A Single Combined Active and Passive Isothermic Heating Protocol Results in a Similar Core Temperature Response as Exercise Alone in Hot Conditions

by

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ABSTRACT

Heat acclimatization can be induced by targeting a core temperature 38.5°C for at least 60 minutes per day lasting 5 to 21 days, complementary to normal exercise activity. However, consistently meeting this threshold on consecutive days may be difficult for athletes. The objective of this study was to evaluate the efficacy of four single-bout heating protocols to reach a core temperature 38.5°C. The study was set up as a non-randomized field study, factoring in the September-October outdoor desert conditions, Tempe, AZ, USA. Environmental conditions were measured using a Kestrel heat stress tracker. Protocols were constituted out of 3 elements: PAS – passive heat exposure in a tent (54±1°C), EH - exercise in hot condition with high intensity interval training (HIIT) outdoors in the heat in a tent with a ventilator (43±1°C), EM – exercise in moderate conditions with HIIT indoors (22±0.4°C). All participants performed protocols in the following order: 1) PAS 60-min; 2) EH-PAS (EH 30-min + PAS 30-min); 3) EH 60-min, and 4) EM 60-min. A cycle ergometer was used for HIIT (2-min warm-up followed by 7x2-min sprints with 2-min relative rest between sprints during the first 30 min and stationary cycling for the second 30 min), with a self-selected workload at 80-100 rpm and similar heart rate (HR) response during exercise testing for EH: 146±10, EM: 142±13, and EH-PAS: 142±13 (*P*>0.05). A total of 10 active male students (25±3 years old) reported no difference between protocols for baseline Tc (*P*=0.37) and HR (*P*=0.28). During the first 30-min, T_c was significantly different between protocols (average ranging from 37.3-37.6°C, P=0.01), but from a practical perspective, differences were limited. During the second 30-min session, the Tc for EH (38.5±0.4°C) and EH-PAS (38.6±0.4°C) were significantly higher from EM (38.1±0.4°C) and PAS alone (37.8±0.4°C), $P<0.001$. The average HR (bpm) was significantly lower in PAS (110±17) and EH (136±13) during the second half of the protocols compared to the EH (151±10) and EM (149±16), $P<0.001$. In conclusion, exercise alone vs. a combination of exercise and passive heating in hot conditions resulted both in a body temperature 38.5°C, but the combination was more efficient since participants exercised for only 30-minutes.

DEDICATION

This thesis work is dedicated to my family and especially my parents, Maria and Ed, who have always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve. This thesis work is also dedicated to my friends (Breezy, Kristin, and Meghan) and my partner who have been a constant source of support and encouragement during the challenges of graduate school and life. I am truly thankful for having you in my life.

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TABLE OF CONTENTS

Page

CHAPTER

TABLE OF CONTENTS

CHAPTER Page

TABLE OF CONTENTS

CHAPTER Page

APPENDIX

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The National Center for Catastrophic Sport Injury Research states that heat-related illnesses are the second leading cause of exertional injury, right behind cardiac-related events, yet are preventable (Kucera, 2019). In order to prevent exertional heat illness (EHI), heat acclimation and cooling protocols are essential for the safety of all athletes and can improve performance in all conditions (Casa et al., 2015). The consequences of exercising in the heat unacclimated can be severe or result in death (Nichols, 2014). Therefore, it is conducive to establish heat and safety guidelines as a means of improving performance.

States with mandated guidelines for preseason high school football heat acclimatization have a lower rate of EHI compared to states that do not provide mandates (Kerr et al., 2019). Mandated guidelines provide a practical approach as to how training sessions should be structured at the beginning of the season to reduce the risk of EHI. In football for example, coaching staff would not allow players to participate in full padding and equipment at the beginning of the season. Rather, players would be able to complete a few practices in limited padding and equipment before being able to complete a session at full strength. Preparing for activities in the heat reduces the incidences of heat illness and is therefore beneficial. Although decreasing the time of practice sessions or limiting the number of sessions can be effective in reducing risk of heat related injuries, at an individual level, athletes still need to prepare for activity in the heat to enable them to perform their best in warmer climates.

Heat stress experienced by the body is caused by a combination of environmental conditions (temperature, humidity, and solar radiation), physical work rate, and the type of clothing the activity is performed in (Sawka et al., 1996). Heat stress paired with exercise increases physiological strain and therefore perceived exertion by the athletes (Willmott et al., 2019). This stress results in a raise in core, skin, and brain temperature which can inflate cardiovascular strain and could impact cerebral function (Bain et al., 2015). These distressing conditions lead to increased heart rate, breathing rate, and altered muscle metabolism which result in fatigue and can prevent athletes from maintaining ultimate power output during activity (Nybo et al., 2014). Maximum athletic performance requires proper oxygen delivery from the blood to the working muscles. The cardiovascular system is challenged by heat direct blood flow to the periphery in an effort to cool the body, though in turn, not efficiently delivering oxygen (Adolph, 1924; No and Kwak, 2016). The practical implications of performing activities in the heat, it that it may reduce speed, and therefore, especially during endurance events such as hiking, running or cycling, a person may be exposed to higher environmental temperatures for longer periods of time in comparison to exercising in moderate conditions (Linsell et al., 2020). In extreme case scenarios, heat stroke can occur if the core temperature exceeds 40.5°C which can result in a coma if untreated by medical services (Armstrong et al., 2007). Apart from optimizing fitness and heat acclimatization, interventions such as ice baths conducted post-exercise and ice slurry ingestions during exercise can assist the body in cooling, though acclimatization can also reduce the chance for heat illness (Armstrong et al., 2007).

Strategies to cool the body before, during, and post exercise may also improve athletic performance and aide in maintaining optimal health (Bongers et al., 2017) and making it more bearable for athletes to perform in a hot environment. Athletic performance can be enhanced through reducing the body's baseline temperature and/or increasing the range of an athlete's core temperature that they are able to recover without suffering from severe heat stress related side effects (Périard et al., 2015). As the temperature and relative humidity of the environment increases, the reliance on evaporation of sweat for heat dissipation becomes greater (Cheuvront and Haymes, 2010). If sweat is not evaporated, the body will not be able to cool down which forces a significant challenge in humid environments. In contrast, evaporation could account for up to 98% of cooling in dry environments compared to 80% in wet environments. However, sweating can induce dehydration which also causes increases in core temperature and in doing so creating a cycle that makes exercising in the heat extremely difficult for athletes (Casa, 1999). Hence, cooling strategies may help combat the thermal load placed on an athlete.

Physiological adaptions from heat acclimation, resulting in the actual heat acclimatization, include changes in core and skin temperature, sweat rate, electrolyte losses, skin blood flow, and overall thermal comfort (Périard et al., 2015). Studies in the past have shown that achieving and maintaining a core temperature of 38.5°C for one hour a day over 6-21 days stimulated heat acclimation (Périard et al., 2015). These adaptations are achieved in both long-term and short-term scenarios and the effects may vary based on the duration completed. Short-term acclimations are usually less than seven days while long-term adaptations normally require greater than fifteen days. Adaptations to exercise in the heat tend to surface rapidly and normally begin on the first day of exposure to warmer temperatures. Previous research has shown that up to 75% of adaptations occur within the first 4-7 days in longer acclimation periods (Shapiro et al., 1998). The variance between short- and long-term acclimation periods differ in sweat rates, or sudomotor function (Cotter et al., 1997).

Short-term protocols will still result in adaptation that can last for up to one week though its affect dissipates after two weeks post-acclimation which is suitable for various scenarios in athletics (Garrett et al., 2011). Research has also been conducted in other protocols such as once- and twice-daily heat acclimation of consecutive and non-consecutive training days, in which results were comparable in improving erogenicity of heat acclimation on aerobic performance in regard to heat stress (Willmott et al., 2018). Adaptations shown between consecutive and non-consecutive heat acclimation could impact potential training schedules for various groups, allowing ample recovery time for athletes and accommodating traveling schedules. Overall, short term protocols are able to provide a sufficient rise in core temperature of 38.5°C or 1.5°C above baseline in order for heat acclimatization to occur (Garrett et al., 2011; Chalmers et al., 2014). There are four routes of inducing adaption, both short and long-term can be divided into categories such as: constant work rate exercise, workload-based exercise, self-paced exercise, and controlled hyperthermia (Périard et al., 2015; Travers et al., 2020; Taylor & Cotter, 2006; Patterson et al., 2004). Nonetheless, questions still exist about the most efficient method to raise core temperature the quickest way to enable athletes to acclimate in the shortest amount of time.

When applying controlled hyperthermia, it may be difficult for athletes to reach the suggested core temperature threshold of 38.5°C to induce adaptations of heat acclimation (Wardenaar et al, 2021). Therefore, more research is needed to compare and assess the efficacy of several types of warming strategies that affect a rise in core temperature bases on single bout interventions. Although previous research has studied the effect of active acclimatization through exercise, passive heating, or a combination of both passive and exercise protocols, previous studies have not compared differences strategies among these strategies (Périard et al., 2015; Taylor et. al., 2006). Therefore, it is unknown whether one heat acclimatization method is more efficient to increase core temperature than one single method alone.

