisms for heat dissipation [39]. Sweating from eccrine sweat glands is caused by the release of acetylcholine from the sympathetic cholinergic nerves when it binds

to muscarinic receptors. As eccrine sweat glands secrete sweat, heat is lost to the surrounding environment as the liquid is converted to water vapor. As Tc and Tsk increase, there is first an increase in the number of active sweat glands, followed by an increased output by each gland to increase evaporative cooling thereby decreasing Tc and Tsk. The effectiveness of sweating as a heat dissipation mechanism is highly dependent on the environmental conditions, with more humid environments having a reduced evaporative capacity, thus, decreasing the efficacy of sweating [40]. If sweat cannot be evaporated, Tsk increases, leading to increased SKBF demands and consequently increasing cardiovascular strain.

An additional mechanism that contributes to heat dissipation through both convection and evaporation is respiratory heat loss (RHL). In humans, RHL contributes to 10-15% of heat loss and increases with hyperpnea [41]. As cooler, drier air enters the respiratory tract, it is warmed and humidified [41]. Heat from the bronchial and pulmonary circulations will be transferred to the air entering the airways and alveoli, and on expiration, this heat will be transferred from the body to the environment through the expired air. At rest and during exercise, ventilation increases with increasing Tc due to increased sensitivity of peripheral chemoreceptors [31,41], which contribute to increased heat dissipation via RHL.

Physiological Responses to Cold Air and Cold-Water Immersion

The influence of cold exposure, both via air temperature and cold water immersion (CWI), on physiological responses has been extensively studied [42–44]. Exposure to cold air and immersion in cold water both present physiological challenges, however, the differences in the biophysical properties between air and water lead to differences in the rate of heat loss. Due to the conductive properties of water and the fact that it is 25x more conductive than air [45,46], an individual will lose heat 3-5x faster while immersed in water leading to much more rapid

declines in core temperature compared to being exposed to cold air [47]. Regardless of the different rates of heat loss, the physiological responses to both types of cold stress include heat conservation and heat production mechanisms. To conserve heat, cutaneous vasoconstriction will occur to increase the insulative shell and prevent heat loss to the external environment. If cutaneous vasoconstriction is not enough to maintain Tc, shivering and NST occur to increase metabolic Hprod [48].

During cold exposure, the first physiological response that occurs (within the first few minutes) is cutaneous vasoconstriction, which decreases SKBF thus convective heat loss to the surrounding environment [43]. There are three primary mechanisms that contribute to the reduction in SKBF that occurs in cold environments. The first is caused by a decrease in Tsk. The decrease in Tsk is sensed by thermoreceptors in the skin which delivers information via afferent nerves to the pre-optic anterior hypothalamus. Efferent signals are then sent from the brain through the intermediolateral cell column of the spinal cord and to sympathetic nerves innervating cutaneous blood vessels. The nerves innervating the vasculature of the skin release norepinephrine and neuropeptide Y, which are responsible for 60% and 20-30% of the cutaneous vasoconstriction, respectively, that occurs during cold exposure[43].

As cooling continues, vasoconstriction increases and the remaining 10-20% of the decrease in SKBF is caused by the local cooling of the blood vessels themselves [43]. The decreased temperature of the skin augments the production of mitochondrial reactive oxygen species, increasing Rho Kinase production[49,50,51]. In the smooth muscle, Rho kinase inhibits myosin light chain phosphatase (MLCP), the enzyme responsible for smooth muscle relaxation. Rho kinase facilitates increases in vasoconstriction through several physiological mechanisms. First, the inhibition of MLCP allows for the myosin light chain to be phosphorylated, which

facilitates smooth muscle contraction and cutaneous vasoconstriction. Also, during cold exposure and because of the increase Rho kinase production, there is an increased sensitivity of the α_2 receptor to norepinephrine, which is mediated through the translocation of α_{2C} receptors from the Golgi bodies to the plasma membrane[52]. The increased sensitivity of the α_2 receptor to norepinephrine will lead to an enhanced cutaneous vasoconstrictor response [43]. Finally, Rho kinase also down-regulates nitric oxide synthase, which subsequently decreases nitric oxide production and the influence of nitric oxide on smooth muscle relaxation[51].

While cutaneous vasoconstriction assists with heat conservation during cold exposure, metabolic rate and heat production are elevated due to shivering and NST [43]. Shivering is defined as "uncontrollable, repetitive and rhythmic muscle contractions" that increases with intensity the longer the exposure to cold [53,54]. Two types of shivering patterns have been observed: burst and continuous [55]. Burst shivering is associated with Type II muscle fibers and often occurs first during cold exposure, followed by sustained shivering which is associated with Type I muscle fibers [56]. During lab studies, shivering can be measured via metabolic changes in oxygen consumption (metabolic cart; increase in VO_2 by 25% for 5 mins) [11,57] and through increased muscle activity (EMG) [53]. The decline in core temperature is the strongest stimulus for the shivering mechanism, however, the initial and faster decrease in Tsk is responsible for the onset of shivering that occurs very quickly after exposure to a cold environment [43,44].

Finally, although not a primary mechanism for heat production in humans during cold exposure, brown adipose tissue (BAT) can increase heat production via NST. Brown adipose cells are richly innervated by sympathetic nerves and the cells contain mitochondria with uncoupling proteins (UCP-1, also known as thermogenin), to produce metabolic heat rather than using ATP synthase [58]. After norepinephrine binds to β_1, β_2, and β_3 receptors on these adipose

cells, it activates G-protein coupled receptors, which in turn increases the activity of adenylate cyclase. Adenylate cyclase converts ATP into cAMP, which activates protein kinase A (PKA) to phosphorylate and activate hormone sensitive lipase (HSL). HSL breaks down triglycerides and fatty acids to go into the mitochondria and undergo beta oxidation and the electron transport chain. The presence of the UCP-1 allows for the passing of H+ ions from the inner membrane space, back into the mitochondrial matrix producing heat without the production of ATP. The disruption of the proton gradient prevents the production of ATP using ATP synthase, so the cell will continue to breakdown fuel to try to produce ATP, but instead it produces heat.

In addition to heat conservation and production mechanisms, anthropometric factors such as body mass and body surface area also contribute to the ability of an individual to tolerate a cold environment and maintain Tc. In fact, most of the variability in the ability to maintain core temperature in a cold environment can be explained by differences in anthropometric factors [54]. Total body mass is strongly related to the risk of hypothermia in those exposed to cold environments in several populations of individuals [48]. The transfer of heat to the environment is highly dependent on the ease by which the tissues lose heat. Although all body tissues provide some degree of thermal resistance to heat conduction from the body, the thermal resistivity of fat is greater than that of either skin or muscle. Subcutaneous fat provides significant insulation against heat loss in the cold, therefore, those with the greatest amount of body fat (increased subcutaneous fat thickness), have the greatest amount of insulation, minimized conductive heat flux, and are better able to maintain core temperature when all other factors are equal [59]. Those with high body surface area to mass ratios have also been shown to have the greatest decrease in core temperature during cold exposure when compared to an individual with a low surface area to body mass ratio [54,60].

These heat production and heat conservation mechanisms are vital in preventing catastrophic and rapid decreases in core temperature during cold exposure, however, wearing the appropriate protective gear (i.e. behavioral thermoregulation) during cold water immersion is also an important consideration [61]. For example, SCUBA divers are often exposed to water temperatures that are far below the water temperature that would be considered thermoneutral for human beings (~34-35°C) [33]. This can be detrimental to physical and cognitive performance and even life threatening [34]. To prevent or delay the onset of hypothermia, SCUBA divers wear wetsuits, semi dry suits or dry suits for protection against CWI [61]. Variables including body mass, body surface area, type of thermal protective suit, water temperature and duration of exposure all play a role in the ability of someone to tolerate CWI. Other factors including the presence of a PFO and physiological mechanisms such as NST (i.e. UCPs) may also play a role in the ability of someone to tolerate CWI.

Influence of the Menstrual Cycle on Thermoregulatory Responses

The menstrual cycle has significant influences on physiological responses in women. Characterized by fluctuations in sex hormones (estrogen, progesterone, follicle-stimulating hormone, luteinizing hormone), the menstrual cycle typically lasts between 21 and 35 days and consists of the follicular phase, ovulation, and the luteal phase [62,63]. These hormones are part of the hypothalamic-pituitary-ovarian system and are regulated via a series of feedback mechanisms [27]. The early follicular phase begins with the first day of menstruation and is when the sex hormones (estrogen and progesterone) are present in their lowest concentrations. As the follicular phase continues, estrogen increases and then peaks near the end of the phase before declining. Ovulation occurs around day 14 (based on a 28 day menstrual cycle) and is characterized by the peak in luteinizing hormone [27]. The luteal phase follows ovulation and in the mid-luteal phase,

progesterone peaks along with a second, yet smaller than progesterone, peak in estrogen and both hormones decline as the luteal phase comes to an end.

An additional and important consideration for studying women involves understanding the influence of various forms of hormonal birth control. According for the Center for Disease Control NCHS Data Brief, ~25% of women between the ages of 18-49 are using either oral contraceptives or some form of long-acting reversible contraceptives [64]. Oral contraceptives contain exogenous and synthetic forms of estrogen and progesterone that suppress the production of endogenous sex hormones. Generally, oral contraceptives contain 3 weeks of the exogenous hormones, followed by one week of placebo pills. While women not utilizing hormonal birth control and women utilizing oral contraceptives have been most widely studied in physiology research, many other types of chemical contraceptives exist and are becoming more widely used. These other contraceptives include types like oral contraceptives with a high hormone vs. low hormone phase such as the hormone patches and vaginal rings. However, there are long-acting reversible contraceptives including hormone intra-uterine devices, Depo-Provera (i.e. medroxy progesterone acetate) shots, and implants that do not have high hormone and low hormone phases as they are continuous release. How these various types of chemical contraceptives influence female physiology including thermoregulation are much less understood and warrant additional investigations [64].

The peaks in hormones, specifically estrogen and progesterone, have been shown to influence the set point for Tc regulation as well as the Tc for the onset of thermoregulatory effector mechanisms, regardless of whether those mechanisms are involved with heat dissipation, conservation, or production across the various phases of the menstrual cycle [3,29]. Tc is increased by ~0.3-0.7°C in the luteal phase vs follicular phase [27] and similar shifts in the regulation of Tc

have been seen in the low hormone (placebo) vs high hormone (exogenous synthetic estrogen and progesterone) phase of birth control/oral contraceptives [28].

In the luteal phase of the menstrual cycle, the onset temperature for heat dissipation mechanisms (i.e. cutaneous vasoconstriction and sweating) will occur at an elevated temperature relative to the follicular phase of the menstrual cycle, or in the high hormone phase vs. low hormone phase of oral contraceptives [28]. While the set point and threshold temperatures are influenced by the ovarian hormones, progesterone and estrogen do not appear to affect the sensitivity of the mechanisms once the Tc threshold has been reached [3].

While less understood relative to the physiological responses to heat, the elevation in sex steroid hormones also influences the mechanisms that are activated during cold exposure [65]. In the case of cold exposure, cutaneous vasoconstriction (both reflex control and local thermal control), shivering and NST all occur at higher Tc in the luteal phase vs. follicular phase. The higher Tc in the luteal phase has implications for the cardiovascular system as well as increases in HR have been reported in the luteal phase vs follicular phase. The higher HR may be partially due to the influence of increased Tc on HR, but may also be a mechanism to increase cardiac output to meet the elevated skin blood flow demands [28].

Additional Factors Influencing Resting Core Temperature

While variations in the external environment contribute to changes in core temperature, circadian rhythm also contributes to variations in measured core temperature over the course of a 24 hr period. The suprachiasmatic nucleus (SCN), which is located at the base of the hypothalamus, is known as the central circadian pacemaker [27,66]. The retina receives light-dark information based on the time of day and external environment, and conveys that information to the SCN, leading to a cascade of synchronized physiological responses. The temperature over the

course of a 24 hr period is influenced by fluctuations in heat production and heat loss and includes three key time points: circadian mesor (average temperature across 24 h), acrophase (time of highest temperature), and the nadir (time of lowest core temperature). The nadir usually occurs between 4-6 am, after which core temperature continues to increase throughout the day. Core temperature peaks 1-4 hr before habitual bedtime, after which core temperature steadily decreases[67] The drop in temperature that occurs in the evening is influenced by both the circadian and sleep processes that affect temperature [68]. To help lower body temperature during sleep, warm sensitive neurons in the brain are less sensitive and cold sensitive neurons are more sensitive, leading to a decrease in heat production and conservation. Interestingly, in circumstances when an individual does not sleep, temperature does not drop to the same extent due to the fact that both the circadian and sleep processes are not contributing to the change in temperature [69]. The fact that core temperature varies throughout a 24 hr period makes it extremely important to control for time of day in studies examining baseline core temperature differences among different individuals.

Fever is another example of a factor that can contribute to elevations in core temperature. During a fever, exogenous or endogenous pyrogens cause the "defense temperature" in the brain to be reset to a higher temperature, which affects the temperature for onset of thermoregulatory mechanisms [70]. Since the set point temperature in the brain is elevated, any temperature that is below the new set point will be interpreted as "hypothermic" and lead to heat generating mechanism to try to increase body temperature. This explains why an individual shivers at the onset of a fever, even though their core temperature is elevated compared to normal conditions. Endogenous pyrogens or cytokines such as interleukins (IL-1, IL-6), tumor necrosis factor (TNF)-α, or monocyte chemoattractant protein-1 (MCP-1) act on a part of the hypothalamus

called the *organum vasculosum* of the *lamina terminalis* (OVLT), which causes the production of a fever. The presence of the cytokines and chemokines in the brain leads to a slowing of the firing rate of the warm-sensitive neurons which causes body temperature to rise [71].

Influence of Heat Production during Exercise on Core Temperature

An important factor to consider when comparing Tc across different groups of exercising individuals is whether there are different amounts of heat produced during the exercise bout. Tc changes during exercise are dependent on the balance of Hprod and heat dissipation, body mass, and body composition [32]. If exercise is prescribed based on an absolute work rate or percentage of VO_{2max}, it could lead to variations in Tc changes due to differences in the amount of Hprod over the course of the exercise bout. If Hprod is not controlled for, it may be that the differences in Tc measured during exercise are due to differences in Hprod, rather than differences in thermoregulation such as differences in the thermoregulatory operating point (i.e., set point determined by the hypothalamus) or differences in the onset/effectiveness of thermoregulatory effector mechanisms (i.e., sweating, SKBF). However, if differences in the change in Tc are seen even with a controlled Hprod, it is possible that the physiological variable (i.e., age, body mass, etc.) is affecting the mechanisms associated with the balance between Hprod and heat dissipation.

Jay et al. (2011) and Cramer and Jay (2019) established the importance of controlling for heat production when comparing core temperature changes between independent groups. The first study evaluated the influence of VO_{2peak} (high vs. low) on thermoregulatory responses to 60 min of exercise at both a fixed heat production (FHP, 540 W) and relative exercise intensity (REL, 60% VO2peak) in men (matched for body weight and body surface area). Interestingly, there was no difference in the change in core temperature over the course of the 60 min exercise

bout between the two groups in the FHP trial, however, during the REL trial, the high VO_{2peak} group had a significantly greater increase in core temperature compared to the low VO2peak group. These results suggest that when comparing thermoregulatory responses among independent groups, using a relative exercise intensity will lead to differences in core temperature because of differences in heat production and thus exercise intensity should be at a controlled level of heat production.

In the initial study, while an absolute value of heat production (540 W) lead to similar core temperature changes during exercise in both groups, Jay et al. (2011) mentioned that all subjects had a similar body weight. While heat production can be used as an absolute amount (i.e., 540 W), heat production can also be used as a relative value and determined based on body weight (W/kg of body weight). To follow up on these findings, Cramer & Jay (2019) conducted a study using men of different body masses at both absolute (500 W, 600 W) and relative levels of heat production, (6.5 W/kg, 9 w/kg). In the absolute heat production trials, the low body mass group had greater changes in core temperature during the exercise bout, however, in the relative heat production trials, the change in core temperature was similar among the groups. Authors concluded that when comparing thermoregulatory responses among independent groups, using a relative heat production for an exercise intensity was the most appropriate study design.

Hprod during exercise can be estimated using partitional calorimetry which takes into account the rate of metabolic energy expenditure and external work rate (watts on the cycle ergometer) [72]. The following formula can be used to estimate rate of metabolic energy expenditure (M) measured in Watts (W)[72]:

$$M = VO_2 \cdot \frac{\left\{\left[\left(\frac{RER-0.7}{0.3}\right)\cdot 21.13\right]+\left[\left(\frac{1.0-RER}{0.3}\right)\cdot 19.62\right]\right\}}{60} \cdot 1{,}000$$

Where VO_2 is the volume of oxygen consumed (L/min), RER is the respiratory exchange ratio, e_c is the energy equivalent for carbohydrate (21.13 kJ) and e_f is the energy equivalent for fats (19.62 kJ).

Hprod can then be calculated as the difference between metabolic energy expenditure (M) and work rate (Wk) in watts (W):

$$H_{prod} = M - Wk$$

Thus, controlling for Hprod during exercise eliminates the confounding influence of different amounts of Hprod on core temperature and cardiovascular responses during exercise when studying different groups of subjects.

Cardiovascular Responses during Heat Stress and Their Influence on Exercise Performance

In addition to affecting thermoregulatory mechanisms, Tc has an influence on the cardiovascular system. During both passive and active heat stress, there are several cardiovascular responses that occur in order to facilitate heat dissipation via increased SKBF while also maintaining arterial blood pressure through increased cardiac output [73]. The increase in cardiac output is caused primarily by an increase in heart rate (HR), which during heat stress, is directly affected by increases in Tc [74]. The direct effect of the increased temperature on the sinoatrial node, as well as the activation of the sympathetic nervous system, both lead to elevations in HR during heat stress [75].