Study Purpose

The purpose of this study was to evaluate the efficacy of different single-bout heat acclimation strategies, both passive and active to reach and maintain an average core temperature of 38.5°C within a period of 60 minutes. The success of this program will result in: (1) determination of the most efficient heat acclimation protocol to increase core temperature and (2) provide education and strategies for further development of heat acclimation.

Hypothesis

A combination of active and passive method of warming will result in a more efficient raise in core temperature above 38.5°C during 60 minutes of duration in healthy, adults (>18 years old) in Phoenix, Arizona than each method individually. Efficiency is defined as the shortest amount of time required to raise the body's core temperature (>38.5°C).

Definition of Terms

- Heat stress – Physiological stress experienced as a result of excessive heat. (Sharkey, 1979)
- Heat stroke – An acute syndrome caused by an excessive rise in core temperature as the result of overloading or failure of the thermoregulatory system during exposure to heat stress. (Gaudio and Grissom, 2016)
- Exertional heat stroke – An acute syndrome caused by an excessive rise in body temperature as the result of overloading or failure of the thermoregulatory system during exposure to heat stress while exercising in a warm environment. (Casa et al., 2005; Armstrong et al., 2007)
- Acclimatization – A physiological change occurring within the lifetime of an organism which reduces the strain caused by stressful changes in the natural climate (e.g., seasonal, or geographical). (Périard et al., 2015; Armstrong and Stoppani, 2002)
- Acclimation – A physiological change that describes the adaptive changes which occur within the lifetime of an organism in response to experimentally induced

changes in particular climatic factors such as ambient temperature in a controlled environment. (Périard et al., 2015; Armstrong and Stoppani, 2002)

Limitations

- No controlled environment chambers
- Particular High Intensity Interval Training (HIIT) protocol used, only representing one type of exercise
- Foods and fluids consumed prior to interventions
- Not conducting VO_2 max test for fitness evaluations
- Insufficient data to determine workload base for each active warming intervention

Delimitations

- Many athletes already live in the heat and may be at least partly acclimatized
- Differences in general fitness levels
- Nutrition and hydration differences between participants
- Only conducted in young, healthy adults (ages 18-30)
- All participants are non-smokers
- All participants not on medications
- Possible limitation with other ethnicities (depending on participants)
- All participants are male

- Strength differences between participants (sprint during protocol requires resistance, individualized)

CHAPTER 2

REVIEW OF LITERATURE

Thermoregulation

Thermoregulation is an organism's ability to maintain its body temperature within a certain range, even when the surrounding area's temperature is vastly different than that of the body (Cisneros & Goins, 2009). Most organisms are able to adapt to new, or unfamiliar, environmental changes through various mechanisms. When exposed to a hotter environment, the body has mechanistic protocols to thermoregulate in a two-fold process: 1) transference of heat between the environment and the human body utilizing convection, conduction, radiation, and evaporation, and 2) self-regulation of homeostasis as a response to different environmental conditions (Cheng et al., 2012). During convection, the body loses heat due to the movement of air or water across the skin (Keim et al, 2002). In conduction, the body loses heat by physical contact with another object. Radiation is a form of heat loss due to infrared rays or electromagnetic waves; this occurs without any physical contact (Cisneros & Goins, 2009). Evaporation, or sweating, is another route in which the body thermoregulates by converting water to gas on the skin, which aids the body in cooling (Cisneros & Goins, 2009). When exposed to an unfamiliar environment, self-regulation responses of the human include vasoconstriction, in which the body decreases blood flow, and shivering in cooler climates to retain body heat (Cisneros & Goins, 2009). Vasodilation and sweating are two processes in which the body can self-regulate in hotter environments to dissipate heat. The efficacy of thermoregulation can vary depending on age, acclimation to the environment, and relative humidity (Mekjavic & Eiken, 2006). For example, young children tend to lose fluids quicker as their sweat rate is different when compared to an adult (Meyer et al, 1994). The elderly population typically has poor self-regulation processes as part of many physiological processes are slower (Van Someren, 2007). Gradual exposure to environments increases acclimatization (i.e., seasonal acclimatization). Environments with lower humidity can promote greater fluid loss due to increased sweat rate (Périard et al., 2015).

For example, an athlete riding a bicycle outdoors with an ambient temperature of 37°C on a sunny day with 20% relative humidity would be exposed to radiation, conduction, and convection. The body will begin the thermoregulation process by recognizing the difference in skin temperature and core temperature from these factors in the environment. Vasodilation occurs to dissipate heat and the body will start to sweat to cool off. If the relative humidity of the environment were increased, the body would still thermoregulate similarly, however, evaporation will occur at a much slower rate than normal due to the increased moisture of the environment.

Heat Stress and Illness

Heat stress occurs when the body cannot rid itself of excess heat (Sharkey, 1979). Physiologically, the human body strives to maintain balance in various environmental climates to prevent disruption of homeostasis. Yet, when the body is overworked and lacks replenishments of fluids, self-regulating processes cannot compensate, especially when physically active in a hot environment or in a climate with elevated relative humidity (Sharkey, 1979). Heat stress is caused by a combination of environmental conditions including, temperature, humidity, solar radiation, and factors such as physical work rate and the type of clothing the activity is performed in (Sawka et al., 2011). Heat stress signs and symptoms may appear as a wide spectrum of mild to severe.

Prolonged heat stress can emerge when the body loses a large amount of water in sweat and is not replenished in a sufficient time frame. The body is unable to regulate core temperature and rises to dangerous levels. Heat stress can appear in an array of signs and symptoms which can be seen below in **Figure 1** (Centers for Disease Control and Prevention, 2017).

It is important to note that heat stroke occurs when the body is unable to cool itself properly. It is diagnosed by a rectal temperature reading of 40°C or greater and can lead to death or permanent disability if not treated quickly; it is presented as confusion, loss of consciousness, hot and dry skin, or excessive sweating, seizures, and increased internal (core) temperature (Binkley et al., 2002). Heat stroke can also be divided into two distinct types: exertional heat stroke and non-exertional heatstroke. Exertional heat stroke (EHS) relates to non-disabled individuals such as athletes, soldiers, and construction workers. Non-exertional heat stroke is reserved for elderly individuals and has comorbidities such as hypertension, obesity, diabetes, alcoholism, and renal disease (Gaudio & Grissom, 2016).

HEAT-RELATED ILLNESSES

WHAT TO LOOK FOR	WHAT TO DO
HEAT STROKE	
• High body temperature (103°F or higher) • Hot, red, dry, or damp skin • Fast, strong pulse • Headache • Dizziness • Nausea • Confusion • Losing consciousness (passing out)	• Call 911 right away-heat stroke is a medical emergency • Move the person to a cooler place • Help lower the person's temperature with cool cloths or a cool bath • Do not give the person anything to drink
HEAT EXHAUSTION	
• Heavy sweating • Cold, pale, and clammy skin • Fast, weak pulse • Nausea or vomiting • Muscle cramps • Tiredness or weakness • Dizziness • Headache • Fainting (passing out)	• Move to a cool place • Loosen your clothes • Put cool, wet cloths on your body or take a cool bath • Sip water **Get medical help right away if:** • You are throwing up • Your symptoms get worse • Your symptoms last longer than 1 hour
HEAT CRAMPS	
• Heavy sweating during intense exercise • Muscle pain or spasms	• Stop physical activity and move to a cool place • Drink water or a sports drink • Wait for cramps to go away before you do any more physical activity **Get medical help right away if:** • Cramps last longer than 1 hour • You're on a low-sodium diet • You have heart problems
SUNBURN	
• Painful, red, and warm skin • Blisters on the skin	• Stay out of the sun until your sunburn heals • Put cool cloths on sunburned areas or take a cool bath • Put moisturizing lotion on sunburned areas • Do not break blisters
HEAT RASH	
• Red clusters of small blisters that look like pimples on the skin (usually on the neck, chest, groin, or in elbow creases)	• Stay in a cool, dry place • Keep the rash dry • Use powder (like baby powder) to soothe the rash

Figure 1. Warning Signs and Symptoms of Heat-Related Illness. Centers for Disease Control and Prevention

High core temperatures that exceed a tolerable threshold could lead to several disruptions in the body such as increased loss of carbon dioxide through lungs and skin, elevated vasodilation, elevated serum muscle and liver enzymes, increased heart rate, rise in skin temperature, damage to organ tissues, disruption to cell volume, metabolism, membrane permeability, organ dysfunction, cell death and brain damage if not treated promptly could lead to death (Adolph & Fulton, 1923; Armstrong et al., 2007). It is unknown why serum muscle and liver enzymes are elevated in unacclimatized populations as the phenomenon occurs in acclimatized individuals (Wyndham et al, 1974).

In one study looking at 30 unacclimated individuals compared to 15 acclimated miners, glutamic oxaloacetate and creatine phosphokinase increased significantly in both groups when exposed to exercise in a hotter environment (Wyndham et al., 1974). However, enzymes such as glutamic oxaloacetate and pyruvate transaminase were 10% higher in the unacclimated group when exposed to exercise at room temperature than the acclimated group (Wyndham et al, 1974). Heat shock proteins bind to denatured or nascent polypeptides as the body’s mechanism for protection in heat stress. Heat shock protein exposure increases during and after exposure to heat stress (Périard et al., 2015).