Elevations in Tc during prolonged steady-state exercise in both a thermoneutral and hot environment can lead to a progressive rise in HR over the course of the exercise bout, a phenomenon known as cardiovascular drift [76,77]. As Tc and body temperature rise, blood flow is redistributed to the skin, decreasing venous return and stroke volume [77]. Initially, cardiac output is maintained due to increased contractility (maintaining stroke volume to an extent) and

elevations in HR. However, as HR continues to increase, diastolic filling time decreases, and stroke volume cannot be maintained, which may influence oxygen delivery and performance at higher workloads.

It is well established that both HR and Tc are physiological variables that affect exercise performance. Several physiological variables, including HR, ventilation, and Tsk have been shown to influence the perception of effort during self-paced exercise and alter exercise performance (St Clair Gibson *et al.*, 2006; Abbiss *et al.*, 2015). During long duration self-paced exercise events, the high rate of metabolic Hprod (in combination with environmental conditions) leads to elevations in Tc, which may impair endurance exercise performance if Tc increases too much [81].

The PFO and Thermoregulation

In addition to the detailed thermoregulatory considerations outlined above, recent work has identified a patent foramen ovale (PFO) as a potential contributor to differences in core temperature and thermoregulation, among other environmental challenges [82]. A foramen ovale is present in normal fetal circulation and is a small tunnel that exists between the right and left atria of the heart [6]. During fetal development the fetal blood is oxygenated through gas exchange with the placenta and does not occur in the lungs. Most of the blood bypasses the pulmonary circulation, and flows directly from the right atria to the left atria, through the foramen ovale [83] and from the pulmonary artery to the aorta via the *ductus arteriosus*. The blood flow from the right atria to the left atria via the foramen ovale is facilitated by the elevated pulmonary vascular resistance due to hypoxic pulmonary vasoconstriction in the fetus. As a result of the increased pulmonary vascular resistance, right atrial pressure exceeds left atrial pressure, which forces the septum primum into the left atrium, causing the foramen ovale to stay open [82].

At birth when the baby starts breathing ambient air, pulmonary vascular resistance decreases, and left atrial pressure exceeds right atrial pressure. This elevation in left atrial pressure causes the septum primum to come into contact with the septum secundum and through the process of fibrosis and the conversion of endothelial cells to mesenchymal cells, the foramen ovale closes [6]. However, in 25-35% of the population, the foramen ovale stays open after birth and is termed a patent foramen ovale [7]. Although the PFO has been associated with higher core temperatures (T_{esoph}; measured via esophageal probe) during exercise and in various environmental conditions [11,12], the reasons for these associations remain elusive.

A higher T_{esoph} (~0.4 °C) in individuals with a PFO in a thermoneutral environment was first reported by Lovering *et al.* in a study investigating differences in pulmonary gas exchange efficiency in those with and without a PFO [84]. In this study, subjects with a PFO (PFO+) had a significantly lower oxygen saturation and a right shifted oxyhemoglobin curve due to this increase in $T_{esoph.}$ Interestingly, this right shifted oxyhemoglobin curve has also been noted in children with congenital cyanotic heart disease [85,86] and in animals with intracardiac shunts [87]. Therefore, a PFO is not the only cardiac abnormality that has been associated with an elevated core temperature and right shifted oxyhemoglobin curve.

While the elevated T_{esoph} in PFO+ subjects was reported by Lovering *et al.*, 2011, the variables that influence T_{esoph} (i.e., time of day, menstrual cycle phase, hydration status, clothing etc.) were not controlled for. Therefore, authors were unable to definitively conclude that the higher T_{esoph} was just associated with the presence of PFO. Follow up studies confirmed the initial findings and found that men with a PFO had elevated T_{esoph} at rest, during active heat stress (VO_{2max} test), as well as during passive heating and cooling compared to men without a PFO (PFO-) [11,12]. Interestingly, T_{esoph} was higher in PFO+ men at rest and T_{esoph} was maintained

at the higher temperature throughout exposure to a given stressor (i.e., exercise, heat, and cold). Whether or not there were differences in resting metabolic rate, levels of inflammatory markers, or even differences in circadian rhythm/sleep wake cycles and whether these differences are contributing to differences in resting T_{esoph} in PFO- vs PFO+ men have yet to be investigated. Thus, additional studies in these areas would fill an important gap.

As discussed above, while RHL only contributes to 10-15% of heat loss [41], there may be differences in RHL between PFO+ and PFO- subjects for a few reasons. First, as blood enters the pulmonary circulation, heat is transferred from the warmer blood to the cooler air in the alveoli. Individuals with a PFO have a volume of blood that goes directly from the right atria to the left atria and this blood does not go through the pulmonary circulation, thus does not participate in RHL. Previous work has shown that this shunted warmer blood may contribute to ~25% or 0.1°C of the temperature difference between PFO+ and PFO- men [12]. Another factor that may contribute to decreases in RHL, and thus higher T_{esoph} in PFO+ men is the fact that PFO+ men have a blunted thermal hyperpnea response [11]. During a bout of passive heating, men with a PFO had blunted thermal hyperpnea and the T_{esoph} threshold for hyperpnea was higher (~0.7°C) than men without a PFO. It may be that the blood flow pattern through a PFO, in combination with a blunted ventilatory response to rising T_{esoph} would decrease RHL in PFO+ individuals, thus contributing to a higher T_{esoph}. Accordingly, investigations in this area would provide additional insight into the differences in core temperatures between PFO+ and PFO- subjects.

Another possible explanation for the elevations in T_{esoph} in PFO+ subjects is decreases in the responsiveness or sensitivity of the physiological responses for heat dissipation (i.e., decreased SKBF, decreased sweat rate, etc.). During the hot water immersion trial, there was a significant difference in percentage of body water lost between subjects, with PFO+ men losing a

lower percentage of body weight (0.7 ± 0.2%) than subjects without a PFO (1.0 ± 0.3%) [11]. The differences in body water lost (i.e., sweating) may account for some of the differences in T_{esoph} between those with and without a PFO, but whether the responsiveness (or sensitivity) of SKBF and sweating responses as functions of Tc and/or Tsk changes during exercise or passive heating contribute to the T_{esoph} differences seen in PFO+ men has yet to be determined. Additional research in this area is warranted.

While these differences in T_{esoph} have been reported in men with a PFO, no study has yet to examine whether these differences in T_{esoph} are seen in women with a PFO at rest or during exposure to any thermal stressors. Progesterone and estrogen affect both the set points for thermoregulation as well as Tc for the onset of heat dissipation and conservation mechanisms, thus, it may be that there is an interaction between the ovarian hormones and PFO influence on Tc and thermoregulatory mechanisms. Studying the influence of a PFO in women independently will fill an important gap in knowledge in this area [26].

Whether or not the previously seen elevations in Tc in PFO+ men influence cardiovascular and other physiological responses during prolonged exercise has also yet to be determined. It may be that the elevations in Tc seen in PFO+ men also have an impact on baseline and exercise HR and other physiological responses. While Tc has been measured in PFO+ men during short duration exercise tests (i.e., VO_{2max} and 10 min graded exercise protocol), it is unknown if these elevations in Tc are enough to negatively influence longer duration, self-paced exercise performance. If individuals with a PFO have higher Tc, and thus a higher HR because of the influence of increased Tc on HR, it may be that this increased cardiovascular strain for a given exercise intensity will lead to a decrease in running speed, thus a decrement in self-paced exercise performance. Any increase in HR at a given speed/work rate

would limit the ability to further increase HR and cardiac output to meet metabolic demands at higher exercise intensities.

In addition to the studies done under thermoneutral conditions and hot water immersion, work done by Davis et al. (2017) reported that men with a PFO can maintain higher T_{esoph} during CWI (19.5 ± 0.9°C) without protective clothing or gear. In addition to maintaining higher T_{esoph}, men with a PFO also shiver (as determined by a 25% increase in $\dot{V}O_2$) for 5 min at higher T_{esoph} compared to men without a PFO. PFO+ men were also able to maintain a higher T_{esoph} than PFO- men even if they did not shiver during CWI. Since it has been previously shown that PFO+ maintain higher core temperatures and initiate heat conservation mechanisms at a higher Tc vs PFO- men during CWI [11], it is unknown if recreational SCUBA divers with a PFO (and those with the highest body mass) would have elevated resting Tc and maintain higher Tc during various dive profiles. SCUBA divers often select various SCUBA gear (known as behavioral thermoregulation) to increase the insulative shell between the skin and the colder environment, thus decreasing heat dissipation while diving. Furthermore, the combination of various SCUBA diving suits, other protective gear (e.g., dry suit and liners, wet suit, hoods, gloves, booties, etc.) and presence or absence of a PFO status has yet to be studied.

In summary, while previous studies have shown higher T_{esoph} in PFO+ men compared to PFO- men during short duration exercise bouts and during passive heating and cooling, there are several significant gaps in our current knowledge about the PFO and thermoregulation. Specifically, we have yet to identify whether similar shifts in temperature occur in women with a PFO. It is also unknown whether the elevation in T_{esoph} previous seen is location specific, or if these temperature differences are also seen in other anatomical locations. We also do not yet know whether differences exist between PFO+ and PFO- men and women in various

physiological measurements including Tsk, RHL, pre- and post-exercise inflammatory markers, heart rate, blood pressure, and perceptual data (rate of perceived exertion and thermal sensation) and whether differences in these measurements contribute to the previously seen differences in T_{esoph}. Finally, while the elevation in temperature has been reported, the implication of this higher temperature on performance and thermoregulation during real world scenarios (i.e., long duration exercise bouts, outdoor self-paced time trial performance, or cold-water exposure during SCUBA diving) has yet to be determined. This dissertation was designed to answer these questions.

CHAPTER III

METHODS

Informed Consent

The University of Oregon Institutional Review Board via Research Compliance Services and the Committee for Protection of Human Subjects formally approved the studies and protocols that constitute this dissertation (Chapters IV-VI). Prior to all studies, I met with all subjects and verbally explained the procedures and risks that were part of the study. Written consent was obtained prior to all study participation and all subjects were provided either a digital or paper copy of the consent form for their records.

Echocardiographic Screening

Patent Foramen Ovale Detection

All subjects who were enrolled in these studies underwent an echocardiographic screening for the presence of a patent foramen ovale. The first step of a PFO screening is to insert an intravenous catheter into a vein in the antecubital fossa. While seated in a phlebotomy chair, subjects had an IV inserted into a vein (see Subject Instrumentation below) and then a 3-lead echocardiogram was applied by the ultrasonographer. Subjects were then positioned in the left lateral decubitus position in a reclining chair and with their head resting on their left arm above their head. This position allowed for the clearest transthoracic ultrasound image of the heart because it allows the heart to move anteriorly and laterally against the subject's ribcage. During the screening, subjects were instructed to wear an oversized t-shirt or were provided a scrub top due to the requirement for the use of ultrasound jelly.

To determine if a subject had a PFO, a technique called transthoracic saline contrast echocardiography was used. To screen for a PFO, two 10 ml syringes were attached to a

stopcock. One syringe was filled with 3 ml of sterile saline, while the other contained 1 ml of air. The air and saline were agitated for ~10 sec, which created a suspension of microbubbles that were then injected into the catheter and subsequently the vein and the microbubbles were visualized in the right side of the heart. Microbubbles appearing on the left side of the heart within 3 cardiac cycles of initial appearance into the right side of the heart was evidence of the presence of PFO [23,88] whereas if bubbles appeared in the left side of the heart after 3 heartbeats, it was assumed that the bubbles traveled through intrapulmonary arteriovenous anastomoses (IPAVA).

We screened for the presence of a PFO under normal resting conditions and upon the release of a Valsalva maneuver. Utilization of a Valsalva release maneuver leads to a surge of blood coming back to the right side of the heart, which allows for increased right-to-left blood flow across the atria if a PFO exists. To perform the Valsalva, subjects were instructed to take in a small breath and then hold their breath for 10-15 seconds while "bearing down" with their upper airway closed. This increases intra-abdominal pressure while reducing blood flow from the inferior cava into the right atrium. Once the Valsalva maneuver was released, there was an increase of blood flow into the right atrium, causing right atrial pressure to exceed left atrial pressure. When performing a Valsalva maneuver to detect the presence of a PFO, the saline contrast was injected just prior to release of Valsalva. Therefore, 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 can be key in detecting a PFO, subjects practiced the Valsalva prior to completion of a Valsalva maneuver in at the same time as a bubble injection.

Pulmonary Function Testing

After echocardiographic screening, the next part of the screening process was to perform a series of pulmonary function tests. These tests were performed in all 3 Chapters (IV-VI) to ensure that all subjects had normal pulmonary function. All tests were conducted according to the 2019 American Thoracic Society/European Respiratory Society (ATS/ERS) standards [89] and were reported as both absolute and percent predicted values using Global Lung Initiative prediction equations.

Forced Vital Capacity

The forced vital capacity (FVC) maneuver is designed to determine the maximal volume of air exhaled from a maximal inspiration, performed with a maximally forced expiratory effort. During the test, the subjects wear a nose-clip and breathe into a low resistance mouthpiece, which is fitted over a MGC Diagnostics PreVent pneumotach connected to the Elite Series Plethysmograph. Subjects are seated in the upright position and are instructed to breathe quietly through the mouthpiece with their feet flat on the floor. After 3-4 normal tidal breaths and at the end of a normal tidal inspiration, subjects are instructed to exhale all the air in their lungs and continue until reaching residual volume (RV). After the subjects breathe out to RV, they are then instructed to take a "big breath in" to total lung capacity (TLC), at which point they maximally exhale or "blast out" all the air in their lungs to residual volume once again for ≥6 sec. The resulting flow-volume loop measured represents their forced vital capacity. In addition to FVC, FEV_1, or forced expiratory volume in 1 sec is also measured by this test. FEV_1 is the volume of air exhaled during the first second of their maximal expiration from a maximal inspiration. In healthy, young individuals the ratio of this volume to their FVC (i.e., FEV_1/FVC ratio) should be

~0.80. The FVC measure requires repeatability and requires 3 measures to meet the repeatability requirements (3 measurements within 150 mL of each other).

Slow Vital Capacity

The slow vital capacity (SVC) maneuver is performed after completion of the FVC maneuver and is very similar to FVC. Subjects are seated in the same position as during the FVC. At the beginning of the test, subjects are once again instructed to take at least four tidal breaths and at the end of the 4th tidal breath, subjects are asked to slowly inhale to total lung capacity (TLC). Once TLC is reached, subjects are asked to "sigh" all the air out of their lungs as opposed to "blast it out" in the FVC maneuver. Unlike in the FVC, the slower and more relaxed nature of the SVC maneuver prevents collapse of the small airways of the lungs which allows for a greater volume of air to be exhale. We performed two measures to have values within 5% of each other.

Subject Instrumentation

Intravenous Catheter

All studies in this dissertation involved the placement of an intravenous (IV) catheter in a peripheral vein in the antecubital fossa. An IV was placed both for PFO screening and on exercise testing days (Chapter IV) for pre-post exercise blood draws (unless subjects preferred to exercise without an IV in, and in this case, blood was drawn with separate vacutainer needle sticks pre- and post-exercise). First, a tourniquet was placed around the upper arm to increase the pressure in the vein below the tourniquet and make the vein more prominent. Upon identification of a vein, the site was cleaned using an alcohol swab. A 22G IV (ProtectIV Safety IV Catheter - radiopaque) was placed and connected to an extension set and one 3-way stopcock. Saline from a prefilled 10 mL syringe was used to ensure proper placement by withdrawing blood from the vein and then flushing saline into the vein to clear the dead space of the extension

set. If there was a blood draw occurring, a small amount of waste was drawn into a 3 mL syringe prior to the collection of the sample. Once the sample was collected, the catheter was flushed, and the extension set was pinched off and stopcock off position was turned to the off position.

Esophageal Temperature Probe

Esophageal temperature (T_{esoph}) was measured in Chapter IV because previous studies showing differences in core temperature saw these differences using esophageal temperature [8,11,12]. Esophageal temperature was measured via an esophageal probe, and it approximates the temperature of the right atrium. Esophageal probes were inserted to a depth of approximately ¼ of the subjects standing height [90]. Prior to insertion, subjects were given a syringe with 1 ml of 2% lidocaine gel to self-administer into the preferred nostril. After a few minutes, the esophageal probe was inserted into the nasal cavity and advanced several centimeters. Once the esophageal probe was visualized in the back of the throat, subjects were given a cup of water with a straw and instructed to take small continuous sips of water to keep the airways closed. As the subjects sipped the water, the esophageal probe was advanced until it was at the appropriate depth. The probe was then taped to the nose to ensure it would not move during exercise testing.

Telemetric Pill

Core temperature was also measured in Chapters IV-VI of this dissertation via a telemetric pill (Jonah core body temperature capsule; Mini Mitter, Bend, OR). In Chapter IV, the telemetric pill was swallowed ~10 hr prior to the start of testing and in Chapters V and VI, the telemetric pill was swallowed ~6 hr prior to the start of testing. The telemetric pill was used in addition to the esophageal probe in Chapter IV to address the question as to whether or not the PFO is a "physiological hot spot" (Filingeri & Chaseling, 2015). Previous studies have shown that PFO+

have elevated esophageal temperature, but some have questioned whether or not this difference in temperature would be seen in other anatomical locations (Filingeri & Chaseling, 2015). The telemetric pill was used in conjunction with the Vital Sense Monitor to read core temperature at the appropriate time points in each study. Compared to esophageal temperature, core temperature measured via telemetric pill is slower to respond and can be ~0.3-0.5 degrees °C warmer due to the location of the measurement [92]. However, due to the duration of the exercise bout in Chapter IV, both methods can provide reliable measures of body core temperature.

Peripheral Oxygen Saturation & Heart Rate

In Chapter IV, heart rate and peripheral arterial oxygen saturation were recorded with a forehead pulse oximeter (Nellcor N600x, Covidien, Dublin, Ireland). The pulse oximetry system was composed of an infrared light emitting diode (LED) and a red-light LED. In Chapter IV, a forehead pulse oximeter used for the estimated of arterial hemoglobin saturation and measurement of heart rate. The pulse oximeter was placed directly above the pupil. In Chapter V, the finger pulse oximeter was placed on the index finger and was used to measure heart rate. The wavelengths of the infrared (940 nm) and red (660 nm) lights are reflected differently, dependent on whether 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) and ~1% is Methemoglobin (which is formed when hemoglobin is oxidized to contain iron in the ferric state). While O_2 and CO are different molecules that bind to the protein hemoglobin, the pulse oximeter interprets both as oxygenated hemoglobin which causes pulse oximetry to overestimate the arterial oxygen saturation by ~2%.

Skin Thermistors

In Chapter IV, subjects were equipped with skin thermistors (MSR Data Logger, Switzerland) on 4 sites on the left side of the body (chest, deltoid, thigh, calf). Prior to attaching the skin thermistors, the site was cleaned with an alcohol swab and then the thermistor was attached in the correct place using medical tape. The 4 sites were used to calculate mean skin temperature using the following equation: Tsk = [0.3*(chest + deltoid)] +[0.2*(thigh + calf)] [93].

Venous Blood Draw and Analyses: Progesterone and Estradiol and FGF-21

An intravenous (IV) catheter was placed into an antecubital vein for blood draws. At both time points (pre- and post-dive), a 15mL venous blood sample was drawn from the IV catheter into serum separator tubes (SST). Prior to being centrifuged (1500 g for 10 min), all samples sat at room temperature for at least 30 minutes to fully clot. Serum was separated and frozen in a -80°C freezer until analyzed. For confirmation of menstrual cycle phase, in the normally cycling female subjects in chapter IV, serum samples were defrosted on ice and estradiol and progesterone concentrations were analyzed via enzyme-linked immunoassay (ELISA; ALPCO Diagnostics, Salem, NH, USA). For chapter VI, samples were defrosted on ice and serum fibroblast growth factor-21 (FGF-21) concentration was analyzed via enzyme-linked immunoassay (ELISA; R&D Systems, Minneapolis, MN, USA).

Statistical Analyses

In Chapter IV, the primary outcome variables of interest were Tc, Tesoph, Tsk, Tb, HR. These variables were measured at baseline and during min 60 of exercise and the effect of a PFO on

these thermoregulatory and cardiovascular variables were analyzed using a two-way repeated measures ANOVA. RPE and TS were analyzed using a Wilcox signed rank test.

In Chapter V, the effect of PFO on Tc and HR was analyzed using a two-way repeated measure ANOVA. For data analysis, subject data was segregated according to sex and analyzed separately in men and in women. Differences in all anthropometric and descriptive data as well as time trial time data were analyzed using Student's t-test.

In Chapter VI, the effect of PFO on Tc pre- and post-dive was analyzed using a two-way repeated measure ANOVA. Differences in all anthropometric and descriptive data between PFO- and PFO+ participants were analyzed using Student's t-test. The relationship between rate of change in core temperature (ΔTc/min) vs anthropometric factors including wetsuit thickness, body mass, body surface area, body mass index and body surface area: mass ratio were analyzed using linear regressions.

In all three chapters, statistical significance was accepted at $p < 0.05$. All data are presented as means $\pm$ SD. All analyses were completed in GraphPad Prism 9.1.2 (GraphPad Software, La Jolla, CA).

CHAPTER IV

INFLUENCE OF A PATENT FORAMEN OVALE ON THERMOREGULATORY & CARDIOVASCULAR RESPONSES DURING EXERCISE AT A CONTROLLED HEAT PRODUCTION

This chapter will be submitted to the *Journal of Physiology* with Aaron W. Betts, Dr. Kaitlyn G. DiMarco, Elizabeth M. Castillo, Dr. Christopher T. Minson, Dr. Nisha Charkoudian, and Dr. Andrew T. Lovering as co-authors. All experimental work was performed either by me independently or by A.W.B., K.G.D., and E.M.C. under my direction. The writing is entirely mine. All co-authors provided editorial assistance.

INTRODUCTION

A foramen ovale is present in normal fetal circulation and is a small tunnel that exists between the right and left atria of the heart [6]. During fetal development the fetal blood is oxygenated through gas exchange with the placenta and does not occur in the lungs. Because of this, most of the blood bypasses the pulmonary circulation, and flows directly from the right atrium to the left atrium, through the foramen ovale [83] and from the pulmonary artery to the aorta via the ductus arteriosus. The blood flow from the right atrium to the left atrium is facilitated by the elevated pulmonary vascular resistance due to hypoxic pulmonary vasoconstriction in the fetus. As a result of the increased pulmonary vascular resistance, right atrial pressure exceeds left atrial pressure, which forces the septum primum into the left atrium, causing the foramen ovale to stay open [82].

At birth when the baby starts breathing ambient air, pulmonary vascular resistance decreases, and left atrial pressure exceeds right atrial pressure. This elevation in left atrial pressure causes the septum primum to come into contact with the septum secundum and through

the process of fibrosis and the conversion of endothelial cells to mesenchymal cells, the foramen ovale closes [6]. However, in 25-35% of the population, the foramen ovale stays open after birth and is termed a patent foramen ovale [7]. Interestingly, the PFO has been associated with higher esophageal temperatures during exercise and in various environmental conditions in men (Tesoph; measured via esophageal probe) [11,12], however, the reasons or mechanisms for the elevated Tesoph remains elusive.

One important factor to consider when comparing core temperature (Tc) across different groups of individuals is whether there are different amounts of heat produced during the exercise bout. Changes in Tc during exercise are dependent on the balance of heat production (Hprod) and heat dissipation, body mass, and body composition [32]. If exercise workload is determined based on an absolute work rate or percentage of VO_{2max}, it could lead to variations in Tc changes due to differences in the amount of Hprod over the course of the exercise bout. If Hprod is not controlled for, then it may be that the differences in Tc measured during exercise are due to differences in Hprod, rather than differences in thermoregulation such as either differences in the thermoregulatory operating point (i.e. set point determined by the hypothalamus) or differences in the onset/effectiveness of thermoregulatory effector mechanisms (i.e. sweating, skin blood flow, etc.). However, if differences in the change in Tc are seen even with a controlled Hprod, it is possible that the physiological variable (i.e. presence/absence of a PFO) is affecting the mechanisms associated with the balance between Hprod and heat dissipation. Whether the elevated Tc & Tesoph previously reported in PFO+ men is due to differences in Hprod is unknown [11,12].

While these elevated Tesoph have been reported in men with a PFO, no study has yet to examine whether these differences in Tesoph & Tc are seen in women with a PFO at rest or

during exercise. However, when studying Tc in women, there are implications of the menstrual cycle to take into consideration [3,27,94]. It is well established that there are fluctuations in Tc over the course of the menstrual cycle and relative to the follicular phase, Tc is elevated by ~0.3-0.7°C in the luteal phase [27] and similar shifts in the regulation of Tc have been seen in the low hormone (placebo) vs high hormone phase of birth control/oral contraceptives [28]. The elevation in progesterone during the luteal phase (or high hormone phase) affects both the set points for thermoregulation as well as Tc for the onset of heat dissipation and conservation mechanisms [3,29], thus, there may be an interaction between the ovarian hormones and PFO influence on Tc and thermoregulatory mechanisms at rest and during exercise [26].

The purpose of this study was to determine whether 1) differences in Tesoph & Tc occur during 60 min of exercise at a controlled Hprod (7 w/kg), 2) differences in Tesoph & Tc also exist in PFO+ women and 3) a PFO is associated with the shifts in Tc measured across either the follicular and luteal phases of the menstrual cycle in naturally cycling women or across high hormone (HH) and low hormone (LH) phases in women on birth control.

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 procedures and provided written approval prior to participating in the study. All experimental procedures were conduction in accordance with the *Declaration of Helsinki 2013* except for registration of this study.

Participants

A total of 41 subjects participated in the study (21 M [10 PFO-, 11 PFO+] and 20 W [10 PFO-, 10 PFO+). Subjects were divided into groups based on their biological sex at birth and no subject

had/was undergoing any hormone replacement therapy treatment. All subjects were healthy, recreationally active, and free of cardiovascular and pulmonary disease. During the first visit to the lab, subjects signed an informed consent and filled out physical activity history questionnaire. Female subjects also completed a menstrual cycle history questionnaire that would be used to assist with scheduling of exercise testing.

PFO Screening

On visit 1 subjects underwent an ultrasound screening for the detection of a PFO. Ultrasound screening has been previously described in detail [95]. 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 visualize optimally all four chambers and interatrial septum and to 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 intravenous 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 at least one 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 [22,23,96,97]. Saline contrast injections were performed during normal breathing, as well as

immediately following the release of a Valsalva maneuver to elevate right atrial pressure transiently and create conditions optimal for the detection of an intracardiac right-to-left shunt. Effective Valsalva maneuvers were confirmed by a transient leftward shift of the interatrial septum. Valsalva maneuvers 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+, whereas 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 [97].

Lung Function

After ultrasound screenings, baseline pulmonary function tests were completed, which included measurements of forced vital capacity (FVC), forced expiratory volume in 1s (FEV1), and slow vital capacity (SVC). Measurements were made with a computerized spirometry system (UltimaPFX, MedGraphics, St Paul, MNV, USA) according to the 2019 American Thoracic Society/European Respiratory Society (ATS/ERS) standards [89]. Predicted values for each test was calculated according to the Global Lung Function Initiative [98].

Baseline Exercise Testing Day

Heat Production Test

The Hprod test has been used in previous studies [32,94] and allowed for the determination of a workload that would elicit a Hprod of 7 w/kg during the hour long cycling protocol. After a 5 min baseline, subjects cycled at 4 different workloads for 5 min each. Initial workload was based on body weight and increased by 20 W for each stage. Metabolic data including VO_2 and RER

were collected and analyzed continuously via a metabolic cart (UltimaPFX, MedGraphics, St Paul, MNV, USA).

Hprod during exercise was estimated using partitional calorimetry which takes into account the rate of metabolic energy expenditure and external work rate (Watts on the cycle ergometer) [72].

Metabolic energy expenditure (W) was calculated using the following formula:

$$M = VO_2 \cdot \frac{\left\{\left[\left(\frac{RER - 0.7}{0.3}\right) \cdot 21.13\right] + \left[\left(\frac{1.0 - RER}{0.3}\right) \cdot 19.62\right]\right\}}{60} \cdot 1000$$

Where VO_2 is the volume of oxygen consumed (L/min), RER is the respiratory exchange ratio, e_c is the energy equivalent for carbohydrate (21.13 kJ) and e_f is the energy equivalent for fats (19.62 kJ).

Hprod was then calculated as the difference between metabolic energy expenditure and work rate in watts:

$$\text{Hprod} = \text{M} - \text{Wk}$$

Peak Oxygen Consumption Test

Following the Hprod test and to characterize subject's maximal aerobic capacity (VO_2peak), subjects completed an incremental exercise test to exhaustion to measure VO_2peak on a cycle ergometer (UltimaPFX, MedGraphics, St Paul, MNV, USA & Lode Excalibur Sport, Lode, Groningen, The Netherlands). Subjects began cycling at 50 W for one minute and resistance was increased by 25 W every minute thereafter until volitional exhaustion. Heart rate (HR) and oxygen saturation were measured continuously throughout the test (Nellcor, Medtronic, Minneapolis, MN). It was assumed that the subject reached their VO_2peak if RER was ≥1.2 and/or HR was ≥ 85% of age-predicted HRmax (220-age).

60 Min Cycling Protocol

The day prior to the 60 min cycling protocol, subjects came to the lab to pick up their telemetric pill, sterile urine cup, and 1 L of water. Subjects were instructed to drink the water starting at ~1800 and finish before going to bed and to ingest the telemetric pill at ~2200 (~10 hr prior to the start of exercise testing). All subjects were asked to wear shorts and a t-shirt for testing and were asked to refrain from exercise and alcohol for 24 h before testing and from caffeine the morning of testing.

The next morning, subjects arrived at the lab at ~0700 with the sterile urine cup with first morning void. Urine specific gravity (USG) was measured to ensure subjects were adequately hydrated (Analog Regractometer; Atago, Tokyo, Japan) and a cut off of 1.025 was used to determine pre-exercise euhydration [99]. If a subject had a USG ≥1.025, they were given an additional 250 mL of water prior to starting exercise. A pregnancy test was conducted for all female volunteers. In addition to USG, nude body weight was measured prior to the start of testing. After a pre-exercise venous blood draw, subjects were equipped with skin thermistors on 4 sites on the left side of the body (chest, deltoid, thigh, calf) which were used to calculate mean skin temperature (Tsk; [93]. Finally, an esophageal probe (if tolerated), was inserted to ~ ¼ of the subjects standing height [90].

Prior to the start of exercise, subjects rested for 5 min on the bike for the collection of pre-exercise data. After 5 mins, subjects began pedaling at the workload that was estimated to elicit the 7 w/kg of Hprod as predicted by the baseline testing day. Workload was adjusted as needed during the first 10 minutes of exercise to ensure the correct Hprod and Hprod was periodically

checked during the remainder of the exercise bout. Subjects were instructed to maintain a pedaling cadence between 70 and 90 rpm.

During exercise, metabolic (VO_2 (L/min) and RER) and ventilation data (VE;L/min), Tc (Jonah core body temperature capsule; Mini Mitter, Bend, OR), Tesoph (CardioCap; Datex-Ohmeda, Louisville, CO), Tsk (MSR Data Logger, Switzerland), HR (Nellcor, Medtronic, Minneapolis, MN), oxygen saturation (Nellcor, Medtronic, Minneapolis, MN), blood pressure (Carescape V100 blood pressure monitor, GE Medical Systems Ltd., United Kingdom), and inspired air temperature, expired air temperature, inspired relative humidity, and expired relative humidity (Vaisala HMT337, Boston, MA) were measured and recorded. Perceptual data including thermal sensation and Rating of Perceived Exertion (RPE) were recorded throughout the 60 min bout using thermal sensation [100] and Borg RPE scales [101]. At the end of exercise, a post-exercise blood sample was taken, and nude weight was measured once more to estimate whole body sweat rate (WBSR).

Calculations

Respiratory Heat Loss

Respiratory heat loss (RHL) was calculated as described by Kenny & Jay [102] and as previously done in our lab [11]. To calculate RHL, inspired air temperature, expired air temperature, inspired relative humidity, and expired relative humidity (Vaisala HMT337, Boston, MA), and minute ventilation (VE) were recorded. Convective heat loss (Cres), evaporative heat loss (Eres), and total respiratory heat loss (Tres) were calculated.

Skin & Mean Body Temperature

Mean Tsk was calculated from the 4 sites and mean whole body temperature (Tb) was calculated using the following equations:

$$Tsk = [0.3*(chest + deltoid)]+ [0.2*(thigh + calf)] \ ^{93}$$

$$Tb = (0.80 * Tc) + (0.20*Tsk) \ ^{103}$$

Venous Blood Draws

Venous blood for sex hormone concentrations (estradiol and progesterone) was drawn via an intravenous catheter or vacutainer stick (depending on subjects' preference) into serum separator tubes (gold or tiger top tubes pre-filled with a polyester-based gel with silica particles that act as a clot activator; Becton-Dickinson, Franklin Lakes, NJ). Blood was allowed to sit for at least 30 minutes to fully clot. After, the blood was spun at 1500g for 10 minutes and serum frozen at -80°C until analysis.

Determination of Menstrual Cycle Phase/Hormone Status

To control for the influence of menstrual cycle phase (MCP) on Tesoph/Tc in female subjects, female subjects completed the 60 min cycling protocol in both menstrual cycle phases. During visit 1, female subjects completed a menstrual cycle history questionnaire. For women naturally cycling, they were tested during the follicular phase (days 2-6) and during the luteal phase (days 19-21). Women utilizing oral contraceptives were tested at the end of week 3 (high hormone) and end of week 4 (low hormone) of pills. Women utilizing a hormonal IUD (LARC; continuous release of hormone) were tested one time at any point during the month. For data analysis, women were broken into two groups based on hormones status: low hormone (LH) or high hormone (LH). The LH group included women in the follicular phase of the menstrual cycle or

those in the placebo week of oral contraceptives. The HH group included women in the luteal phase of the menstrual cycle, and either those in week 3 of oral contraceptives, or those utilizing hormonal IUDs. To confirm MCP in those not utilizing any type of hormonal birth control, venous blood was drawn for analysis of serum estradiol and progesterone during the pre-exercise blood draw. Menstrual cycle phase was verified utilizing estradiol and progesterone enzyme-linked immunoassays (ALPCO Diagnostics, Salem, NH).

Statistical Analyses

All statistical analyses were conducted in GraphPad Prism 9.4.1. For data analysis, subject data was segregated according to sex and analyzed separately in men and in women. Tc, Tesoph, Tsk, mean Tb, RHL and HR were all analyzed via a two-way repeated measures ANOVA (PFO status X time). A nonparametric Friedman test was used to compare RPE and thermal sensation among conditions. All data are presented as means ± SD besides RPE and thermal sensation data, which are presented as medians and ranges.

As a sub-analysis, we chose to examine whether thermoregulatory and cardiovascular responses to 60 min of exercise at 7 w/kg of Hprod varied over the course of the menstrual cycle/oral contraceptives. This sub-analysis included 12 women who were either naturally cycling (no birth control) or were utilizing oral contraceptives. Those utilizing LARCs (n= 6) were not included in this analysis.

RESULTS

Anthropometric, lung function, and aerobic fitness data are presented in **Table 1**. There were no differences in any of these variables between groups of the same sex.

Table 1. Anthropometric and lung function data. Values are mean ± SD for n subjects.