At Risk Populations for Heat Stress and Illness

Several populations are at risk, including children, senior citizens, construction workers, active military soldiers, and athletes. In the past, children were thought to be at a disadvantage for thermoregulation due to the greater body surface area to body weight ratio (Falk & Dotan, 2008). However, this population relies heavily on dry-heat dissipations such as convection, conduction, and radiation as their sweat rate is much lower and diffusely space than that of an adult’s body (Dotan, 2021; Inbar, 2004; Falk & Dotan 2008; Rowland, 2008). The disadvantage to these methods is when ambient temperatures exceed the skin temperature for this population as children can reach a higher core temperature quicker than adults (Wagner et al, 1972; Leppäluoto, 1988; Sohar and Shapiro, 1965). During physical activity, children tend to create greater heat production in comparison to adults due to their lower exercise economy and therefore making them more efficient (Falk & Dotan 2008; Rowland 2008).

As humans age, the ability to thermoregulate and dissipate heat properly diminishes significantly. This occurs because the mechanistic routes that the body takes to thermoregulate are affected via metabolic heat production, reduced skin blood flow, smaller increases in cardiac output, reduced sweat gland output, and less redistribution of blood flow from renal and splanchnic circulation (Kenney & Munce, 2003). The elderly population also utilizes polypharmacy, including diuretics, beta-blockers, and anticholinergics which can block the increased cardiac output and sweating needed to cool the body (Gaudio & Grissom, 2016). Seniors can also not adapt or acclimate as quickly compared to young adults. Like the elderly, those with high-risk conditions mentioned above can also suffer from effects from prescription drugs which can alter the body's ability to cool properly.

Construction workers accounted for 36% of occupational heat-related deaths from 1992 to 2016 within the United States alone (Dong et al., 2019). Construction workers and laborers can be subjected to various environmental conditions. They can quickly become dangerous if safe working conditions, protocols, and education is not set in place. According to the CDC, Department of Human Health Services, and National Institute for Occupational Safety and Health (NIOSH), continuous work in hotter environments is not advisable. As the environmental conditions increase in ambient temperatures and humidity, sufficient rest breaks are advised periodically to allow the body to cool down (*Criteria for a Recommended Standard: Occupational Exposure to Heat and Hot Environments*, n.d.)

Another population that can be affected by heat illness includes active-duty military personnel. Members of the military branch can be subjected to a wide variety of environmental conditions depending on the location of the military base and missions.

They must be able to adapt and adjust to a new environment within a condensed time frame while at the same time being prepared mentally and physically for any scenario that may arise (Parsons et al., 2019). Like construction workers, they must perform numerous tasks in extreme conditions such as ceremonial duties, exercise, and protecting and serving the community, all while in uniform and carrying 60-100 pounds (27.3-45.4 kilograms) of gear each day.

The most notable population to be affected by heat illness is athletes. More than 9000 high school athletes are treated for heat-related illnesses in the United States, primarily pre-season football players (Yard et al., 2010). Athletes can range from high school, collegiate, and elite-level athletes such as those in the National Football League (NFL), Major League Baseball (MLB), Olympians, triathletes, and cyclists. Like active-duty military personnel, athletes can be subjected to a wide variety of environmental conditions regarding traveling to new locations for competitions. Locations with high relative humidity may be difficult for an athlete’s body to evaporate or sweat. Exercising in the heat on the human body places a strain on the body’s ability to thermoregulate adequately (Coris et al., 2004). As the body is attempting to cool, vasodilation occurs, which can strain the heart to pump and circulate blood, affecting cardiac output properly. An increase in cardiac output affects the body’s ability to continue maximal exercise performance. Additionally, athletes may be unable to properly thermoregulate due to the type of clothing or uniform they practice and compete. Helmets and padding that are necessary for safety can also play a factor in the body's ability to dissipate heat. Athletes are also more likely to become dehydrated quickly, leading to several kinds of heat-related illnesses.

Treatment for Heat Stress and Illness

Cooling the body is detrimental to survival outcomes in heatstroke victims. Long-lasting effects of heat stroke, if not treated quickly, increases morbidity and mortality rate, which can happen up to one week after its occurrence. Starting the cooling process as soon as possible can also impact the survival rate. Protocols can range from ice packs on the body, sitting in front of a fan (with or without water), splashing tap water on the face, hand and feet immersion, and total body immersion in cold water depending on the heat stress or illness severity (Casa et al., 2007). However, the cooling rate can also vary between protocols. Therefore, an efficient cooling protocol is suggested to cool the body greater than 0.15°C per minute (Kenney & Munce, 2003).

According to the National Health Statistics Report from the Center for Disease Control and Prevention (CDC), from 2006 to 2010, there were 3332 deaths due to heatstroke in the United States alone (Berko, 2014). The gold standard to treat exertional heat illness (EHI), such as heat stroke where the core temperature exceeds 40°C, is a cold-water immersion. This involves submerging the body up to the neck in an ice water bath in which the temperature ranges from 1.7-15°C within a 30-minute timeframe of diagnosis in an attempt to bring the body's core temperature below 39°C (Casa et al., 2007; Nye et al., 2016). The goal is to cool the body back to a baseline or normal temperature range as quickly as possible to prevent long-lasting damage of excessive heat on the body.

Survival rates of EHS are usually 100% without medical complications, though they solely depend on how quickly one can be diagnosed and how efficient the cooling protocol is at returning the body to normal conditions. It is suggested to cool the body within the first 10-minutes of collapse. Within a sports setting, EHS patients have a 4.46 increased risk of developing medical complications if not treated immediately (Filep et al., 2020). Medical complications can appear within 24 hours after EHS diagnosis, which can include lactic acidosis, hyperkalemia, acute renal failure, rhabdomyolysis, and dissemination of intravascular coagulation (Binkley et al., 2002).

Heat Acclimatization

A way to prevent heat-related illness is to acclimatize or adjust to the hotter environment (Sharkey, 1979). Benefits of gradual heat acclimation on the body for exercise include increased thermal comfort, reduced heart rate, increased sweat rate, decreased skin temperature, decreased plasma volume, which leads to an increase in exercise capacity

(Périard et al., 2015). Similar to how athletes acclimate to higher elevations using the "Live high/train low" model, the same can be conducted in hotter and humid climates (Lorenzo et al., 2010).

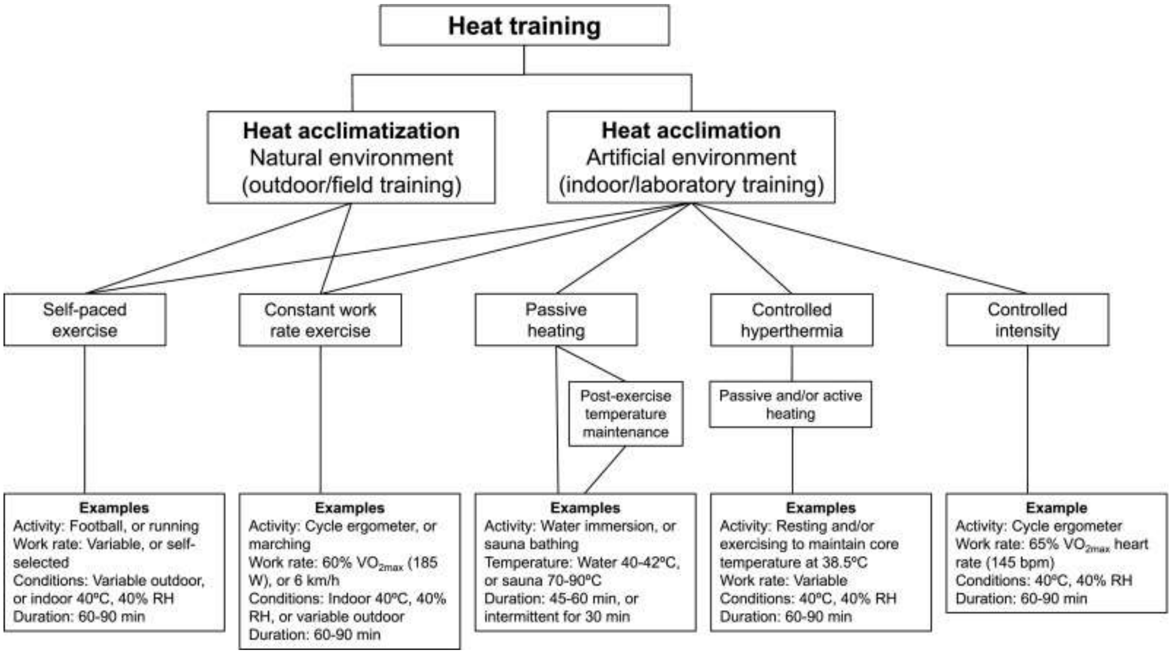

Figure 2. Overview of methods for heat acclimation and heat acclimatization. Various combinations of temperature and humidity are possible in each setting, as well as the use of portable heaters and/or wearing additional or specific type of clothing (Daanen et al, 2018).