	PFO- M (n = 10)	**PFO+ M (n = 11)**	**PFO- W (n = 10)**	**PFO+ W (n = 10)**
Age (yrs.)	23 ± 6	26 ± 5	25 ± 5	26 ± 8
Height (cm)	180.3 ± 4.6	179.6 ± 4.9	166.7 ± 5.3	166.7 ± 6.6
Weight (kg)	77.9 ± 6.1	77.7 ± 9.2	64.7 ± 10.3	64.5 ± 8.4
BSA (m^2)	2.0 ± 0.1	2.0 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
BMI	24.0 ± 1.6	24.1 ± 2.9	23.2 ± 3.0	23.5 ± 2.2
FVC (L)	5.7 ± 0.6	5.9 ± 0.9	4.2 ± 0.5	4.2 ± 0.6
FVC (% pred.)	109.9 ± 12.5	113.7 ± 11.3	106.3 ± 15.9	111.7 ± 13.5
FEV1 (L)	4.6 ± 0.5	4.8 ± 0.9	3.4 ± 0.4	3.4 ± 0.5
FEV1 (% pred.)	103.6 ± 13.6	108.7 ± 15.2	113.8 ± 17.6	107.2 ± 14.2
FEV1/FVC	0.82 ± 0.9	0.81 ± 0.8	0.80 ± 0.1	0.82 ± 0.1
FEV1/FVC (% pred.)	93.3 ± 4.6	94.9 ± 8.7	99.2 ± 22.8	94.2 ± 4.8
VO_{2peak} (ml/kg/min)	39.1 ± 5.5	40.1 ± 5.4	34.3 ± 7.5	35.5 ± 4.4
Peak power output (W)	313 ± 45	336 ± 39	233 ± 39	225 ± 31

The number of subjects for each hormone status and the estradiol and progesterone concentrations are outlined in **Table 2**. Estradiol and progesterone were significantly higher in the luteal phase vs follicular phase in the naturally cycling women.

Table 2. PFO+ and - subject numbers for each birth control group and each sex hormone concentration. *denotes $p < 0.05$ vs follicular phase.

	PFO- W	**PFO+ W**
No birth control	**6**	**2**
Oral Contraceptives	**2**	**4**
IUD	**2**	**4**
	Estradiol (ng/ml)	**Progesterone (pg/ml)**
Follicular (n = 8)	91.9 ± 40.6	1.2 ± 0.8
Luteal (n = 8)	166.0 ± 90.0*	7.9 ± 4.5*

Average Hprod, workload, $\%VO_{2peak}$, % peak power output, USG, and environmental conditions are presented in **Table 3.** There were no differences between any of the groups (of the same sex and hormone status) in average Hprod, external workload, or $\%VO_{2peak}$. All subjects arrived at the lab adequately hydrated (all had USG < 1.025). There were no differences in ambient temperature or humidity between PFO+ and PFO- men or women in the HH phase ($p > 0.05$). However, mean ambient temperature was higher in the PFO- LH trial and ambient humidity was elevated in the PFO+ LH trial ($p < 0.05$).

Table 3. Exercise trial data. Values are mean ± SD for subjects.

			High Hormone		Low Hormone	
	PFO- M (n = 10)	**PFO+ M (n = 11)**	**PFO- W (n = 10)**	**PFO+ W (n = 10)**	**PFO- W (n = 7)**	**PFO+ W (n = 5)**
Ambient Temp	22.2 ± 0.6	22.3 ± 0.5	21.9 ± 1.0	22.3 ± 0.6	22.5 ± 0.3*	21.7 ± 0.5
Rel. Humidity	40.5 ± 12.8	37.7 ± 9.9	37.9 ± 7.2	41.6 ± 10.1	33.6 ± 6.5	44.3 ± 7.9*
USG	1.016 ± 0.006	1.017 ± 0.005	1.016 ± 0.005	1.016 ± 0.005	1.012 ± 0.004	1.016 ± 0.005
Workload (W)	115 ± 17	113 ± 20	95 ± 20	90 ± 18	89 ± 17	95 ± 23
Hprod (W/kg)	7.1 ± 0.3	7.1 ± 0.2	7.0 ± 0.2	7.1 ± 0.3	6.9 ± 0.2	7.0 ± 0.1
%VO_{2peak}	64.7 ± 9.8	63.1 ± 9.4	74.3 ± 14.2	70.5 ± 9.9	73.3 ± 14.7	68.4 ± 9.5
% Peak Power Output	37.4 ± 7.3	33.6 ± 5.5	41.4 ± 8.2	40.3 ± 8.4	40.4 ± 9.0	39.4 ± 12.0
ΔBW (kg)	-0.6 ± 0.2	-0.6 ± 0.2	-0.5 ± 0.3	-0.3 ± 0.1	-0.4 ± 0.2	-0.2 ± 0.1
% ΔBW	-0.7 ± 0.2	-0.7 ± 0.2	-0.8 ± 0.3	-0.5 ± 0.2	-0.6 ± 0.3	-0.3 ± 0.2
ΔTc (°C)	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.3	0.8 ± 0.4	0.8 ± 0.2	0.9 ± 0.5

PFO+ vs PFO- Men

Tc was significantly higher in PFO- men vs PFO+ men at rest and over the course of the exercise bout (**Fig 1A**, main effect, $p < 0.05$). In both groups and as expected, there was a main effect of exercise time on Tc ($p < 0.05$). There was no difference in Tesoph between PFO+ and PFO- men (**Fig 1B,** $p > 0.05$), however there was a main effect of time on Tesoph ($p < 0.05$). There was no effect of PFO on Tsk or Tb (Table $p > 0.05$) but there was a main effect of exercise time on Tsk and Tb ($p < 0.05$). There was no influence of PFO on HR (**Table 4**, $p > 0.05$), but there was a main effect of exercise time on HR ($p < 0.05$). There was also no difference in absolute or percent body weight loss over the course of the hour between PFO+ and PFO- men, suggesting that sweat loss was not different between the groups (**Table 3**, $p < 0.05$).

In men, there was no influence of PFO on RHL ((**Table 5**), $p > 0.05$), but there was a main effect of exercise time on all components of RHL (Cres, Eres, and Tres) which were greater than pre-exercise at all 3 time points during exercise (min 0-10, min 25-30, min 55-60, $p < 0.05$). Additionally, RHL was higher from min 55-60 vs min 0-10 ($p < 0.05$) due to slight increases in VE.

PFO+ vs PFO- Women

There was no effect of PFO on Tc (**Fig. 1C/1E**), Tesoph (**Fig. 1D/1F**), Tsk (**Table 4**); Tb (**Table 4**), HR (**Table 4**), or components of RHL (**Table 5**) at rest or during exercise ($p > 0.05$ for all) in women in the HH group or women in the LH group. There was a main effect of time for Tc, Tesoph, Tb, and HR where values at min 60 were greater than pre-exercise ($p < 0.05$ for all). There was a main effect of exercise time on all components of RHL (Cres, Eres, and Tres) which

were greater than pre-exercise at all 3 time points during exercise (min 0-10, min 25-30, min 55-60, $p < 0.05$) for both HH and LH groups. In the HH group, RHL (Eres and Tres) at min 25-30 was higher than min 0-10 ($p < 0.05$).

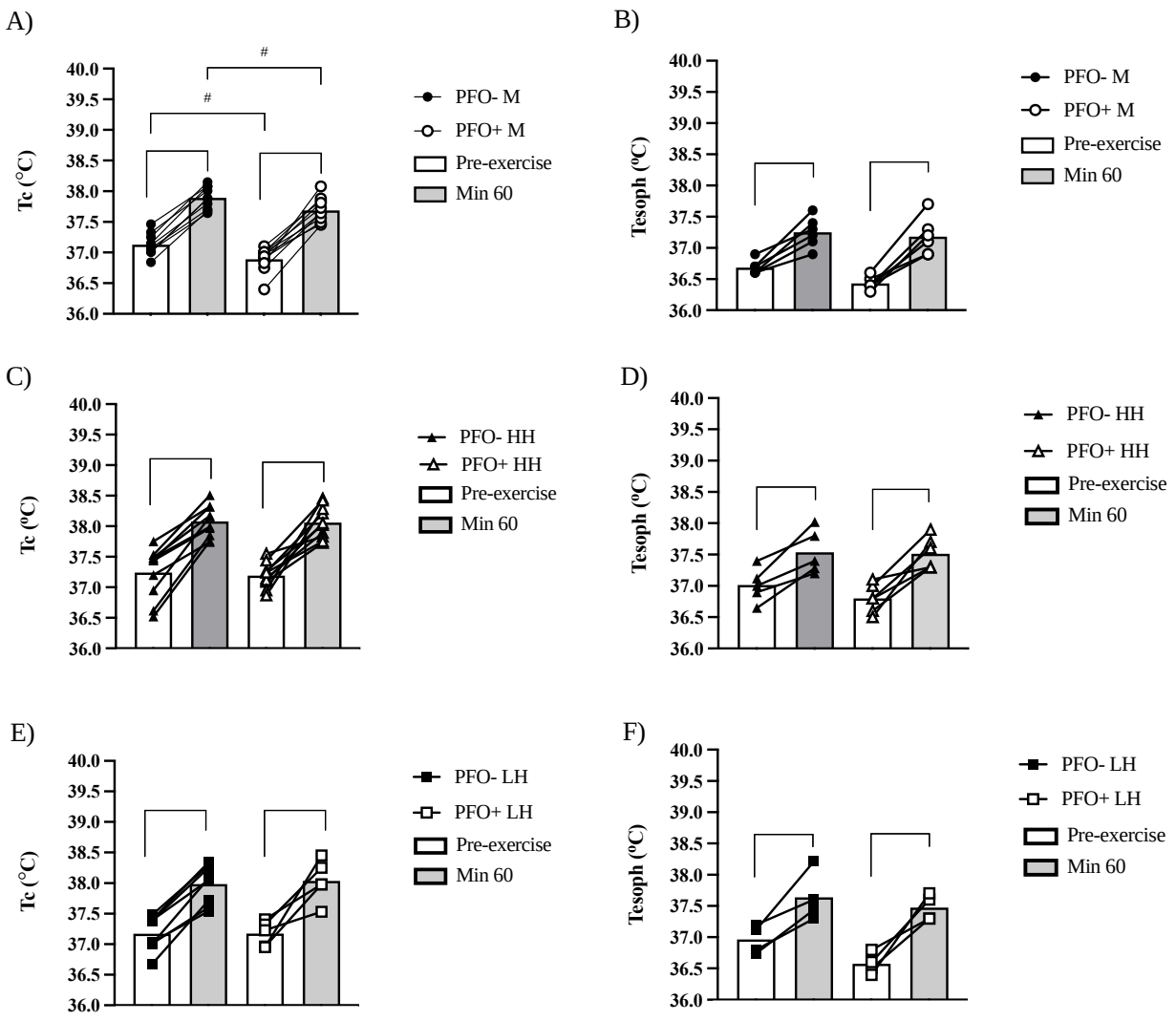


Figure 1. Core temperature (Tc) and esophageal temperature (Tesoph) at pre-exercise and at min 60. **Panel A**: Tc in PFO+ vs PFO- men, **Panel B**: Tesoph in PFO+ vs PFO- men, **Panel C**: Tc in PFO+ vs PFO- women in the HH group, **Panel D**: Tesoph in PFO+ vs PFO- women in the HH group, **Panel E:** Tc in PFO+ vs PFO- women in the LH group, **Panel F**: Tesoph in PFO+ vs PFO- women in the LH group. * denotes main effect of time, $p < 0.05$, # denotes main effect of PFO, $p < 0.05$. Not all subjects could tolerate the esophageal probe, so the following subject numbers were included for the analysis: men n = 12 (6 PFO+, 6 PFO-); HH group n = 11 (5 PFO-, 6 PFO+); LH group n = 8 (4 PFO-, 4 PFO+).

Table 4. Cardiovascular and thermoregulatory variables pre-exercise and min 60. *denotes main effect of exercise, $p < 0.05$.

				High Hormone		Low Hormone	
		PFO- M (n = 10)	**PFO+ M (n = 11)**	**PFO- W (n = 10)**	**PFO+ W (n = 10)**	**PFO- W (n = 7)**	**PFO+ W (n = 5)**
HR (bpm)	Pre-Exercise	64 ± 9	69 ± 8	76 ± 10	78 ± 9	72 ± 12	68 ± 8
	Min 60	131 ± 18*	130 ± 12*	158 ± 16*	146 ± 25*	156 ± 29*	142 ± 26*
Tsk (°C)	Pre-Exercise	30.6 ± 0.8	30.9 ± 0.9	30.7 ± 1.1	29.9 ± 0.8	30.3 ± 0.7	29.9 ± 1.2
	Min 60	30.9 ± 1.3	31.1 ± 1.0	31.0 ± 1.2	30.1 ± 1.3	30.3 ± 0.8	30.2 ± 1.0
Tb (°C)	Pre-Exercise	35.8 ± 0.3	35.7 ± 0.3	35.9 ± 0.4	35.8 ± 0.2	35.8 ± 0.2	35.7 ± 0.3
	Min 60	36.5 ± 0.3*	36.4 ± 0.2*	36.7 ± 0.7*	36.4 ± 0.3*	36.4 ± 0.3*	36.5 ± 0.4*
RPE	Pre-Exercise	6 (6-7)	6 (6-9)	6 (6-6)	6 (6-6)	6 (6-6)	6 (6-6)
	Min 60	14 (12.5-18)	13 (11-17)	14 (11-17)	15 (11-19)	14 (11-7)	14 (12-17)
Thermal Sensation	Pre-Exercise	4 (3-5)	3.5 (1.5-4)	4 (3.5-4.5)	4 (3.5-5)	4 (3-5)	3.5 (2-4)
	Min 60	5 (4.5-6.5)	5.5 (4-7)	6 (4.5-7)	6 (5-7)	6 (4.5-6.5)	5.5 (5-7)

Table 5. RHL and VE pre-exercise and during exercise in men and women. Values are mean ± SD for n subjects. * denotes $p < 0.05$ vs pre-exercise, + denotes $p < 0.05$ vs min 0-10.

				High Hormone		Low Hormone	
		PFO- M (n = 10)	**PFO+ M (n = 11)**	**PFO- W (n = 10)**	**PFO+ W (n = 10)**	**PFO- W (n = 7)**	**PFO+ W (n = 5)**
Cres (W)	Pre-Ex.	0.78 ± 0.35	0.83 ± 0.32	0.66 ± 0.21	0.88 ± 0.35	0.43 ± 0.14	0.80 ± 0.36
	Min 0-10	4.76 ± 0.99*	4.61 ± 0.87*	4.70 ± 1.53*	4.55 ± 1.17*	3.80 ± 1.02*	3.96 ± 2.16*
	Min 25-30	4.96 ± 1.07*	4.94 ± 0.75*	5.05 ± 1.75*	4.89 ± 1.51*	4.17 ± 1.42*	5.29 ± 1.56*
	Min 55-60	5.12 ± 1.19*+	5.18 ± 0.82*+	5.07 ± 2.02*	4.79 ± 1.63*	4.23 ± 1.50*	5.01 ± 2.16*
Eres (W)	Pre-Ex.	1.58 ± 0.71	1.69 ± 0.66	1.39 ± 0.44	1.79 ± 0.72	0.87 ± 0.28	1.64 ± 0.72
	Min 0-10	9.71 ± 2.00*	9.40 ± 1.75*	9.23 ± 3.43*	8.35 ± 3.09*	7.76 ± 2.09*	7.12 ± 4.97*
	Min 25-30	10.12 ± 2.17*	10.08 ± 1.52*	10.07 ± 3.84*#	9.12 ± 3.96*#	8.50 ± 2.91*	8.79 ± 4.85*
	Min 55-60	10.43 ± 2.43*+	10.57 ± 1.66*+	10.08 ± 4.40*	8.95 ± 4.13*	8.63 ± 3.05*	8.66 ± 5.65*
Tres (W)	Pre-Ex.	2.36 ± 1.07	2.52 ± 0.98	2.05 ± 0.64	2.67 ± 1.07	1.30 ± 0.41	2.45 ± 1.08
	Min 0-10	14.47 ± 2.99*	14.00 ± 2.62*	13.92 ± 4.73*	12.90 ± 4.05*	11.56 ± 3.11*	11.08 ± 7.03*
	Min 25-30	15.08 ± 3.24*	15.02 ± 2.28*	15.12 ± 5.47*	14.01 ± 5.36*	12.66 ± 4.34*	14.16 ± 6.27*
	Min 55-60	15.55 ± 3.62*+	15.75 ± 2.49*+	15.15 ± 6.35*	13.74 ± 5.67*	12.86 ± 4.55*	13.67 ± 7.71*
	Pre-Ex.	11.24 ± 2.81	12.11 ± 2.48	10.42 ± 1.88	12.36 ± 2.79	9.11 ± 1.39	11.63 ± 2.06
	Min 0-10	39.35 ± 4.87*	40.14 ± 7.65*	39.58 ± 8.57*	38.79 ± 5.76*	36.10 ± 6.34*	39.03 ± 5.63*

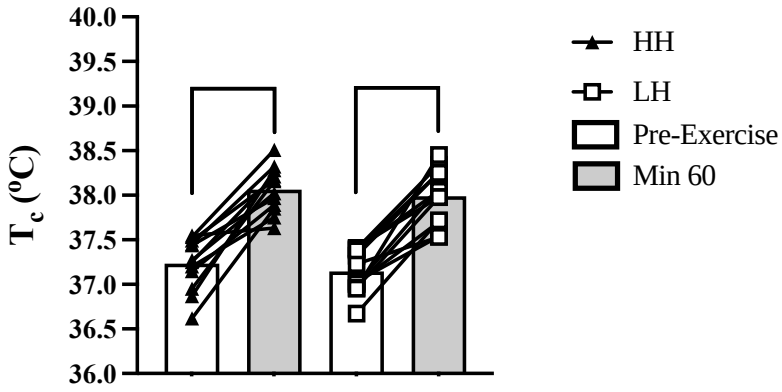
VE (L/min)	Min 25-30	43.70 ± 3.70*+	46.02 ± 7.22*+	43.64 ± 9.48*+	43.51 ± 8.41*+	40.63 ± 10.04*+	42.98 ± 7.70*+
	Min 55-60	44.70 ± 4.88*+	46.54 ± 7.43*+	43.50 ± 9.90*+	44.08 ± 9.23*+	40.74 ± 8.79*+	44.54 ± 9.00*+

Influence of Hormone Status

There were no differences in the cardiovascular or thermoregulatory responses to 60 min of exercise at 7 w/kg of Hprod between in HH phase and LH phase in women naturally cycling or utilizing oral contraceptives ($p > 0.05$). Tc and Tesoph are presented in **Figure 2** and HR, Tsk, Tb, RPE, and TS are presented in **Table 6**.