Acclimatization can occur naturally or can be induced in a laboratory setting (acclimation), though the routes to induce heat adaptation to vary (Daanen et al, 2018). There are four routes of inducing adaption, both short and long-term can be divided into categories such as, constant work rate exercise, workload-based exercise, self-paced exercise, and controlled hyperthermia (Périard et al., 2015; Travers et al., 2020; Taylor & Cotter, 2006; Patterson et al., 2004). The most common method is the regular work rate exercise which requires an activity to be performed at a consistent rate for a specified duration (Périard et al., 2015). This has been utilized for occupational and the military population and has been effective to improve performance in a hotter environment (Horvath & Shelley, 1946; Lorenzo et al, 2010; Nielson et al, 2015), although it has been argued that this can lead to a decrease in physiological strain as adaptation develop in a progressive manner which also hinders the ability for further adaptation (Taylor, 2000; Taylor 2004). This could result in varied physiological strain depending on an individual's fitness level. In contrast, the workload-based activity requires an athlete to reach a certain heart rate level and will rise as an athlete's fitness level increases; however, it may be more challenging to maintain a high core temperature and lower over time which could impact training (Travers et al., 2020). Self-paced exercise will have participants follow predetermined work and rest intervals but can modify intensity to their comfort level (Travers et al., 2020). This method has also been implemented by the military for unacclimatized individuals prior to deployment in warmer environments (Nelms & Turk, 1972). The hindrance to this method is determining how much heat stress or thermal load the athlete is experiencing (Taylor & Cotter, 2006). The goal of controlled hyperthermia is a forced increase in core temperature 1.5°C above baseline or over 38.5°C while observing and monitoring from a distance. This method has been shown to create a more drastic adaptation to activity in the heat and may result in adaptions for a longer time after the initial introduction period of acclimation (Patterson et al., 2004). The continuous monitoring of core temperature allows for verification that an athlete is experiencing an increased thermal load to adapt to activity in the heat. Monitoring will also assist the heat illness risk in the adverse scenario that the core temperature increases too much during acclimation protocols.

Heat Acclimatization Protocols

Current protocols in several states to prevent heat illness are structured at the beginning of the season in such a way as to minimize the risk for exertional heat illnesses. In football, for example, the coaching staff will not allow players to participate in full padding and equipment at the beginning of the season. Instead, players complete a few practices in limited padding and equipment before completing a session in full uniform. Preparing for activities in the heat reduces the incidences of heat illness and is therefore beneficial. Although decreasing the time or limiting the number of practice sessions can help at a population level, athletes still need to prepare for activity in the heat.

According to the Korey Stringer Institute, a dedicated program in preventing sudden deaths in sports, Arizona is ranked 21st out of 51 states (including the District of Columbia) for safety and policy scores, with a 54% in the year 2020. However, in a report conducted by the same institute in 2019, Arizona was above the average regarding exertional heat stroke in safety and policy.

The National Athletic Trainers' Association positive statement gives several recommendations for heat acclimation guidelines to prevent EHI as shown in **Figure 3** (Casa et al., 2015). Before the start of the season, medical screenings should occur under the supervision of a trained physician to identify if any athletes may be at higher risk or have a history of EHI. Acclimation should occur over 7 to 14 days which involves gradually increasing the intensity and duration of practices and the amount of gear. Other recommendations include for athletes to stay home if feeling ill, maintaining euhydration during practice, and providing plenty of breaks for athletes to recover and hydrate during pre-season practice in hot environments.

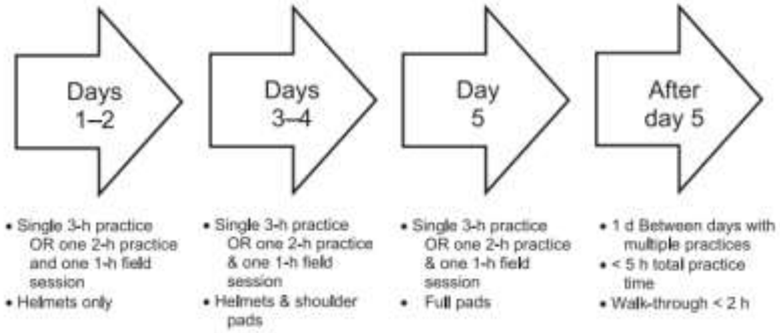

Figure 3. National Collegiate Athletic Association heat acclimatization guidelines (Casa et al, 2015)

Within the state of Arizona, current heat acclimation protocols include several key strategies. On days 1-5, practices are formal, with no more than one session per day and not to exceed 3-hours in length. During days 1-2, only a helmet should be worn during practices if applicable. Between days 3-6 helmets and shoulder pads should be worn. On day six, all necessary protective equipment should be worn. Days 6-14, two practice sessions per day can be applied to all sports, though a one-hour session walk must follow through the next day. On double practice days, practices should not exceed 3 hours in length or 5 hours in total for the day. Practices should be separated by at least 3 hours in a cool environment between sessions (Belval, 2017).

While current protocols within the state of Arizona focus on solutions to prevent EHS, they lack routes and protocols to induce heat adaptation which can improve athletic performance in hotter climates and further reduce the risk of EHS and other illnesses. Areas of improvement include monitoring the environmental conditions using a wet bulb globe temperature or a heat index for pre-season practices held outdoors. The state does not have any policies for modification of work, specifically in areas of rest ratios and access to fluids ad libitum. There seems to be a lack of designated shaded areas for rest breaks during formal practice sessions. Another area of improvement would be the inclusion of a cold-water immersion tub onsite during practices in warmer conditions (Belval, 2017).

The current best practice to achieve heat acclimation is to train in conditions similar to that of competitions and increase body temperature and sweat rate over consecutive training days in a 1-two-week timeframe (Périard et al., 2015). Various protocols can range as short as five to seven days or even longer with 10-14 days of exercise training to reduce the risk of EHS. Studies in the past have shown that achieving and maintaining a core temperature of 38.5°C for one hour a day over 6-21 days stimulated heat acclimation (Périard et al., 2015). These adaptations are achieved in both long-term and short-term scenarios, and the effects may vary based on the duration completed. Short-term acclimations are usually less than seven days, while long-term adaptations requiring greater than 15 days.

Adaptations to exercise in the heat tend to surface rapidly and usually begin on exposure to warmer temperatures. Previous research has shown that up to 75% of adaptations occur within the first four to seven days in more extended acclimation periods (Shapiro et al., 1998). The variance between short- and long-term acclimation periods differ in sweat rates or sudomotor function (Cotter et al., 1997).

While various methods and routes exist to induce heat acclimatization, it is still unknown whether one method is more efficient than others. All routes of heat acclimation can produce results as outlined by **Figure 4** (Périard et al, 2015). Typically, extending the amount of heat exposures improve core temperature adaptation (Daanen et al., 2018), though most benefits from heat adaptation plateau after six days.

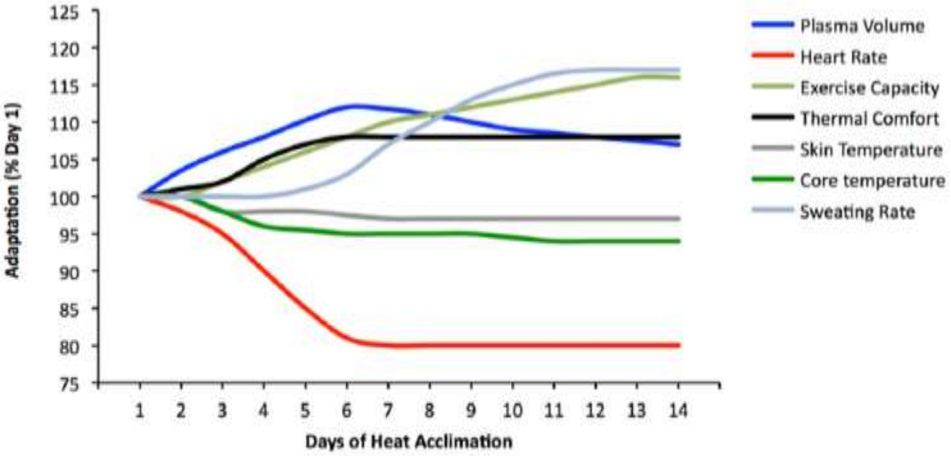

Figure 4. Timeline of induction in human adaptations to heat stress. The degree of these adaptations is affected based several factors such as an individual's initial level of acclimation, the environmental conditions (i.e., dry or humid), exercise intensity, and approach for acclimation (Périard et al, 2015).

Previous research has been conducted using a mixed methods and various lengths of heat acclimation protocols. In one study using sauna exposure, a form of controlled hyperthermia method, in combination with an exercise at a controlled rate of 65% of VO_2 max resulted in a reduction in resting core temperature and heart rate, exercise core temperature and heart rate, and skin temperature in females (Mee et al., 2018). Although this study is promising in the use of a combination method for heat acclimatization, females tend to have a higher elevated core temperature threshold during menstrual cycles (Inoue et al., 2005). Another limitation to providing a controlled hyperthermia approach prior to exercise is decreased thermal comfort and exercise performance (Linsell et al., 2020).

Other studies have combined methods of exercise with controlled hyperthermia in the form of sauna or hot water immersion have also been promising in males (Scoon et al., 2007; Stanley et al., 2015; Zurawlew et al., 2016).

Scoon et al. performed a cross-over study for eight weeks, with a 3-week washout period, examining the effects of physiological adaptations of sauna bathing to enhance endurance performance (Scoon et al., 2007). Six male competitive long-distance runners and triathletes (23± 3yr, 81 ± 5 kg body mass) were randomized into two groups of three for a cross-over design. Following their normal endurance training sessions, participants sat in a sauna (89.9 ± 2.0°C) for 31 ± 5 minutes over 12.7 ± 2.1 occasions (Scoon et al., 2007). Measurements such as blood pressure, and heart rate were recorded 5-minutes before, during, and post sauna intervention. Following the bouts of sauna exposure, participants performed a run to exhaustion test on a treadmill with zero slope at their current best 5-kilometer time (~12-15 minutes to elicit fatigue). Following the performance test, blood volumes were measured before and during the 10,15, 20, and 25-minutes. Plasma volume was calculated as well. The researchers found an increase in plasma volume (7.1%) and an increase in red cell volume (3.5%), which aligns with the current literature on physiological adaptations from a heat acclimation protocol (Scoon et al., 2007, Périard et al., 2015). When researchers evaluated the efficacy of this intervention in terms of exercise performance, participants had an increase of 32% in time to exhaustion after sauna exposure (Scoon et al., 2007). Scoon et al. reported that this is equivalent to approximately a 2% decrease in time for a 5000-meter timed trial (Scoon et al., 2007). Stanley et al. also examined post-exercise sauna exposure on plasma volume expansion and documented changes in heart rate-based measurements (Stanley et al., 2015). Researchers followed seven male, well-trained cyclists (23.3 ± 4 years, body mass 78.0 ± 6.6 kg) for 35 consecutive days. Participants reported total time spent and subjective intensity of their training (rate of perceived exertion) throughout the study's duration. Following the participants' daily training schedule, a post-exercise sauna exposure was introduced on days 18-28 of the study for 30-minutes in total duration each time (87°C, 11% RH). Measurements such as blood pressure, tympanic temperature, and thermal comfort and sensation were recorded 5 minutes before, during, and after the sauna intervention. Participants also completed a sub-maximal cycling test which consisted of 125 watt and a cadence of 100 rpm for 5-minutes total to assess heart rate during and in the recovery period following the test (10-minutes post). This test was performed periodically throughout the duration of the study (days 1, 8, 15, 22, 25, 29, and 35). As a result this research team reported an increase in plasma volume (17.8%) during and following post-training sauna exposure (Stanley et al., 2015). Plasma volume increased largely within the first 4 days of exposure compared to the average values during the pre-sauna exposure training days (Stanley et al., 2015). The research team noticed that post-exercise sauna exposure elicits physiological adaptations that represent heat acclimation. It was also observed that plasma volume decreased to pre-intervention levels after the final sauna exposure. In addition, trivial to small changes in peak heart rate or peak power output were observed over the duration of the study (Stanley et al., 2015). Zurawlew et al. examined the effects of hot water immersion after exercise to improve exercise performance in temperate and hot conditions. Seventeen physically active, non-acclimatized males participated were randomized to either the control (n=7, 23 ± 3 years, body mass 72.1 ± 5.8 kg) or intervention group (n=10, 23 ± 3 years, body mass 69.5 ± 6.9 kg) for 6 days (Zurawlew et al., 2016). Experimental trials were conducted before and after intervention days, consisting of a 40-minute submaximal run, 60-minutes of rest, followed by a timed trial 5-kilometer on a treadmill. Participants were subjected to these experimental trials in both temperate (18.0 ± 0.1 °C, 42.5 ± 3.6% RH) and hot conditions (33.0 ± 0.3 °C, 40.2 ± 0.7% RH) (Zuralew et al., 2016). Before testing days, participants performed a submaximal exercise which consisted of running on a treadmill at 65% VO_{2max} in a temperate environment (18 °C) dressed in running shorts, socks, and shoes. Following the exercise protocol, participants were subjected to either the controlled thermoneutral water immersion (34.1 ± 0.4 °C) or hot water immersion (39.9 ± 0.3 °C) up to the neck for 40-minutes (Zurawlew et al., 2016). Participants were allowed to remove themselves from the water immersion intervention if they felt any discomfort. During these experiments, measurements such as body temperature (T_{re}), heart rate, physiological strain, sweating response, and blood samples were obtained. As a result, heat acclimation was observed in the hot water immersion group by an increase in sweat rate by the fourth day (1.08 ± 0.30 L/hr, P=0.02) and increased immersion time day the third day (P=0.04) (Zurawlew et al., 2016). All participants in the control group were able to complete the 40-minute water immersion, and no changes in sweat rate were observed (0.39 ± 0.08 L/hr) (Zurawlew et al., 2016). In terms of experimental trials, the hot water immersion group showed improvement in heat strain in hot and temperate conditions and the 5-kilometer timed trial test in the heat. No changes were found in the control group regarding the experimental trials.

Practically speaking, heat acclimation protocol designs may pose some challenges to athletes, such as cold temperate environments, costs of travel to heat camps, or access to passive heating methods such as heat chambers (Casadio et al., 2017). Current recommendations for heat acclimation alternatives include post-exercise sauna exposure, although no studies have studied the efficacy to induce thermoregulation adaptations or performance enhancement (Casadio et al., 2017).

Previous work conducted by the Athleat Field Lab has suggested that a 5-day acclimation protocol may be sufficient while maintaining exercise capacity (Wardenaar et al, 2021), yet it is still unclear if a combined method of approaches in athletes is more efficient when compared to other methods for heat acclimatization.

CHAPTER 3

METHODS

Study Design

The research design was non-randomized field study, factoring in the natural environment at the end of summer in the desert. The study was to evaluate four warming strategies for feasibility and efficacy in bringing the body's core temperature to 38.5°C. Each participant completed all interventions outdoors including one intervention in a thermoneutral environment. Time and changes in core temperature were monitored as measures of efficiency. The study was performed at Arizona State University (ASU) in Tempe, Arizona in September-October of 2020. The research protocols were reviewed and approved by the ASU IRB (#20193446). See Appendix A for flow chart of the study design after including participants.

Participants

For this study, n=10 healthy, uninjured, active, male athletes (18-30 years old) were considered. Participants were non-smokers, using no medications were athletically fit (training approximately five to ten hours per week). Subjects were primarily recruited through ASU club sports, but recruitment was open for outside participants as well from gyms in the surrounding area. Participants were recruited through email and poster advertisements. The study was approved, by the ASU IRB (1273622) and all participants read and signed informed consent. All participants received $90 after completion of the study. Participants were screened for any contraindications with the physical activity readiness PAR-Q questionnaire (Appendix B) and for the use of E-Celsius core temperature capsule (Appendix C).

Procedures

Participants came into the research facility at their respective time slots. The number of ten participants was chosen for this crossover study after evaluating other heat acclimation studies and their sample sizes used to show significant adaptions. (Burdon et al., 2013; Chinevere et al., 2008; Patterson et al., 2004).

Participants were randomly assigned a study ID number (1-10) by a researcher team member. The number identified the participant, water bottles, urine sample cups, skin temperature devices, and elastic chest strap device. The investigators identified participants by their ID numbers to avoid bias in associating results with names.

Each participant was sent an email reminder from the research team about the study days and scheduled time. Once at the research facility, each participant’s core temperature capsule was synchronized with a monitor. They were then asked to use the facility bathroom stall and provide a urine sample. They voided their bladders completely into large specimen containers. Participants were then taken to a designated area to attach monitored devices (elastic chest strap and skin temperature buttons) and collected a semi-nude pre-exercise weight. Once each participant completed an intervention, a second urine sample was collected in the same method, followed by a second semi-nude post-exercise weight in their designated area. Height of the participants was collected and recorded once during the time of the study. A research assistant measured the weight of the urine voided (pre and post) and transferred it into a 30 mL test tube to measure urine specific gravity (USG).

Heating Strategies

During each visit to the lab, participants were asked to perform in the following 60-minute interventions:

Passive heating (PAS): The passive heating strategy consisted of sitting in a heated tent at approximately 38-40°C. Participants were allowed to enter and exit the tent as desired throughout the intervention, but the objective was to have them remain in the environment for as much time as possible in the 60-minutes. Participants wore light, athletic clothing in this environment.

Exercise in heat and passive heating (EH-PAS): Participants completed a HIIT protocol that consisted of stationary biking at 80-90 RPM for two minutes followed by 5-10 two-minute sprints (100 RPM) at higher resistance and 2 minutes of relative rest at 80-90 RPM for a total of 30 minutes in a heated tent (35°C) with standardized clothing and watertight coverall suit. After reaching a target core temperature of 38.5-39.5°C (101.3-103.1°F), participants were instructed to keep a constant work pace to maintain their temperature within the target until they have reached 30 minutes of exercise duration. Participants then removed the coverall suit and were led to a 30-minute PAS as outlined above.

Exercise in a Hot Environment (EH): Participants completed a HIIT protocol as outlined above in a heated tent (35°C). After reaching a target core temperature, participants were instructed to keep a constant work pace to maintain their temperature within the target until they had reached 60 minutes of exercise duration. Participants wore a watertight coverall suit for insulation during this intervention.