Figure 2. Core temperature (Tc; panel A) and esophageal temperature (Tesoph; panel B) pre-exercise and at min 60 in the high hormone and low hormone phases. * denotes main effect of time, $p < 0.05$

A)



B)

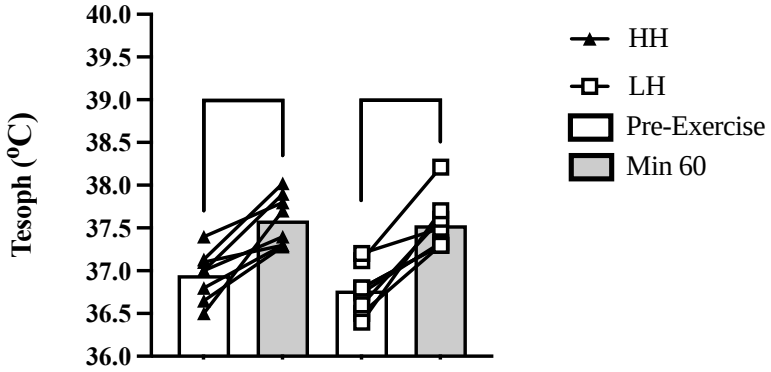


Table 6. Cardiovascular and thermoregulatory variables pre-exercise and at min 60. *denotes main effect of exercise, $p < 0.05$.

		Low Hormone Phase	**High Hormone Phase**
HR (bpm)	Pre-Exercise	71 ± 10	74 ± 10
	Min 60	149 ± 28*	148 ± 26*
Tsk (°C)	Pre-Exercise	30.1 ± 0.9	30.3 ± 1.2
	Min 60	30.3 ± 0.9	30.6 ± 1.0
Tb (°C)	Pre-Exercise	35.7 ± 0.3	35.8 ± 0.4
	Min 60	36.4 ± 0.3*	36.6 ± 0.3*
RPE	Pre-Exercise	6 (6-6)	6 (6-6)
	Min 60	14 (11-19)	14 (12-17)
Thermal Sensation	Pre-Exercise	4 (3.5-5)	4 (2-4)
	Min 60	6 (5-7)	6 (4-7)

DISCUSSION

Our study was the first to study the influence of a PFO on thermoregulatory and cardiovascular responses to 60 min of exercise at a controlled Hprod in men and women. While previous studies had found that the presence of a PFO was associated with higher Tesoph in men at rest, during exercise, and during passive heating and cooling [8,11,12], the mechanisms responsible for this elevation in temperature were largely unknown. Previous investigations had not controlled for Hprod during exercise, therefore we aimed to determine whether the varying amounts of Hprod contributed to these differences in Tesoph reported. Additionally, these previous studies did not include women, therefore, whether similar differences in Tc & Tesoph exist between PFO+ and PFO- women was unknown. Surprisingly, and contrary to previous results, we found that men without a PFO have a significantly elevated Tc compared to men with a PFO and no difference in Tesoph between the two groups. We also found that there was no difference in either Tc or Tesoph between women with and without a PFO, regardless of hormone status.

While the two previous investigations reported elevated Tesoph in PFO+ men [11,12], the first study reporting differences in temperature between PFO+ and PFO- subjects did not report baseline Tesoph differences, but reported that those with a PFO had higher Tesoph at maximal exercise, which contributed to the lower oxygen saturation (despite same arterial PO_2) in those with a PFO [8]. While our results agree with the results from the initial investigation, the work done by Lovering et al., (2011) did not control for time of day, hydration status, menstrual cycle phase, etc., in the participants, therefore whether the difference in temperature was due to differences in metabolic Hprod or heat dissipation mechanisms is unknown.

The previous studies investigating the influence of a PFO on Tc in men utilized esophageal probes [11,12]. The idea of a PFO being a potential "physiological hot spot" had been

suggested [91] and while Tesoph is the gold standard for rapid and dynamic changes during exercise [91,104], it is unknown if these different in temperature would exist in other anatomical locations (i.e. gastrointestinal) in those with and without a PFO. To address this question, a goal of this current investigation was to study core temperature responses in more than one anatomical location using both esophageal probes and telemetric pills. In our study, since not all subjects were able to tolerate the esophageal probe, we were not able to collect Tesoph in all subjects. In the subset of men, 6 PFO+ and 6 PFO- were able to tolerate the probe during exercise. Interestingly, there was no difference in Tesoph between PFO+ and PFO- men ($p < 0.05$). It is important to note that despite both not being statistically significant, pre-exercise Tc and Tesoph were ~0.2 °C higher in PFO- men than PFO+ men.

It is possible that the temperature differences seen between groups of men is the result of variability in the methods used for measurement of Tc. Both Tesoph and Tc (telemetric pill) measurements will be affected by the location of the device. While Tesoph is the gold standard for temperature measurement, it is very sensitive to location and can be affected by factors such as ventilation [91,105]. On the other hand, telemetric pill measurements can be affected by the location of the pill along the gastrointestinal tract and gut motility [104,106,107]. While we attempted to control for this issue by placing the esophageal probes to a widely accepted depth (~1/4 standing height, Mekjavic & Rempel, 1990) and instructed all subjects to swallow the telemetric pill 10 hr prior to the start of exercise [107] it is possible that variability in temperature measurements between our study and previous investigations could be due to the anatomical location of the sensors or the individual anatomy of each subject.

One factor that we did not control for in this study (or previous studies) is the influence of *individual* circadian rhythms on Tc. To control for the influence of circadian rhythm on core

temperature measurements, most investigations have experimental testing sessions occurring at the same time of day (i.e. between 0800 and 1000). While we tried to control for the influence of circadian rhythm on pre-exercise Tc measurements by having all subjects arrive to the lab at ~0700 and starting exercise by ~0800, it is possible that individual variability in wake time and sleeping patterns contributed to the differences in Tc between groups. In most humans, during a normal sleep cycle, the nadir (time of lowest core temperature) usually occurs between 4-6 am, after which core temperature continues to increase throughout the day [68,69]. It is possible that the differences in Tc across studies are driven by circadian rhythm differences and not the PFO, *per se*. To gain more insight on this possible mechanism, future studies comparing pre-exercise Tc among different individuals should control for, or at minimum record, normal wake times in their subjects.

Our investigation was the first to measure Tsk and calculate mean Tb in those with and without a PFO. While we found that men without a PFO had higher Tc compared to men with a PFO, there was no difference in mean Tb between the groups. The slightly higher Tsk (albeit not statistically significant) contributed the fact that mean Tb was the same between PFO+ and PFO- men despite differences in Tc. Since previous investigations did not measure Tsk, it is unknown, but possible that the mean Tb between the men in the previous studies was the same and the differences in Tc were offset by differences in Tsk.

We saw no influence of PFO on RHL. Davis et al. (2017) demonstrated that PFO+ men have a blunted ventilatory response to heat stress evidenced by a higher Tesoph threshold for thermal hyperpnea and a higher end tidal CO_2 at end of hot water immersion. In addition, another factor that could contribute to differences in RHL the fact that the shunted blood traveling across the PFO bypasses the lungs and therefore does not participate in respiratory system cooling.

However, under the environmental conditions in this investigation, an increase in VE by ~4.5 L/min would increase total RHL by ~ 1 W, therefore, due to the difference in VE needed to influence RHL, it should not be surprising that we did not see PFO driven differences. As expected, there was a significant increase in RHL due to the increase in ventilation during exercise compared to pre-exercise.

We found no influence of the presence of a PFO on Tc or any other measure of thermoregulation (Tsk, Tb, HR, RHL) during 60 min of exercise at a Hprod of 7 w/kg in women. Surprisingly, we did not see a significant shift in Tc in women between the HH and LH phases as we would have expected. The influence of menstrual cycle phase and oral contraceptives on Tc shifts has been extensively studied and reported with higher Tc by 0.3-0.5 °C measured in the luteal/high hormone phase vs follicular/placebo phase [3,27,28,108,109]. While many studies report higher Tc, we are not the only study to report insignificant temperature shifts [94]. One possible reason for not seeing temperature shifts is the timing of the core temperature measurements. In our investigation we recorded pre-exercise Tc after the subjects had been instrumented and were seated on the cycle ergometer. It is possible that if we had taken basal body temperature measurements or had subjects rest (supine) for a longer period of time prior to the baseline measurements we would have seen a temperature shift [28]. While we attempted to control for all variables influencing Tc, it is possible that pre-exercise measurements were influenced by effects of unknown illnesses, sleep patterns, or diet and masked the influence of progesterone on pre-exercise Tc [110,111].

While an increase in Tc is commonly reported in the luteal phase of the menstrual cycle (biphasic temperature pattern), it is also important to note that monophasic patterns of basal body temperature are common and not all women experience the shift in temperature during ovulation

[111,112]. Additionally, while no subjects in this study had an anovulatory cycle (confirmed via measurements of estradiol and progesterone), it may be that we missed the progesterone peak and that there is a dose-dependent relationship between progesterone and Tc [27,94].

The lack of differences in RHL across the menstrual cycle is not a surprising finding. While progesterone has been shown to be a ventilatory stimulant and increase baseline VE [113] it has been previously shown that submaximal exercise VE is not influenced by menstrual cycle phase, despite significant increases in progesterone. (Beidleman *et al.*, 1999; Macnutt *et al.*, 2012). Unlike previous studies, we did not find a significant difference in pre-exercise VE between the high hormone (10.8 ± 2.1 L/min) and low hormone (10.2 ± 2.1 L/min) phases. The reasons for this due to the fact that pre-exercise measurements were taken on a cycle ergometer immediately prior to the start of exercise and the anticipatory effect of exercise/central command may have influenced baseline VE values [114], overriding the influence of sex hormones on resting ventilation. However, as previously mentioned, an increase in VE by ~4.5 L/min would increase total RHL by ~ 1 W. In our investigation and others, the difference in pre-exercise VE ranges from 0.6 L/min to 1.5 L/min [115,116], which is not enough of an increase in VE to contribute to a meaningful difference in RHL.

We also chose to include women (n = 6) utilizing hormonal intrauterine devices (IUD) in our study. While the influence of the menstrual cycle and oral contraceptives on thermoregulation have been studied more extensively, data are lacking on how an IUD and a constant release of a progestin only contraceptive may influence these responses [29,117]. However, recent data have shown that the prevalence of women utilizing IUDs is increasing and the number actually exceeds those using oral contraceptives and use is more often considered and utilized especially in populations such as the military for logistical reasons [29,118]. Since LARCs

frequently lead to amenorrhea [119] (and did in all of our subjects), we decided to test women with an IUD one time during the month. Similar to recently published results on women exercising in a warm environment [120], the small subset of women with IUDs experienced similar changes in Tc (0.7 °C) compared to women using OCs or no birth control (n = 14) in the HH or LH phase (0.8°C for both) during exercise in a thermoneutral environment (22 °C, 39% rh) further demonstrating that women with an IUD do not have impaired ability to thermoregulate compared to naturally cycling women or women using oral contraceptives.

Contrary to our initial hypothesis, we found that men without a PFO have a higher Tc but no difference in Tesoph compared to PFO+ men. This was the first investigation to study the influence of a PFO on Tc/Tesoph in women and we found no influence of PFO on any thermoregulatory variable at rest or during 60 min of exercise at 7 W/kg. Our results indicate that those (men and women) with a PFO do not have an impaired ability to thermoregulate under the conditions studied. Interestingly, regardless of PFO status, sex or hormone status, the average increase in Tc was ~0.8-0.9 °C (**Table 3**, $p > 0.05$). This is supported by the fact that there is no difference in estimated sweat losses (**Table 3**, $p > 0.05$) or RHL between groups (**Table 5**, $p > 0.05$). While numerous factors may contribute to the variability in core temperature at rest, the fact that there is no difference in the change in Tc supports the fact that those with a PFO may not be at an increased risk of a heat related illness. Future studies should investigate other factors that would contribute to differences in pre-exercise core temperature to try to understand the mechanisms leading to these discrepancies reported in both Tc/Tesoph across several studies.

CHAPTER V

SELF-PACED TIME TRIAL EXERCISE AND CORE TEMPERATURE RESPONSES IN THOSE WITH AND WITHOUT A PATENT FORAMEN OVALE

This chapter was submitted to Medicine & Science in Sports & Exercise (MSSE) with Aaron W. Betts, Dr. Kaitlyn G. DiMarco, Dr. Tyler S. Kelly, Dr. Nisha Charkoudian, and Dr. Andrew T. Lovering as co-authors. All experimental work was performed either by me independently or by A.W.B., K.G.D., and T.S.K. under my direction. The writing is entirely mine. All co-authors provided editorial assistance.

INTRODUCTION

Physiological variables including skin temperature, heart rate (HR), and ventilation have all been shown to alter the perception of effort during a given exercise task [121,122]. An additional factor that has been shown to influence self-paced exercise performance is the narrowing of the core-to-skin thermal gradient that is seen during exercise, specifically during exercise performed in the heat. During exercise, individuals may titrate their work rate based on an ideal HR, core temperature or skin temperature[79,80,122]. As the exercise intensity becomes too high to maintain these physiological variables within a narrow range, subjects will decrease their exercise intensity, leading to a compromised performance and in the case of running, slower times. Therefore, any factor that can alter these physiological responses to exercise (i.e., HR, body temperature, ventilation, etc.), would likely lead to alterations in self-paced exercise performance.

It has been previously shown that men with a patent foramen ovale (PFO+) have elevated Tc (measured via esophageal probe) at rest and during exercise compared to men without a PFO (PFO-) [12]. Similar differences in Tc between those with and without a PFO have been seen

during bouts of passive heating and cooling [11]. The mechanism for this shift to a higher Tc both at rest and during thermal stressors in PFO+ men is unknown but may be related to differences in thermoregulatory mechanisms (i.e., decreased skin blood flow or sweating) or differences in circulating inflammatory cytokines that may alter body temperatures due to their pyrogenic action, among other possibilities. However, it has yet to be established whether these higher Tc have implications for either the health or exercise performance of those with a PFO.

PFO+ men have also been shown to have higher resting HR compared to PFO- men, but no differences have been shown during exercise [12]. The higher HR at rest may be partially explained by the influence of higher body temperatures on both the sinoatrial (SA) node and the activation of the sympathetic nervous system. While no differences in HR were seen during exercise, the exercise stages were only ~2.5 minutes long and at various relative exercise intensities (25%, 50%, 75% and 90% of VO_2peak) [12]. Whether or not there would be differences in HR when individuals are able to regulate their own exercise intensity has yet to be established.

Although the influence of a PFO on cardiovascular and thermoregulatory responses have been studied in men during short duration exercise (i.e. VO_2peak test and 10 min graded exercise protocol) [12] and during passive heating/cooling [11], it is unknown whether or not these Tc and HR differences would be seen during a self-paced exercise bout in a field setting. Additionally, no studies investigating the influence of a PFO on Tc and HR at rest or during exercise have included women, therefore, it is unknown if women with a PFO have higher Tc and higher HR compared to women without a PFO and if this would affect self-paced exercise performance. If PFO+ subjects have higher Tc and HR at rest, it may lead to elevations in Tc and HR during a self-paced exercise bout (i.e. increased physiological strain) and cause these individuals to decrease their exercise intensity (i.e. running speed), ultimately compromising exercise

performance. Since ~25-35% of the population has a PFO [7,20,23] the findings of this study have important implications for understanding factors that may influence Tc and HR and therefore, self-paced exercise performance. The purpose of our study was to determine whether individuals with a PFO have elevated Tc and HR pre-exercise and post-self-paced, outdoor, 5k time trial. We hypothesized that PFO+ subjects would have higher Tc and higher HR at rest and immediately following the 5k compared to PFO- subjects, and therefore slower 5k times.

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 informed consent prior to participating in the study. All experimental procedures were conduction in accordance with the *Declaration of Helsinki 2013* except for registration of this study.

Participants

A total of 44 subjects participated in the study (22 M [12 PFO-, 10 PFO+] and 22 W [14 PFO-, 8 PFO+). Anthropometric data are presented in **Table 7**. All subjects were healthy, recreationally active, and free of cardiovascular and pulmonary disease confirmed with ultrasound and spirometry. Due to the nature of the study, it was not possible to control for menstrual cycle phase in female subjects, however, self-reported menstrual cycle phase was documented. For data analysis and to determine if there were differences in Tc, HR, or 5k time trial time as the result of hormone status women were broken into two groups: low hormone (LH) or high hormone (HH). The LH group included women in the follicular phase of the menstrual cycle or those in the placebo week of oral contraceptives. The HH group included women in the luteal phase of the menstrual cycle, those in the hormone weeks of oral contraceptives, or those

utilizing hormonal intrauterine devices. Subjects reported to the lab on 3 different days for data collection (outlined below).

Table 7. Descriptive and lung function data. * $p < 0.05$ vs PFO- M.