Exercise in Moderate Conditions (EM): Participants completed a HIIT protocol as outlined above in the setting of a moderate environment indoors (20°C; 68°F). After reaching a target core temperature, participants were instructed to keep a constant work pace to maintain their temperature within the target until they had reached 60 minutes of exercise duration. Participants wore a watertight coverall suit for insulation during this intervention.

Interventions were separated by one week to minimize the effect on heat acclimation over time. No effects on heart rate, rectal temperature, skin temperature, or VO_2 and total sweat loss using this strategy (Barnett and Maughan, 1993).

Measurements

Measurements were taken within 10-minutes prior to each intervention. The following is a list of measurements taken during the study: core temperature, skin temperature, heart rate, fluid intake, urine samples (pre and post), height, body weight (pre and post), and environmental factors (temperature, humidity, saturated vapor pressor, actual vapor pressure). Measurements collected during interventions were also used to calculate body mass change, sweat rate, and estimated energy expenditure. An explanation of how each measurement was performed can be found in the following sections.

Physiological Measurements

Core Temperature (T_c)

A portable telemetry system (E-Celsius, BodyTemp, France) was used to measure core body temperature (T_c), which is a safe and reliable method to examine at rest and during exercise (Gant et al., 2006). E-Celsius tablets have an accuracy of +/- 0.2°C (+/- 0.36° F) (BodyCap USA, 2020). Subjects ingested an individually calibrated telemetric temperature pill on the evening preceding the exercise, to avoid interaction with fluid ingestion during testing (Wilkinson et al., 2008). T_c is measured at 15-second intervals. Baseline T_c was determined as the average of 30-seconds before beginning each test. Average T_c was calculated based on all output during tests. The highest value of the continuous T_c measurements was presented as peak T_c and increment in T_c (ΔT_c) was calculated as peak T_c minus baseline T_c.

Skin Temperature (T_{sk})

T_{sk} was assessed using wireless temperature recorders (iButton DS1+2L, Dallas Semiconductor Corp, USA) set to acquire temperature samples at 20-second intervals with a resolution of 0.0625°C (Smith et al., 2010). iButton device can operate in ambient temperature of -55 °C to +100 °C. It has an accuracy of ± 0.5°C within the range of 0°C to +70°C. In the ranges of -40°C to 0°C and +70°C to +85°C, the accuracy decreases to 1.0°C (Maxim Integrated Products, 2002). For T_{sk}, the temperature recorders were attached to the skin using Opsite transparent film (Opsite Flexigrid, Smith & Nephew Medical Limited, Hull, England). T_{sk} was measured at four locations (neck, right shoulder, left hand, and right shin) and T_{sk} mean was calculated using a weighted average of the four sites (Standard, 2008). Each site had a weighted coefficient according to each site; neck, right shoulder, and right shin of 0.28 with the left-hand coefficient of 0.16. Baseline T_{sk} was measured as the average of three consecutive measurements directly before each intervention. Average T_{sk} was calculated based on all output during tests. The highest value of the continuous T_{sk} was presented as peak T_{sk} and increment in T_{sk} (ΔT_{sk}) will be calculated as peak T_{sk} minus baseline T_{sk}.

Heart Rate, breathing rate and activity

Participants wore an elastic chest strap device (Bioharness-3, Zephyr Technology, Annapolis, USA) that included a 3-axis accelerometer providing a heart rate (HR), breathing rate (BR), and an activity score. HR was measured at 1-second intervals directly before the start of the selected interventions and continuously until the end of the interventions. Baseline HR was measured as the average of the three lowest values within 30-seconds prior to beginning interventions. Average HR was calculated based on all output during tests. The highest value of the continuous HR was presented as peak HR and increment in HR (Δ HR) was calculated as peak HR minus baseline HR. HR baseline and HR peak were delineated from the output.

Fluid intake

Participants were allowed to drink water at room temperature (20°C) ad libidum during the warming phase. All drinks, within 30 minutes before the start and 15 minutes after each test or training session that the subject planned to consume were labeled and weighed on a scale (PT 1400, Sartorius AG, Göttingen, Germany). Directly after each session, all products or bottles were weighed to determine how much the participant consumed.

Urinalysis

All subjects provided an all-out urine sample before and after each intervention. The most important aim of collecting the urine sample was to make sure the bladder was empty to measure clean body weight. Participants voided their bladders completely into a urine bottle and transferred into specimen cups. A 30 mL urine sample was used and analyzed at a standard sample temperature of 20°C to determine urine specific gravity (Pocket refractometer, ATAGO, Tokyo, Japan). A urine specific gravity of ≥1.020 g/mL is indicative for underhydration (McDermott et al., 2017).

Body Height and Weight

Directly before starting interventions, height was measured once via a stadiometer to the nearest 0.1 cm. Body weight was measured semi-nude in dry underwear after voiding before and after each intervention. Participants then changed into sporting clothes. Body weight was taken with a digital scale in kilograms (kg).

Environmental Measurements

During each experiment, ambient temperature and relative humidity was measured. After this, saturated vapor pressure and actual vapor pressure were calculated to measure the moisture of the environment using the following formula (US Department of Commerce, 2019) where T_{air} equals the air temperature (°C) and T_{dew} equals the dew point temperature (°C):

$$\text{Saturated Vapor Pressure} = 0.61365^{(17.502 * \mathrm{T_{air}})/(240.97 + \mathrm{T_{air}})}$$

$$\text{Actual Vapor Pressure} = 0.61365^{(17.502 * \mathrm{T_{dew}})/(240.97 + \mathrm{T_{dew}})}$$

Environmental measures will be logged every 1-minute and will be reported as mean ± SD for each environment for interventions. Two separate measurements will be reported for EH-PAS (Tent 1 and Tent 2, respectively) as each participant spent a total of 30-minutes in both environments for this intervention.

Calculations

Body mass change and Sweat rate

Sweat rate (mL/h) was calculated as a combination of semi-nude body mass, fluid intake, and urine output data using the following formula (Baker, 2017):

$$\text{Sweat rate (mL/h)} = \frac{(\text{pre}-\text{exercise body mass}-\text{post exercise body mass} + \text{fluid intake} - \text{urine output})}{\text{Exercise duration}}$$

The relative change in body mass (in %) between the measurement immediately before the start and directly after each test or training session was calculated. The weight of the collected urine excretion before exercise and directly afterwards (within 15 minutes) after ending each intervention measured within 0.1-gram accuracy (PT 1400, Sartorius AG, Göttingen, Germany) at one decimal point (kg) was inserted in the formula to estimate sweat rate.

Estimated Energy Expenditure

Work rate, the absolute intensity expressed in metabolic equivalents (METs), was estimated based upon a linear regression approach derived from the 3-axis accelerometer, HR, and BR. METs were calculated for energy expenditure (in terms of kcals) by using the following formula (Olzinski, 2019):

$$\text{Energy Expenditure (kcal)} = \text{METs} * \text{body weight (kg)} * \text{hours of exercise}$$

Statistical Analysis

Results were presented as mean ± SD. The primary variables are time to reach ≥38.5℃ core body temperature as well as baseline, peak, increment, and average core temperature for the 60-minute trials (slope and time increments per 10 minutes). Additionally, the total outcomes were reported for total fluid intake and sweat rate. Primary variables were assessed in 30-minute increments to assess differences in the EH-PAS intervention. Averages were reported for environmental conditions (temperature, relative humidity, saturated vapor pressure, actual vapor pressure), skin temperature, and heart rate.

Differences between treatments was assessed using repeated measures ANOVA and post hoc analyses or their non-parametric counterparts. For all tests significance was set for $P < 0.05$.

CHAPTER FOUR

RESULTS

A total of ten athletes participated in all four interventions (club athletes, n=5; ROTC, n=1; other, n=4) in the WUCD study. Data was normally distributed, and results are presented as mean and SD. Data for weight and BMI was not normally distributed and therefore presented as median and IQR. The population consisted of male athletes ages 24.5 ± 3.4 years, with an average height of 180 ± 11 cm and weight of 91.9 ± 24.6 kg [median (IQR): 84.5 kg (81.4-89.4)]. All athletes reported to be healthy, free from injuries, with a 5-10 hours of training per week. Characteristics on participants can be found in **Table 1.**

Table 1. Characteristics of participants

Participants (*n*)	10
Gender	Males
Age (y) mean, SD	24.5 ± 3.4
Height (cm) mean, SD	180.0 ± 11.1
Weight (kg) mean, SD	91.9 ± 24.6*
BMI (kg/m^2) mean, SD	28.4 ± 6.3**
Category	
Club Athlete	5
ROTC	1
Other	4
Hours training per week (range)	5-10

*Weight (kg) median (IQR): 84.5 (10.55), **BMI (kg/m^2) median, (IQR): 25.5 (10.1)

Table 2. Environmental measurements presented as mean and ± SD for different interventions and significant differences.

Conditions	**PAS**^{a}	**EH-PAS**^{b}	**EH**^{c}	**EM**^{d}	**P**
Ambient Temperature (°C)					
Average	53.8 ± 1.1 $^{b1, c, d}$	**Tent 1**: 43.3 ± 1.1 $^{a, b2, d}$ **Tent 2**: 54.0 ± 2.3 $^{b1, c, d}$	42.8 ± 1.4 $^{a, b2, d}$	22.4 ± 0.4 $^{a, b1, b2, c}$	<0.001 †
Peak	58.0 ± 1.7 $^{b1, c, d}$	**Tent 1**: 50.8 ± 1.5 $^{a, b2, d}$ **Tent 2**: 58.4 ± 2.1 $^{b1, c, d}$	49.1 ± 3.1 $^{a, b2, d}$	22.89 ± 0.5 $^{a, b1, b2, c}$	<0.001
Relative Humidity (%)					
Average	13.5 ± 2.2 $^{c, d}$	**Tent 1:** 18.3 ± 6.6 $^{c, d}$ **Tent 2**: 20.4 ± 9.2 ^{d}	10.0 ± 1.8 $^{a, b1, d}$	34.7 ± 0.5 $^{a, b1, b2, c}$	<0.001 †
Peak	30.9 ± 13.2 ^{d}	**Tent 1:** 39.5 ± 26.1 $^{b2, c}$ **Tent 2**: 47.7 ± 21.1 $^{b1, c}$	12.2 ± 1.6 $^{a, b1, b2, d}$	41.7 ± 4.7 ^{c}	0.04 †‡
Saturation Vapor Pressure (kPa)					
Average	15.3 ± 1.0 $^{b1, c, d}$	**Tent 1:** 9.0 ± 0.6 $^{a, b2, d}$ **Tent 2:** 15.4 ± 1.7 $^{b1, c, d}$	8.7 ± 0.7 $^{a, b2, d}$	2.7 ± 0.1 $^{a, b1, b2, c}$	<0.001 †
Peak	18.4 ± 1.4 $^{b1, c, d}$	**Tent 1:** 13.0 ± 0.9 $^{a, b2, d}$ **Tent 2:** 18.7 ± 1.9 $^{b1, c, d}$	12.0 ± 1.9 $^{a, b2, d}$	2.8 ± 0.1 $^{a, b1, b2, c}$	<0.001
Actual Vapor Pressure (kPa)					
Average	2.0 ± 0.3 $^{b1, c, d}$	**Tent 1:** 1.3 ± 0.2 $^{a, b2, c, d}$ **Tent 2:** 2.3 ± 0.4 $^{b1, c, d}$	0.8 ± 0.1 $^{a, b1, b2}$	2.7 ± 0.1 $^{a, b1, b2, c}$	<0.001 †
Peak	3.4 ± 0.4 $^{b1, c, d}$	**Tent 1:** 1.8 ± 0.2 $^{a, b2, c, d}$ **Tent 2:** 4.0 ± 0.3 $^{b1, c, d}$	1.2 ± 0.3 $^{a, b1, b2}$	2.8 ± 0.1 $^{a, b1, b2, c}$	<0.001 †

b1 = Tent 1 of EH-PAS environment
b2 = Tent 2 of EH-PAS environment
† Greenhouse-Geisser utilized with P-value set at 0.05
‡ Repeated Measures ANOVA without Bonferroni Correction with P-value set at 0.05

Environmental Conditions

The environmental conditions as mentioned in **Table 2** were significantly different between intervention for average ambient temperature (P<0.001), peak ambient temperature (P<0.001), average relative humidity (P<0.001), peak relative humidity (P=0.04), average saturation vapor pressure (P<0.001), peak saturation vapor pressure (P<0.001), average actual vapor pressure (P<0.001), and peak actual vapor pressure (P<0.001). Tent 2 of EH-PAS environment was the hottest in peak ambient temperature (58.4 ± 2.1 °C) followed by the PAS environment (58.0 ± 1.7 °C) and third was EH environment (49.1 ± 3.1 °C). EM was the coldest environment as it was conducted indoors as opposed to the other three interventions which were conducted outdoors.

Conditions of tent one of EH-PAS were comparable to that of the conditions for EH. No significant differences noted amongst the environmental conditions for tent 1 of EH-PAS and EH in terms of average ambient temperature (P=0.09), peak ambient temperature (P=0.07), average saturation vapor pressure (P=0.08), and peak saturation vapor pressure (P=0.07). Conditions of tent two of EH-PAS were comparable to that of the conditions for PAS. No significant differences noted amongst environmental conditions for tent 2 of EH-PAS and PAS in terms of average ambient temperature (P=0.50), peak ambient temperature (P=1.00), average saturated vapor pressure (P=0.50), peak saturated vapor pressure (P=1.00), average actual vapor pressure (P=0.20), and peak actual vapor pressure (P=0.06).

Table 3. Hydration and Estimated Energy Expenditure presented as mean and ± SD for different interventions and significant differences.

	PAS^{a}	**EH-PAS^{b}**	**EH^{c}**	**EM^{d}**	**P**
Hydration status					
Urine Specific Gravity					
Pre-Exercise	1.012 ± 0.008	1.011 ± 0.010	1.013 ± 0.009	1.011 ± 0.008	0.81
Post-Exercise	1.018 ± 0.007	1.008 ± 0.006	1.011 ± 0.008	1.012 ± 0.009	0.06 †
Saliva Osmolality					
Pre-Exercise	71.1 ± 29.8	67.5 ± 18.4	71.0 ± 13.2	73.2 ± 10.9	0.86
Post-Exercise	67.7 ± 23.8 $^{b, c, d}$	90.9 ± 27.7 ^{a}	95.7 ± 33.9 ^{a}	101.9 ± 31.6 ^{a}	0.04 ‡
Sweat Rate (mL/hr)	1071 ± 551 $^{b, c}$	1737 ± 416 $^{a, d}$	1698 ± 657 ^{a}	1289 ± 298 ^{b}	<0.001
Fluid Intake (mL)	747 ± 802	1084 ± 750	1020 ± 837	563 ± 604	0.07
Absolute Body Weight Change (%)	0.38 ± 0.36 $^{b, c, d}$	0.92 ± 0.59 ^{a}	1.16 ± 0.81 ^{a}	0.92 ± 0.60 ^{a}	0.002 ‡
Energy Expenditure (kcal)	228 ± 85 $^{b, c, d}$	443 ± 125 $^{a, c}$	510 ± 121 $^{a, b}$	492 ± 107 ^{a}	<0.001

Hydration Status

Variables pertaining to the athletes’ hydration status and fluid balance among each intervention are described in **Table 3.** No significant difference was found between conditions, although fluid intake during EH-PAS and EH produced comparable results (1084±750 and 1020±837 mL, respectively), while PAS (747±802 mL) and EM (563±604 mL) participants drank 250 and 500 mL less, respectively.

Urine Specific Gravity (USG) values were measured pre-exercise and post-exercise resulting in USG Values listed in **Table 3**. The values ranged from 1.003 (well-hydrated) to 1.032 (severe dehydration), which were recorded before and after each intervention. No significant difference was found pre- and post each intervention (P=0.81, P=0.06 respectively).

Calculated sweat rate was the highest for an EH-PAS method (1737 ± 416 mL/hr), which was significantly different compared to PAS and EM (P=0.004; P=0.001, respectively). The sweat rate in the EH was slightly lower in volume of 1698 ± 657 mL/hr and was significant when compared to the PAS intervention (P=0.048).

On average, EH method produced the largest difference of absolute body weight loss of 1.16 ± 0.81%, being significantly different from PAS 0.38 ± 0.36% (P=0.01), but not from the other interventions (P >0.05). An EH-PAS method also produced a significant difference of absolute body weight loss of 0.92 ± 0.59 when compared to PAS method (P=0.02). No differences noted between absolute body weight change between EH-PAS and EM (P=0.96)

Estimated Energy Expenditure

Estimated total energy expenditure (EE) was significantly different between the four interventions (PAS: 228 ± 85 kcal, EH-PAS: 443 ± 125 kcal, EH: 510 ± 121 kcal, and EM: 492 ± 107 kcal, respectively; $P < 0.001$). After pairwise comparisons, statistical significance was found for total EE between PAS and EH-PAS ($P < 0.001$), EH ($P<0.001$), and EM ($P<0.001$). EH was also significantly different when compared to an EH-PAS method ($P = 0.002$).

Table 4. Physiological outcomes presented as mean and ± SD for different interventions and significant differences.