	PFO- M (n = 12)	**PFO+ M (n = 10)**	**PFO- W (n = 14)**	**PFO+ W (n = 8)**
Age (yrs)	23.8 ± 6.1	24.0 ± 5.7	25.1 ± 4.0	26.4 ± 5.4
Height (cm)	178.4 ± 5.1	182.5 ± 2.7*	164.2 ± 6.2	166.6 ± 6.8
Weight (kg)	73.7 ± 10.1	79.4 ± 9.6	64.7 ± 11.6	65.1 ± 11.0
BSA (m^2)	1.9 ± 0.1	2.0 ± 0.1	1.6 ± 0.2	1.7 ± 0.2
BMI (kg/m^2)	23.1 ± 2.9	23.9 ± 3.1	23.8 ± 3.0	23.4 ± 2.9
FVC (L)	5.5 ± 2.9	6.2 ± 0.5*	4.3 ± 0.6	4.7 ± 0.4
FVC (% pred.)	99.5 ± 10.6	107.2 ± 11.9	114.6 ± 12.8	127.0 ± 14.8
FEV1 (L)	4.5 ± 0.6	4.9 ± 0.8	3.5 ± 0.4	3.7 ± 0.4
FEV1 (% pred.)	97.5 ± 10.1	100.8 ± 17.3	109.9 ± 12.4	115.6 ± 15.5
FEV1/FVC	0.83 ± 0.05	0.78 ± 0.09	0.83 ± 0.06	0.80 ± 0.05
FEV1/FVC (% pred.)	97.3 ± 5.5	92.9 ± 10.2	95.6 ± 6.6	90.9 ± 6.1
VO_{2peak} (mL/kg/min)	41.1 ± 7.3	42.7 ± 6.7	39.4 ± 6.3	38.3 ± 6.3
Hemoglobin (g/dL)	15.4 ± 1.5	14.9 ± 1.1	12.2 ± 1.1	11.8 ± 1.8

Screening Visit

On visit 1 subjects underwent an ultrasound screening for the detection of a PFO. Ultrasound screening has been previously described in detail [95]. 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 visualize optimally all four chambers and interatrial septum and to 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 intravenous 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 at least one 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 [22,23,96,97]. Saline contrast injections were performed during normal breathing, as well as immediately following the release of a Valsalva maneuver to elevate right atrial pressure transiently and create conditions optimal for the detection of an intracardiac right-to-left shunt. Effective Valsalva maneuvers were confirmed by a transient leftward shift of the interatrial septum. Valsalva maneuvers 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+, whereas all others without left sided contrast or late appearing bubbles (>5 heart beats) were categorized as PFO−. Using this approach, we have shown that we have the sensitivity to accurately detect PFO presence in the general healthy population [97].

Lung Function

After ultrasound screenings, baseline pulmonary function tests were completed, which included measurements of forced vital capacity (FVC), forced expiratory volume in 1s (FEV1), and slow vital capacity (SVC). Lung function measurements were made to ensure no subjects had impaired lung function and then respiratory limitations would not play a role in any of the

differences in performance. Measurements were made with a computerized spirometry system (UltimaPFX, MedGraphics, St Paul, MNV, USA) according to American Thoracic Society/European Respiratory Society (ATS/ERS) standards [123]. Hemoglobin concentration was also measured to confirm no subjects were anemic.

Peak Oxygen Consumption Test

To characterize participants' maximal aerobic capacity subjects completed an incremental exercise test to exhaustion to measure VO_2peak on a cycle ergometer (UltimaPFX, MedGraphics, St Paul, MNV, USA, Lode Excalibur Sport, Lode, Groningen, The Netherlands). Subjects began cycling at 50 W for one minute and resistance was increased by 25 W every minute thereafter until volitional exhaustion. HR and oxygen saturation was measured continuously throughout the test. It was assumed that the subject reached their VO_{2peak} if RER was ≥1.2 and/or HR was ≥ 85% of age-predicted HR max.

Five-kilometer (5k) Run

Subjects were asked to refrain from caffeine and large meals for 4 hours and from heavy exercise for 24 hours prior to the time trials. Leading up to the experimental trial, all subjects ran a familiarization 5k on the same course they would run on for their experimental (EXP) trial (within ~1 month). On the day of their EXP trial, subjects arrived at the lab ~6 hr prior to testing to ingest the telemetric pill (Jonah core body temperature capsule; Mini Mitter, Bend, OR). Subjects returned to the lab at ~1600 for the 5k run. Upon arrival, subjects rested in a chair for a minimum of 10 mins prior to the collection of baseline resting Tc and HR.

For a warmup, subjects walked ~1 mile from the lab to the start of the 5k. Pre-5k Tc and HR was collected after the 1 mile walk and prior to the start of the 5k. The 5k started at ~1630 every day. Post-5k TC and HR were measured immediately after the subjects completed the run. Environmental conditions (ambient temperature (°C) and relative humidity (%), were measured continuously during the 5k using a Kestrel (Nielsen Kellerman Co, Boothwyn, PA). To control for weather and heat acclimation status in our participants, all testing was completed in May-June 2022 & 2023 in Eugene, OR.

Statistical Analyses

The effect of PFO on Tc and HR was analyzed using a two-way repeated measure ANOVA. For data analysis, subject data was segregated according to sex and analyzed separately in men and in women. Differences in all anthropometric and descriptive data as well as time trial time data were analyzed using Student's t-test. Statistical significance was accepted at $p < 0.05$. All data are presented as means ± SD. All analyses were completed in GraphPad Prism 9.1.2 (GraphPad Software, La Jolla, CA).

RESULTS

There were no differences in ambient environmental conditions, any anthropometric measures (except height in men) or VO_{2peak} between PFO- and PFO+ subjects of the same sex (i.e. PFO+ men vs PFO- men and PFO+ women vs PFO- women) (**Tables 7 & 8**). PFO+ men were significantly taller than PFO- men (182.5 ± 2.7 cm vs 178.4 ± 5.1 cm, $p = 0.03$). Five women (3 PFO-, 2 PFO+) were in the LH phase and 17 women were in the HH phase (11 PFO-, 6 PFO+).

Table 8. Time trial environmental conditions and baseline physiological data. * denotes $p < 0.05$ vs PFO- M.

	PFO- M (n = 12)	PFO+ M (n = 10)	PFO- W (n = 14)	PFO+ W (n = 8)
Ambient Temperature (°C)	24.4 ± 3.0	23.6 ± 2.5	24.9 ± 2.7	22.6 ± 2.8
Relative Humidity (%)	54.0 ± 8.0	50.8 ± 8.1	47.5 ± 4.9	45.0 ± 5.6
Heat Index (°C)	24.5 ± 3.9	23.7 ± 2.9	24.7 ± 2.6	22.4 ± 2.8
Run Time (min)	20.9 ± 2.0	23.9 ± 3.8*	24.1 ± 3.8	25.9 ± 2.1
Arrival Tc (°C)	37.4 ± 0.3	37.4 ± 0.5	37.5 ± 0.3	37.5 ± 0.2
Arrival HR (bpm)	70 ± 11	78 ± 15	72 ± 12	67 ± 13

Thermoregulatory, Cardiovascular and Performance Data

PFO+ vs PFO-

There was a main effect of time on both Tc and HR in all 4 groups (**Fig 3 & 4**), with post-TT Tc and HR elevated vs pre-TT ($p < 0.0001$ for all). In the men, there was a significant interaction effect of PFO x time ($p = 0.0436$) with PFO+ men having a greater increase in Tc (1.7 ± 0.3 °C) from pre- to post-time trial compared to PFO- men (1.4 ± 0.3 °C). Additionally, there was no difference in HR at any time point between the groups of men ($p = 0.5476$). There was no difference in Tc ($p = 0.3606$) or HR ($p = 0.3602$) between PFO+ and PFO- women at any time point.

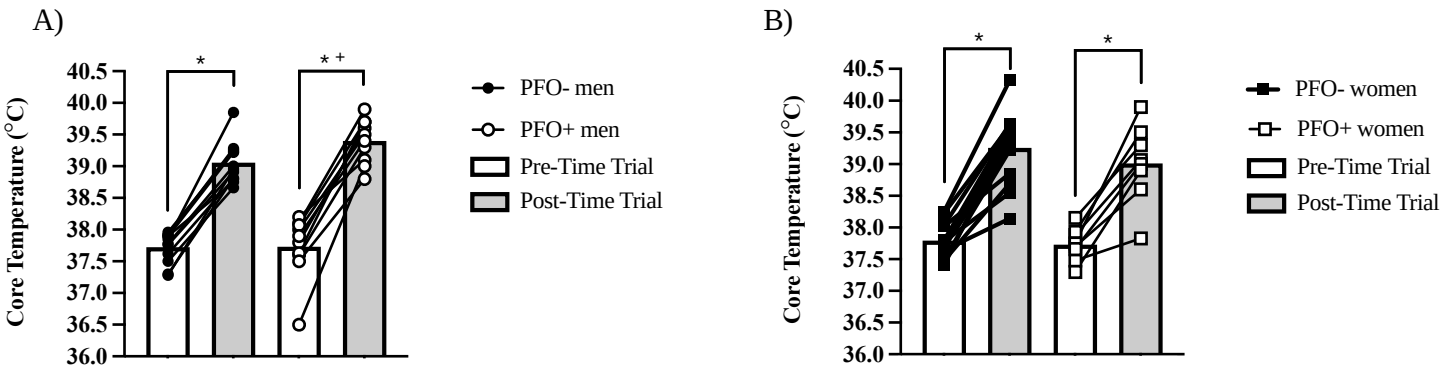


Figure 3. Core temperature pre- and post-5k time trial in men (panel A) and women (panel B). $^{+}$denotes significant interaction (time x PFO status, $p < 0.05$). * denotes $p < 0.05$ vs pre-time trial.

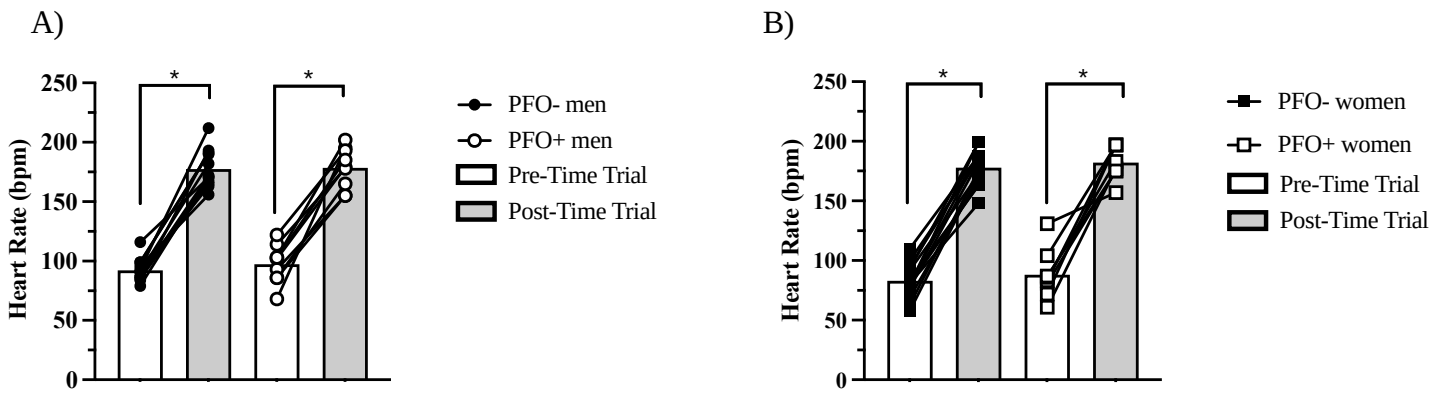


Figure 4. Heart rate pre- and post-5k time trial in men (panel A) and women (panel B). *denotes $p < 0.05$ vs pre-time trial.

For time trial duration, the PFO+ men ran significantly slower than the PFO- men (20.9 ± 2.0 vs 23.9 ± 3.8 min, $p = 0.03$, **Fig. 5**). There was no difference in time trial duration between PFO+ and PFO- women ($p = 0.23$, **Fig. 5**).

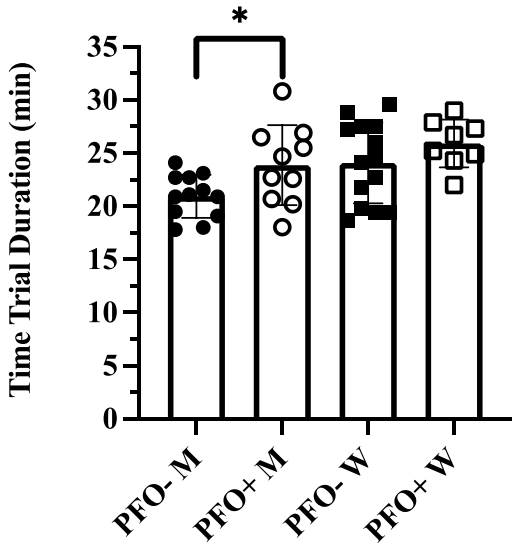


Figure 5. Time trial duration for men and women (PFO+ and PFO-). * denotes significant difference between PFO- and PFO+ men with PFO+ men running significantly slower than PFO- men.

Low Hormone vs High Hormone Phases in Women

There was no difference in Tc ($p = 0.8873$) or HR ($p = 0.1301$) or time trial time ($p = 0.67$) between the LH and HH groups **(Fig. 6)**.

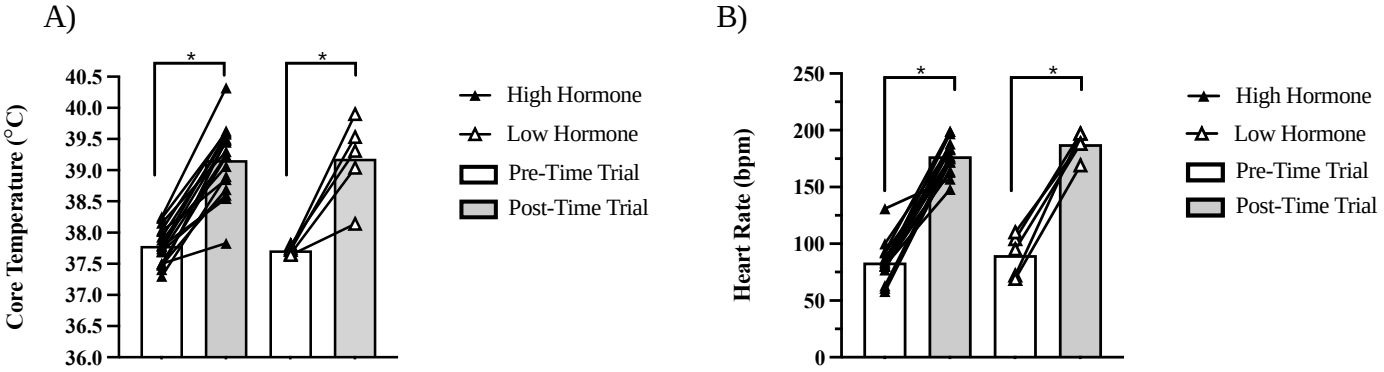


Figure 6. Core temperature (panel A) and heart rate (panel B) pre- and post-5k time trial in women in groups dependent on self-reported menstrual cycle phase or phase of hormonal birth control. *denotes $p < 0.05$ vs pre-TT.

DISCUSSION:

Our study was the first to examine the influence of the presence of a PFO on Tc and HR during a self-paced outdoor 5k time trial. Previous studies have examined the influence of a PFO on Tc responses at rest, during short duration exercise, and during passive heating and cooling, however, no research to date has included women. In the current investigation, PFO+ men had a slower 5k time and a greater increase in Tc during the 5k vs PFO- men, but there were no differences in time trial time or Tc responses between the PFO+ and PFO- women. The other main finding was that the presence of a PFO did not affect HR at rest or immediately post self-paced exercise.

The parallel differences in average change in Tc and average running time for the two groups of men raises the interesting question of whether the higher Tc post-time trial in the PFO+

men group were the cause, or the consequence, of the longer running times. Interestingly, our results, unlike previous data, show that PFO+ men have a greater increase in temperature for a given stressor. In the current study, the greater increase in Tc in the PFO+ men could be a consequence of longer running times (more time for heat storage), higher exercise intensity (increased metabolic heat production), decreased effectiveness of heat dissipation mechanisms, or some combination of the three. In the PFO+ men, there was also one individual with the largest increase in Tc who had a faster running speed thus a shorter running time. It is plausible that in this individual, the greatest increase in Tc was due to high rates of metabolic heat production since Tc changes during exercise are highly dependent on metabolic rate [92]. However, due to the design of the present field study, we are not able to identify the specific biophysical and/or physiological mechanism(s) for this finding.

Interestingly, our results, unlike previous data from our laboratory, show that Tc at rest was not different between PFO+ and PFO- men and as previously mentioned, that PFO+ men had a greater increase in temperature for a given stressor. While the reason(s) for this discrepancy between the results of the present and previous investigations are unknown, it could be related to differences in methodology used to measure Tc and the timing of the measures. In the current investigation, due to the nature of the exercise task (time trial / outdoor field study), Tc was measured using a telemetric pill, whereas in all previous investigations Tc was measured via an esophageal probe [11,12]. The question has been raised about whether the PFO may be a "physiological hot spot" [91] and while esophageal temperature is the gold standard for measuring rapid and dynamic changes in arterial blood temperature and the temperature of the blood leaving the aorta, it is not necessarily indicative of changes in whole body thermal balance. While the results of the current study suggest that the differences in temperature seen previously

may be limited to esophageal temperature, future studies should include measurement of esophageal temperature and other indices of Tc in the same individuals at rest and during exercise to determine whether the PFO is truly a "physiological hot spot".

While differences in Tc had been previously reported between PFO+ and PFO- men, whether similar differences in Tc exist in women with a PFO had yet to be investigated. There were no differences in baseline Tc, or in the Tc responses to self-paced 5k time trial exercise between the PFO+ and PFO- women. In the current study, while we noted menstrual cycle phase, we were unable to control for menstrual cycle phase. However, it has been shown that while menstrual cycle phase influences baseline temperature, with higher Tc reported in the luteal vs follicular phase (or low hormone vs high hormone phase for oral contraceptives) in a given female [3,124], the change in Tc for a given exercise bout is not always different between the two phases [27]. Recent data has shown that during 60 min of exercise at a controlled Hprod (175 w/m^2 and 275 w/m^2) that there is no difference in the change in core temperature (both esophageal and rectal) across the menstrual cycle phases[120]. Therefore, while the hormone status may have affected baseline Tc, it would not affect the change in temperature during a given exercise stressor. In the current data for women, we showed that there was no influence of hormone status on baseline Tc, HR, or on the cardiovascular/thermoregulatory responses to the time trial.

In the previous studies and current study, men with a PFO have different Tc at rest and/or during various thermal stressors. It is possible that the differences in baseline Tc seen in previous studies were driven by differences in circadian rhythm, inflammatory markers, or another physiological mechanism. However, with respect to the current data, it has been shown that a key variable in the individual Tc responses to an exercise task is metabolic heat production [32,72]. In the previous exercise study [12], exercise was assigned as a relative exercise intensity at each stage

($\%VO_{2peak}$) and in the current study, exercise intensity was self-selected, and likely varied over the course of the 5k run. Since there was no difference in starting Tc between the PFO+ and PFO- men in the present data, it may be that the mechanism responsible for these varying thermal responses is differences in metabolic heat production and/or heat storage during the current exercise bout, rather than differences in thermoregulatory mechanisms (i.e. differences in skin blood flow, sweating, etc.). While there was no statistically significant difference in mean body mass between the PFO+ and PFO- men, the PFO+ men were, on average, 5.7 kg heavier than the PFO- men, which may have affected heat storage during the 5k. Future studies should examine thermal and cardiovascular responses to exercise in those with and without a PFO at a controlled heat production to determine whether varying amount of heat production is contributing to the differences in Tc seen in these groups.

Self-paced exercise performance has also been shown to be influenced by HR responses. In the current study, HR values at rest and in response to exercise, were not different across groups and did not appear to be affected by the presence of a PFO in either men or women. The elevation in HR at rest in the previous study may have been partly the result of the higher Tc in those individuals [75], and not the presence of a PFO, *per se*. In terms of cardiovascular responses to exercise, this positive aspect of these findings suggests that the presence of a PFO did not increase cardiovascular strain during self-paced running exercise in our subjects, which suggests that those with a PFO frequently participating in athletic activities, both recreationally and competitively, may not have elevated cardiovascular strain (and associated risk).

Limitations

While the aim of the current study was to determine whether the presence of a PFO influences Tc and HR responses during an outdoor self-paced 5k, there are a few limitations to address. First, since the 5k runs were completed on several days (over the course of 2 years), there was some variability in the temperature and humidity during which the subjects ran in. While we aimed to collect all data during the same time of year to control for seasonal fluctuation in temperature, it is possible that variations in temperature day to day affected thermal sensation and rating of perceived exertion, and therefore running speed in our groups of men. However, there was no difference in mean temperature or humidity between the PFO+ men (23.6 ± 2.5°C and 50.8 ± 8.1% rh) and PFO- men (24.4 ± 3.0°C and 54.0 ± 8.0% rh). Also due to the nature of the study, we were unable to measure Tc at several time points during the 5k, thus we are unable to determine the rate of change of Tc throughout the run, and whether individuals titrated their running speeds based on variations in rate of change in Tc. Finally, while this study has applicability to real world 5k performance, a more controlled chamber study while measuring and controlling for additional variables (i.e., RPE, thermal sensation, hydration status/urine specific gravity, environmental conditions) would have allowed for a better understanding of the factors contributing to the differences in Tc responses seen between the PFO+ and PFO- men during self-paced exercise performance.

CONCLUSIONS

In the present work, men with a PFO had a greater increase in Tc compared to men without a PFO during a self-paced outdoor 5k time trial. There was no difference in 5k performance or in Tc at any time point between PFO+ and PFO- women. Whether or not the differences in Tc in the men are a result of differences in metabolic heat production or differences in the effectiveness of

heat dissipation mechanisms is unknown. These findings may have important implications for those with a PFO performing exercise tasks at a high intensity, however, follow up work is necessary to clarify the specific mechanisms involved.

CHAPTER VI

CONTRIBUTION OF NON-SHIVERING THERMOGENESIS TO THE MAINTENANCE OF CORE TEMPERATURE IN SCUBA DIVERS WITH AND WITHOUT A PATENT FORAMEN OVALE

This chapter was submitted to the Journal of Science and Medicine in Sport with Kaitlyn G. DiMarco, Joel E. Futral Rachel Lord, Justin Edwards, Otto Barak, Željko Dujić, Igor Glavičiç, Ivana Miloš, Ivan Drvis, and Andrew T. Lovering as co-authors. All experimental work was performed either by me independently or by K.G.D., A.T.L., J.E.F., R.L., and J.E., under my direction. The writing is entirely mine. All co-authors provided editorial assistance.

INTRODUCTION

According to the Sports and Fitness Industry Association, SCUBA diving is a sport that is enjoyed by ~6 million around the globe and ~2.5 million people in the United States [125]. SCUBA divers are often exposed to water temperatures that are far below the water temperature that would be considered thermoneutral for humans (~34-35°C) [33], which can be detrimental to physical and cognitive performance and even life threatening [34,126]. The influence of cold water immersion (CWI) on physiological responses has been extensively studied [42–44]. Exposure to cold water during can lead to decreases in core temperature (Tc) if the heat conservation/production mechanisms are not sufficient to overcome the heat losses and maintain Tc. To conserve heat, cutaneous vasoconstriction will occur to increase the insulative shell and prevent heat loss to the external environment [65]. If cutaneous vasoconstriction is not enough to maintain Tc, shivering and non-shivering thermogenesis (NST) occur to increase metabolic heat production [48].

NST, also known as cold-induced metabolism, is facilitated by brown adipose tissue (BAT) and the activation of the sympathetic nervous system [127]. Mitochondrial uncoupling protein, or UCP-1 is the key protein responsible for NST during cold exposure. Fibroblast growth factor 21 (FGF21) is an adipokine that has been recently shown to upregulate UCP-1 activity and be predictive of the NST during cold exposure in humans [128,129]. It has also been shown that baseline FGF-21 concentrations are related to the change in Tc during cold exposure (cold air and cold water immersion), with high levels of FGF-21 being associated with increases in Tc and low levels of FGF-21 being associated with decreases in Tc [130]. It has also been recently reported that FGF-21 concentration was elevated in SCUBA divers immediately post-dive (~30 mins) [131] compared to pre-dive in water that was ~13-14°C. That study did not measure Tc. Therefore, whether the divers with the highest concentrations or greatest change of FGF-21 were better able to maintain Tc was not determined.

In addition to heat conservation/production mechanisms, anthropometric factors such as body mass (BM), body surface area (BSA), body surface area to mass ratio (BSA/BM) and body mass index (BMI) also contribute to the ability of an individual being able to tolerate a cold environment and maintain Tc. In fact, most of the variability in the change in Tc during cold exposure can be attributed to differences in anthropometric factors and body composition [43]. Total body mass has been shown to be highly related to the risk of hypothermia in those exposed to cold environments in several populations of individuals [48] and those with the highest body surface area to mass ratios tend to have the greatest decreases in Tc [43,59,103]. While both physiological responses and anthropometric factors can assist with maintenance of Tc during cold exposure, wearing the appropriate protective gear (known as behavioral thermoregulation) during CWI is also an important consideration [61]. SCUBA divers are often exposed to very cold

waters, but to prevent or delay the onset of hypothermia, SCUBA divers wear wetsuits (of varying thicknesses), semi-dry suits or dry suits for protection against CWI [61].

Previous work has shown that men with a patent foramen ovale (PFO+), a small hole between the atria of the heart, have higher Tc (as measured by esophageal probe) at rest and during cold water immersion (CWI; 19.5 ± 0.9°C) compared to men without a PFO (PFO-) [11]. Interestingly, during the CWI, PFO+ men were able to maintain higher Tc regardless of whether they shivered. As previously mentioned, an additional physiological mechanism that can contribute (albeit minor) to the maintenance of Tc is NST. It may be that the individuals who were able to best maintain Tc had increased NST. Whether or not PFO+ individuals have a greater amount of NST during CWI compared PFO- individuals, which may contribute to their ability to maintain higher Tc has yet to be determined.

The purpose of this study was to increase the understanding of the factors that influence Tc changes during SCUBA diving and included three main aims: 1) to determine the influence of a PFO on Tc responses during two dive profiles; 2) determine influence of various anthropometric measures and wetsuit thickness on Tc in SCUBA divers; 3) determine whether both baseline or the change in FGF-21 is associated with Tc responses during SCUBA diving. For **Aim 1)** we hypothesized that individuals with a PFO would have the highest Tc at rest and post-SCUBA dive; **Aim 2)** we hypothesized that FGF-21 would be increased in SCUBA divers from pre- to post-dive and the change in Tc during the dive would be related to baseline concentrations of FGF-21; **Aim 3)** we hypothesized that those with the highest body mass index and body mass would have the smallest change in Tc and those with the highest body surface area to body mass ratio would have the greatest change in Tc.

METHODS

This study received approval from the University of Oregon's Office for Protection of Human Subjects (RB# 07302018.031) and University of Split School of Medicine (#2181-198-03-04-19-0052). 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 2013* except for registration of this study.

Participants

A total of 31 divers participated in the study that was completed in Bol, Croatia in July and September 2022. In the July dives, 8 PFO- (0 F) and 11 PFO+ (3 F) divers participated, whereas in the September dives 6 PFO- (1 F) and 6 PFO+ (0 F) divers participated. All participants were healthy, recreationally active, and free of cardiovascular and pulmonary disease. During the first visit to the lab, participants signed an informed consent and filled out physical activity history questionnaire. Anthropometric data and wetsuit info is presented in **Table 9**.

Table 9. Participant Characteristics and Wetsuit Information

	July Dive			September Dive		
	PFO- **(n = 8)**	**PFO+** **(n = 11)**	**Group** **(n = 19)**	**PFO-** **(n = 6)**	**PFO+** **(n = 6)**	**Group** **(n =12)**
Sex	8 M	8 M, 3 F	16 M, 3 F	5 M, 1 F	6 M	11 M, 1 F
Age (yrs.)	45.3 ± 10.8	41.8 ± 15.0	43.3 ± 13.2	45.0 ± 12.1	44.2 ± 13.4	44.6 ± 12.2
Height (cm)	181.9 ± 4.3	176.0 ± 9.1	178.5 ± 7.9	176.5 ± 8.1	186.7 ± 7.7	181.8 ± 9.7
Weight (kg)	87.0 ± 12.5	78.7 ± 16.5	82.2 ± 15.1	86.5 ± 28.4	86.4 ± 9.1	82.5 ± 14.3
BSA (m^2)	2.1 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	2.0 ± 0.3	2.1 ± 0.1	2.0 ± 0.2
BMI	26.2 ± 3.4	25.2 ± 2.9	25.7 ± 3.6	27.6 ± 9.1	24.9 ± 3.1	24.8 ± 3.2
Suit Thick. (mm)	7.0 ± 2.0	7.5 ± 2.0	7.0 ± 2.0	7.0 ± 1.0	7.0 ± 1.0	7.0 ± 1.0
# of Hoods	n = 7	n = 9	n = 16	n = 6	n = 6	n = 12
# of Gloves	n = 4	n = 7	n = 11	n = 3	n = 4	n = 7
# of Booties	n = 6	n = 11	n = 17	n = 6	n = 5	n = 11

PFO Screening

On visit 1 participants underwent an ultrasound screening for the detection of a PFO. Ultrasound screening has been previously described in detail [95]. Initial agitated saline contrast studies were performed with participants 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 visualize optimally all four chambers and interatrial septum and to 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 intravenous 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 at least one microbubble in the left atrium or ventricle in any frame during the subsequent 20 cardiac cycles served as the criterion that participants were either positive for an intracardiac right-to-left shunt (i.e., PFO) or demonstrated the transpulmonary passage of contrast [22,23,96,97]. Saline contrast injections were performed during normal breathing, as well as immediately following the release of a Valsalva maneuver to elevate right atrial pressure transiently and create conditions optimal for the detection of an intracardiac right-to-left shunt. Effective Valsalva maneuvers were confirmed by a transient leftward shift of the interatrial septum. Valsalva maneuvers 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 participants were classified as PFO+, whereas 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 [97].

Dive Protocol

Participants completed one of two dive protocols. In July 2022, participants dove to 18 m of seawater for 47 min (SHALLOW) and in September 2022 participants dove to 30 m of seawater for 20 min (DEEP). These dive protocols were chosen because the time and depth of the dives would not require a decompression stop when the divers were returning to the surface.

The dives took place in both the morning (~1000) and afternoon (~1600). Regardless of time of day of the dive, participants ingested the core temperature pill (Jonah core body temperature capsule; Mini Mitter, Bend, OR) ~6-10 hr prior to the start of the dive. Participants returned to the lab later for the experimental dive protocol. Prior to boarding the boat, participants underwent an intravenous blood draw and information regarding the wetsuit worn (i.e. thickness, brand, gloves, boots, hoods, etc.) was recorded on all participants.

Participants then boarded the boat and were escorted to the dive site by study staff. Just prior to entering the water and after donning all gear, baseline Tc was measured (Jonah core temperature capsule; Mini Mitter, Bend, OR) and recorded. Participants completed their pre-determined dive profile before returning to the boat where post-dive Tc was measured and recorded within a few minutes of surfacing while wearing all gear. Once all participants had returned to the boat, participants returned to the lab for a post-dive blood draw (1-2 hr post dive). During the dives, all

participants were equipped with a dive computer (Scubapro M2, El Cajon, CA) to ensure participants followed the appropriate dive protocol and for measurement of water temperature/depth.

Blood Draws and Analysis of FGF-21

An intravenous (IV) catheter was placed into an antecubital vein for blood draws. At both time points (pre- and post-dive), a 15mL venous blood sample was drawn from the IV catheter into serum separator tubes (SST). Prior to being centrifuged (1500 g for 10 min), all samples sat at room temperature for at least 30 minutes to fully clot. Serum was separated and frozen in a -80°C freezer until analyzed. Samples were defrosted on ice and serum FGF-21 concentration was analyzed via enzyme-linked immunoassay (ELISA; R&D Systems, Minneapolis, MN, USA).

Statistical Analyses

The effect of PFO on Tc pre- and post-dive was analyzed using a two-way repeated measure ANOVA. Differences in all anthropometric and descriptive data between PFO- and PFO+ participants were analyzed using Student's t-test. The relationship between rate of change in core temperature (ΔTc/min) vs anthropometric factors including wetsuit thickness, body mass, body surface area, body mass index and body surface area: mass ratio were analyzed using linear regressions. Statistical significance was accepted at $p < 0.05$. All data are presented as means ± SD. All analyses were completed in GraphPad Prism 9.1.2 (GraphPad Software, La Jolla, CA).

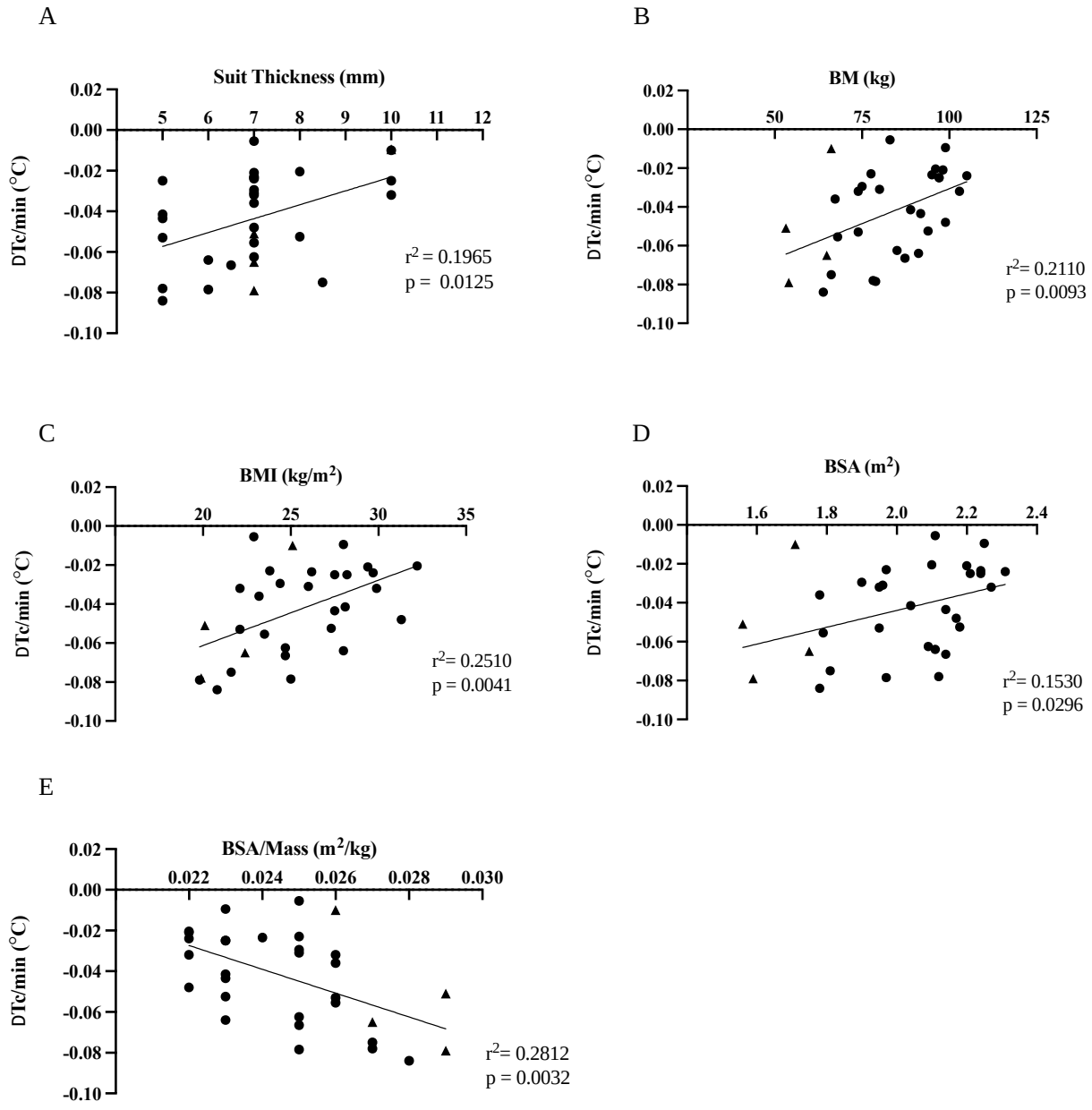
RESULTS

There were no differences in any anthropometric measures or wetsuit thickness between PFO- and PFO+ groups in either dive profile (**Table 9**). The bottom and surface water temperatures were ~17°C/26 °C and ~18°C/23 °C for the SHALLOW and DEEP dives, respectively.

There was a main effect of time on Tc with post-dive temperatures being significantly lower than pre-dive temperatures in both dive profiles ($p < 0.0001$ for both). There was no difference in the absolute ΔTc from pre- to post-dive between the two profiles (SHALLOW ΔTc = -0.9 ± 0.5 °C vs DEEP ΔTc = -0.8 ± 0.4 °C, $p = 0.73$), however, due to the difference in dive time, there was a significant difference in the rate of ΔTc over the course of the dives, with the DEEP dive having a faster decrease in Tc (-0.04 ± 0.02 °C/min) vs the SHALLLOW dive (-0.02 ± 0.1 °C/min; $p < 0.01$). There was no effect of the presence of a PFO on Tc at any time point during both the SHALLLOW ($p = 0.6186$) and DEEP dive ($p = 0.5847$).

The linear regressions between all anthropometric variables and the rate of change in core temperature (ΔTc/min) are displayed in **Fig. 7**. There was a positive and significant linear relationship between ΔTc/min and all anthropometric factors including wetsuit thickness (**Fig. 7A**; $r^2 = 0.1965$, $p = 0.0125$), body mass (**Fig. 7B;** $r^2 = 0.2110$, $p = 0.0093$), body mass index (**Fig. 7C;** $r^2 = 0.2510$, $p = 0.0041$), and body surface area (**Fig. 7D;** $r^2 = 0.1530$, $p = 0.0296$). There was a significant, negative linear relationship between body surface area to mass ratio and ΔTc/min (**Fig. 7E**; $r^2 = 0.2812$, $p = 0.0032$).

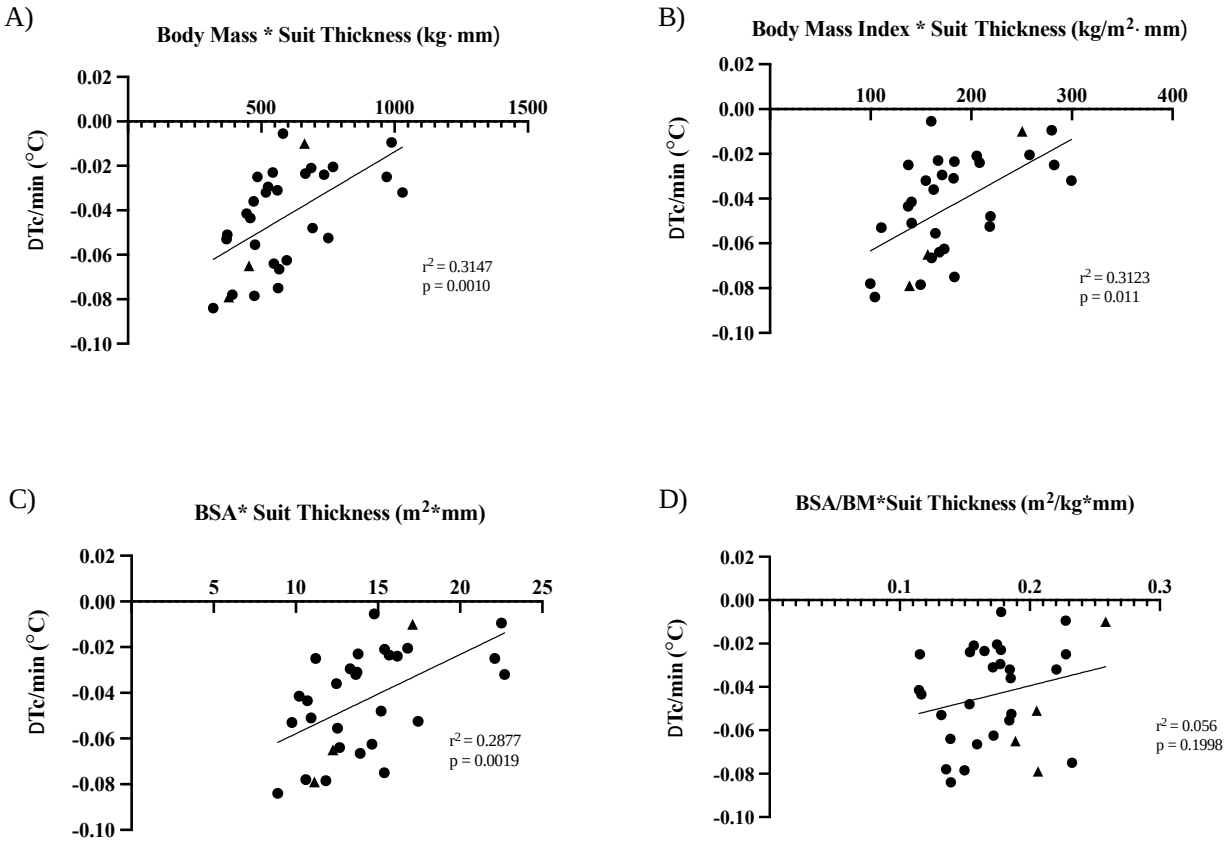
Figure 7. Relationship between anthropometric variables and rate of change in core temperature during the dives. Female subjects are designated by the triangle symbols and male subjects are designated by the circle symbols.



In addition to examining the relationship between each anthropometric variable and ΔTc, we wanted to examine the combined influence of the anthropometric variables and wetsuit thickness

on ΔTc/min as they would act together to provide various levels of thermal protection and prevent decreases in Tc during the dive. The data are presented in **Fig. 8A-D**. When multiplied by wetsuit thickness (mm), body mass (**Fig. 8A** $r^2 = 0.3147$, $p = 0.0010$), BMI (**Fig. 8B** $r^2 = 0.3123$, $p = 0.0011$), and body surface area (**Fig. 8C** $r^2 = 0.2877$, $p = 0.0019$) all had positive and significant relationships with the ΔTc/min. There was no relationship between body surface area/mass*thickness and ΔTc/min (**Fig. 8D** $r^2 = 0.056$, $p = 0.1998$).

Figure 8. Combination of anthropometrics and wetsuit thickness on the change in core temperature during the SCUBA dive. Female subjects are designated by the triangle symbols and male subjects are designated by the circle symbols.



There was no main effect of time or PFO on the FGF-21 response to SCUBA diving in either dive profile ($p > 0.05$ for all). Additionally, in our group of participants, there was no relationship between baseline FGF-21 and the ΔTc pre- to post-SCUBA diving.

DISCUSSION

Our study was the first to examine the influence of a PFO on Tc responses in SCUBA divers. Surprisingly, in our current study, there was no difference in Tc pre-dive or post-dive between our PFO+ and PFO- participants (in both dive profiles). Previous studies from our lab have shown that men with a PFO have higher Tesoph at rest and during CWI in a cold tub (19.5 ± 0.9°C; Davis *et al.*, 2015, 2017). Interestingly, in the studies by Davis et al., men with a PFO maintained higher Tesoph compared to PFO- men, regardless of whether they shivered or not. We hypothesized, based on previous results, that our PFO+ participants would have higher Tc pre-dive and that higher Tc would be maintained post-dive. Three primary differences exist between the previous study and the current data which could explain the differing results. First, in the cold tub study, all study visits occurred at ~0800. In the current investigation, study visits occurred both the morning (~1000) and afternoon (~1500). It may be that the Tesoph differences between those with and without a PFO are related to circadian timing differences, therefore when data is collected at various times throughout the day, the influence of circadian rhythm on Tc overrides the influence of PFO on Tc. Second, in the cold tub study participants only wore swim trucks, whereas in this current study participants wore the wetsuit of their choosing (variable thicknesses). It may be that the differences in Tc exist between PFO+ and PFO- participants, but the variability in wetsuit thicknesses (ranging from 5-10 mm) chosen by the participants masked the differences in Tc.

The third potential reason for the differences in Tc results between the two studies is the method for Tc measurement. Davis et al. (2017), measured Tc via esophageal probe, whereas in our current study, we used telemetric pills for the measurement of Tc. Other studies have also reported elevated Tesoph in PFO+ participants vs PFO- participants both at rest and during exercise [8,12]. It was previously suggested that the PFO may act as a "physiological hot spot" and it was questioned as to whether or not those differences in Tc would be seen in several anatomical locations [91]. Interestingly, a recent investigation examining the influence of PFO on Tc responses during a self-paced 5k run also using telemetric pills reported that there were no differences in baseline Tc (*Bradbury et al, in submission*) in those with and without a PFO. While the results of these various investigations support the idea about a PFO being a potential "physiological hot spot", whether these temperature differences exist only via esophageal probe measurement warrants further investigation.

Many physiological responses influence an individual's ability to maintain Tc during cold exposure [65]. It is well defined that during cold exposure BAT activity and NST increase, however, the extent to which it aids in heat production, thus maintenance of Tc, may be minor. Interestingly a previous study showed increased FGF-21, a marker of NST, in a group of professional SCUBA divers immediately post-dive [131]. In our study, we saw no difference in FGF-21 pre-dive to post-dive. The differences between these results may be due to the temperature of the water, acclimatization status of the divers, or timing of the blood draws. The professional divers had at least 7 years of experience where they dove at least 50-100x/year, however, our participants were recreational divers. It may be that the increase in FGF-21 in the professional divers was an effect of cold acclimatization where those divers were regularly exposed to cold water. Increases in the amount BAT and BAT activity has been shown to increase

following cold-acclimatization in several investigations [65,132,133]. Additionally, the dives in the current study took place in July and September, when our participants would not be acclimatized to a cold environment.

While cold acclimatization status may have played a role, it is also possible that the temperature of the water in our study (~17-18°C) was not enough of an acute cold stimulus to elicit increases in NST. FGF-21 concentration was increased after 30 min in ~13-14 °C water [131], which would have been a greater thermal stressor compared to our study. We are not the only study to report no increase in FGF-21 during acute cold exposure [130] and some even report decreases in FGF-21 [134]. It has also been reported that the change in Tc during cold exposure was significantly related to the concentrations of baseline FGF-21[130], however, we did not see that relationship in our data. It may also be that our participants did not have an increase in FGF-21 (decreased NST) due to an increased reliance on shivering for heat production [130]. From a methodological perspective, it is also possible that our blood draws occurred too late post-dive (1-2 hr post-dive) to measure the increase in FGF-21, however, the exact reason(s) for the discrepancy in these results remain unknown.

The third aim of our study we aimed to quantify the contribution of anthropometrics (i.e. BM, BSA, BSA/Mass, BMI), wetsuit thickness (indicative of behavioral thermoregulation), and the combination of the two on Tc responses. As expected, those with the thickest wetsuit, highest BM and BMI, and lowest BSA/BM ratio had the lowest rate of decrease in Tc during the dive. To further the analysis, we looked at the combination of these anthropometric factors and wetsuit thickness to quantify thermal protection for cold water exposure (**Fig. 8**). The practical implications of these results are that although someone with a high BSA/BM ratio may have a greater decrease in Tc compared to someone with a low BSA/BM ratio, these changes in

temperature can be mitigated by selecting a thicker wetsuit. On the other hand, an individual with a higher body mass will be able to select a thinner wetsuit and be able to maintain Tc. Generally, when selecting wetsuits for diving, divers are given a range of thickness that would work well for the expected temperature of the water. Our results suggest and confirm that in addition to the temperature of the water, anthropometric characteristics such as BSA/Mass ratio, BM, and BMI of the diver should also be taken into consideration to prevent larger drops in Tc and optimize safety during SCUBA diving.

Limitations

The primary limitation for this study the timing of our blood draws for measurement of FGF-21. It is possible that there were increases in FGF-21, but our blood draws occurred too late after the end of the dive for us to measure the increase. Additionally, while the purpose of this study was not to study sex differences in these responses, few (13%) female participants enrolled in this study. Whether or not these findings are generalizable to the whole population is unknown.

Conclusion

In conclusion, the presence of a PFO did not alter the Tc responses to SCUBA diving in two separate dive profiles. There was also no relationship between the FGF-21 response and Tc responses during the dive. The results of this study further support the fact that wetsuit thickness as well as anthropometric data are the keys factors in determining the Tc response to cold water immersion and SCUBA diving.

CHAPTER VII

CONCLUSION

MAIN FINDINGS

Previous studies have found that the presence of a PFO in men influences baseline core temperatures[11,12], suggesting that a PFO may be an important factor contributing to the variability in core temperature seen among a group of individuals. The studies in this dissertation aimed to explore whether various factors including differences in heat production during exercise were a contributing factor to those previous seen differences and if those differences in baseline Tc influences real world exercise performance (self-paced outdoor 5k and SCUBA diving).

Surprisingly, in chapter IV, we showed that men without a PFO have a higher core temperature at rest and after 60 min of exercise at a controlled Hprod (7 w/kg) compared to men with a PFO. This investigation was also the first to study core temperature in women with and without a PFO and we found that there were no differences in Tc between PFO+ and PFO- women, regardless of menstrual cycle phase or birth control status. We also showed that there were no differences in other thermoregulatory or cardiovascular responses (Tsk, Tb, RHL, HR, sweat rate, and HR) to 60 min of exercise. Also, surprisingly in this study, we did not see an elevation temperature in the luteal or high hormone phase of the menstrual cycle relative to the follicular phase. Several reasons for this may exist including variability in activity prior to coming to lab (driving vs biking vs walking), thus leading to variability in arrival and pre-exercise core temperature. Additionally, while shifts in Tc in those using oral contraceptives has been well documented[3], the variability in doses of the hormones in the OCs may be responsible for the differences in our results compared to previous investigations.

In chapter V, we aimed to determine if the previously seen difference in baseline Tc affect Tc responses and running time during a self-paced outdoor 5k time trial. We studied both men and women and found that there were no differences in baseline Tc in either group. Despite having significantly slower running times compared to PFO- men, the PFO+ men had a greater increase in temperature over the course of the 5k. There was no difference in performance or Tc responses between PFO+ and PFO- women. The reason for the differences in Tc responses in the PFO+ men may be due to two reasons. First, one subject had the fastest running time and greatest increase in Tc over the course of the 5k, therefore the increase in Tc may have been driven by high levels of heat production. Second, although there was no significant difference in mean body mass between PFO+ and PFO- men, the PFO+ men did weigh on average ~5.7 kg more than PFO- men, which may have contributed to greater heat storage.

In chapter VI, we examined the influence of the presence of a PFO on core temperature responses during sea water dives in men and women wearing wetsuits. We demonstrated that during two different dive profiles, the presence of a PFO did not have an influence on baseline or post-SCUBA diving core temperatures. This was a surprising finding considering a previous investigation had shown that men with a PFO are able to maintain higher Tc during cold water immersion [11]. Our study showed that the anthropometrics such as body mass, body surface area, body mass index, in addition to wetsuit thickness contribute significantly to the core temperature responses during SCUBA diving.

SUMMARY AND FUTURE DIRECTIONS

Surprisingly, and contrary to our hypotheses in all chapters in this dissertation, those (specifically men) with a PFO did not have higher Tc compared to those without a PFO. While the reasons for the discrepancies in results from previous studies are unknown, a primary reason

may be due to circadian differences among individuals. Since temperature fluctuates with habitual sleeping habits, future investigations should study individuals at time that is based on time after normal wake time (2 hr after waking), rather than a specified clock time (i.e. ~0800).

The major limitations in chapter V include that while performed in a real-world outdoor scenario, a more controlled chamber study while measuring and controlling for additional variables (i.e., RPE, thermal sensation, hydration status/urine specific gravity, environmental conditions) would have allowed for a better understanding of the factors contributing to the differences in Tc responses seen between the PFO+ and PFO- men during self-paced exercise performance.

In Chapter VI, the primary limitation for the study was the timing of our blood draws for measurement of FGF-21. It is possible that there were increases in FGF-21, but our blood draws occurred too late after the end of the dive for us to measure the increase. Additionally, while the purpose of this study was not to study sex differences in these responses, few (13%) female participants enrolled in this study. Whether or not these findings are generalizable to the whole population is unknown.

This dissertation answered key questions about the factors contributing to the differences in temperature seen between those with and without a PFO and whether the previously documented differences affect Tc responses in real world scenarios. Interestingly, and contrary to our hypotheses, we found that those with a PFO did not have a higher Tc or Tesoph compared to those without a PFO in all three studies in this dissertation while all previous work had found that men with a PFO have higher Tesoph compared to men without a PFO[8,11,12]. The reasons for the discrepancy in findings between current and previous work are unknown, however, may be due to multiple factors contributing to the normal biological variability we see in Tc in humans.

For example, future studies examining differences in baseline Tc should record and possibly control for individual circadian rhythms. Controlling for the time after waking up, rather than clock time would help us understand additional factors contributing to the range of Tc in a group of people. For example, future studies examining differences in baseline Tc should record and possibly control for individual circadian rhythms. Controlling for the time after waking up, rather than clock time would help us understand additional factors contributing to the range of Tc in a group of people. Studies examining 24 hour core temperature in PFO+ vs PFO- individuals would also give additional insight into the mechanisms causing the variability in resting temperature measured between the groups.

The key and important findings from the present dissertation is that there are no differences in the change in Tc (or Tesoph) between those with and without a PFO in response to exercise when Hprod is controlled for, suggesting that there is no influence of the absence or presence of a PFO on the ability to thermoregulate in humans. It was previously thought that PFO+ men *may* be at an increased risk for a heat injury or illness due to the increase in baseline Tesoph. While the current results conflict previous findings an additional important finding from this dissertation is that since the elevation in Tesoph/Tc is not present under every circumstance (time of day, stressor, etc.), those with a PFO are likely not at an increased risk of a heat event. However, since in most cases, exercise in the real world does not occur at a controlled Hprod, additional follow up work is needed to truly determine the influence of a PFO on performance. While the current study found that men with a PFO have a greater increase in Tc for a slower running time, future studies should consider the influence of a PFO on the decrement in performance in the heat (using thermoneutral as a control) pre- and post-heat acclimation to rule out any influence of heat acclimation on the previously seen differences in Tc. Findings from

these studies would truly help answer the question as to whether or not those with or without a PFO respond differently to the heat, either acutely via decrement in performance, or chronically with differences in the heat acclimation process.

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