	PAS^{a}	EH-PAS^{b}	EH^{c}	EM^{d}	P
Core Temperature (°C) ◊					
Baseline	37.3 ± 0.3	37.3 ± 0.2	37.3 ± 0.2	37.1 ± 0.5	0.37 †
Average	37.5 ± 0.4 $^{b, c}$	38.2 ± 0.2 $^{a, d}$	38.2 ± 0.1 ^{a}	37.9 ± 0.2 ^{b}	<0.001
Increment	0.8 ± 0.2 $^{b, c}$	1.8 ± 0.6 ^{a}	1.6 ± 0.5 ^{a}	1.3 ± 0.6	<0.001
Peak	38.1 ± 0.5 ^{b}	39.1 ± 0.4 $^{a, d}$	38.9 ± 0.3 ^{d}	38.4 ± 0.3 $^{b, c}$	<0.001
Heart Rate (bpm)					
Baseline	73 ± 13.1	86 ± 24.5	79 ± 15.6	80 ± 20.9	0.28
Average	98 ± 15.2 $^{b, c, d}$	142 ± 12.3 ^{a}	146 ± 9.7 ^{a}	142 ± 13.3 ^{a}	<0.001
Increment	60 ± 15.9 $^{b, c, d}$	101 ± 29.8 ^{a}	102.6 ± 14.4 ^{a}	94 ± 22.5 ^{a}	<0.001
Peak	134 ± 20.5 $^{b, c, d}$	186 ± 9.7 $^{a, d}$	181 ± 9.1 ^{a}	174 ± 12.8 $^{a, b}$	<0.001 †
Skin Temperature (°C) □					
Baseline	33.5 ± 1.18	34.7 ± 0.56 ^{d}	34.4 ± 0.56 ^{d}	32.7 ± 0.73 $^{b, c}$	0.004
Average	39.1 ± 0.28 $^{b, c, d}$	38.2 ± 0.45 $^{a, c, d}$	36.8 ± 0.49 $^{a, b, d}$	33.8 ± 0.51 $^{a, b, c}$	<0.001
Increment	6.7 ± 1.50 $^{c, d}$	5.4 ± 0.90 $^{c, d}$	3.60 ± 0.58 $^{a, b, d}$	2.17 ± 0.58 $^{a, b, c}$	<0.001
Peak	40.3 ± 0.58 $^{c, d}$	40.1 ± 0.71 $^{c, d}$	38.0 ± 0.43 $^{a, b, d}$	34.9 ± 0.65 $^{a, b, c}$	<0.001

Sample size n=10
◊Core Temperature n=7
□Skin Temperature n=5
P-value set at 0.05 based on Sphericity Assumed.
Differences between categories are indicated with lowercase letters with respect to intervention (repeated-measures ANOVA; $p<0.05$, with post-hoc Bonferroni correction)

Physiological Outcomes

Total heating protocol (0-60 minutes)

EH-PAS and EH produced comparable results in terms of average, increment, and peak T_c. When compared to PAS, an EH-PAS intervention produced significant results in terms of average (P=0.02) and peak T_c (P=0.03) while EH produced significant results in terms of average (P=0.04) and increment T_c (P=0.01). A total of 8 participants were able to reach or exceed the targeted core temperature of 38.5°C in the EH-PAS intervention compared to seven participants in a EH method. **Figure 5** shows peak T_c expressed as box plots with all individual responses spanning over the four interventions. No significance noted for baseline T_c among interventions (P=0.37).

When looking at HR across all four interventions, a similar pattern is visible as shown for T_c, confirming a relationship between HR and body core temperature. The peak HR in PAS was significantly lower than during the other interventions involving exercise (EH-PAS P<0.001; EH P<0.001; EM P=0.01). **Figure 5** shows the peak HR expressed as box plots with all individual responses spanning over the four interventions. No significance noted between baseline HR among interventions (P=0.28). **Figure 5** also shows the outcomes for T_c and HR across all interventions with 10-minute increments.

Baseline skin temperatures were noted to be significant (P=0.004), though only baseline T_{sk} for EM was significant when compared to EH-PAS (P=0.001) and EH (P=0.003).

Baseline T_{sk} for EM was not significant when compared to PAS (P=0.28). A PAS method yielded the highest results in terms of average, increment, and peak T_{sk} (39.1 ± 0.28°C, 6.7 ± 1.50°C, and 40.3 ± 0.58°C respectively). An EH-PAS intervention produced the second highest average (38.2 ± 0.45°C), increment (5.4 ± 0.90°C), and peak T_{sk} (40.1 ± 0.71°C). All interventions produced a statistically significant result amongst each other in terms of average, increment, and peak T_{sk} (P<0.001).

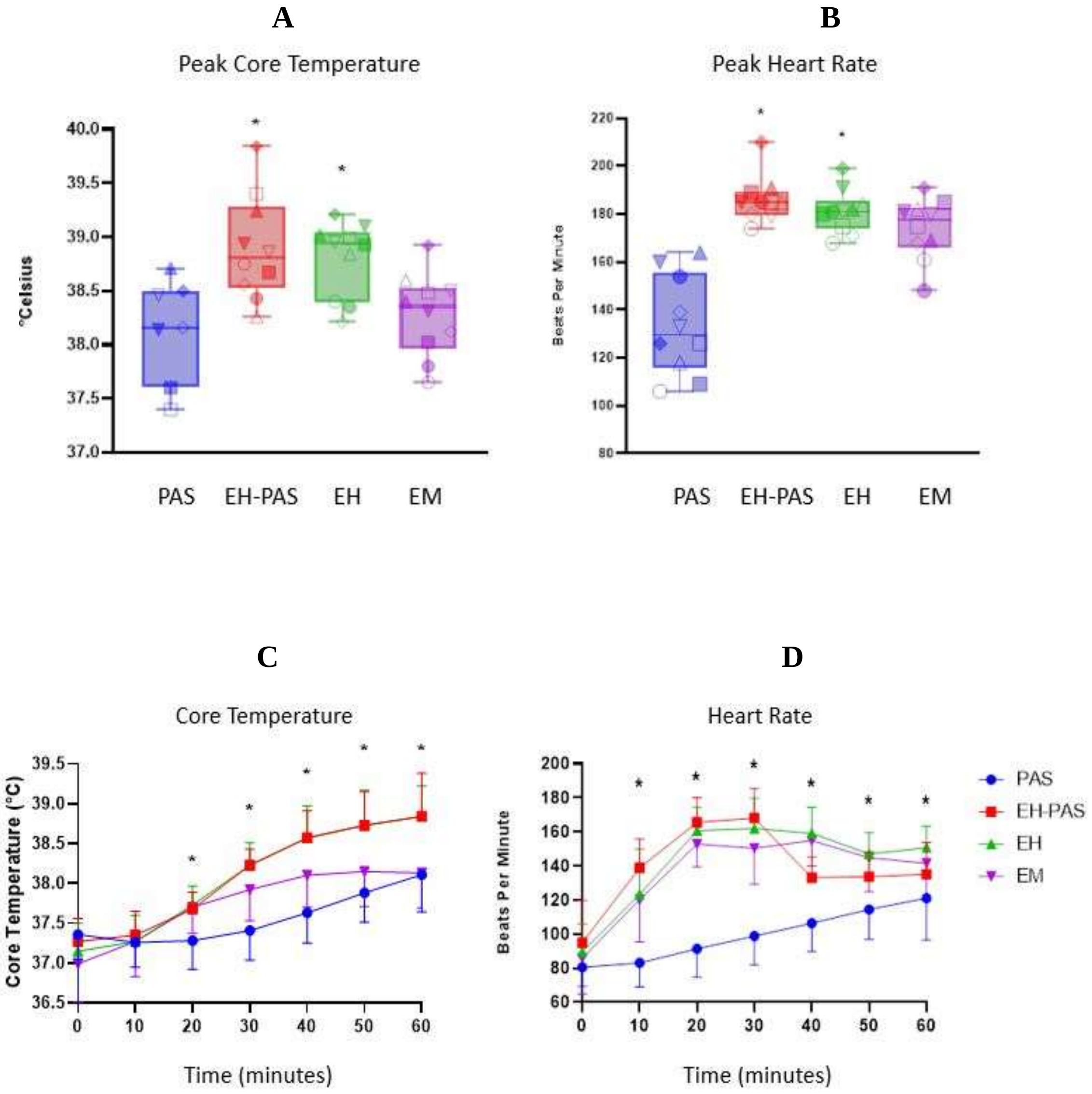

Figure 5. Boxplots and individual responses for A: Peak T_c and for B: Peak HR (n=10), and outcomes over one-hour time of participants during interventions for C: Average T_c and D: Average HR. n= 10; *$P < 0.01$. Error bar represents 95% CI for A and B standard deviation for C and D

Table 5. Mean T_c (°C) ± SD reported in 10-minute intervals during one-hour period for all interventions. *P < 0.01

Intervention	0	10	20*	30*	40*	50*	60*
PAS	37.4 ± 0.3	37.3 ± 0.3	37.3 ± 0.4	37.4 ± 0.4	37.6 ± 0.4	37.9 ± 0.4	38.1 ± 0.5
EH-PAS	37.3 ± 0.3	37.4 ± 0.3	37.7 ± 0.2	38.2 ± 0.2	38.6 ± 0.3	38.7 ± 0.4	38.8 ± 0.5
EH	37.2 ± 0.4	37.3 ± 0.3	37.7 ± 0.2	38.2 ± 0.3	38.5 ± 0.4	38.6 ± 0.4	38.7 ± 0.4
EM	37.0 ± 0.5	37.3 ± 0.4	37.7 ± 0.3	37.9 ± 0.4	38.1 ± 0.4	38.2 ± 0.4	38.1 ± 0.4

Table 6. Mean ± SD HR reported in 10-minute intervals during one-hour period for all interventions. *P < 0.01

Intervention	0	10*	20*	30*	40*	50*	60*
PAS	80.4 ± 11.1	83.0 ± 14.1	91.3 ± 16.6	98.8 ± 17.1	106.4 ± 16.7	114.4 ± 17.4	121.1 ± 24.8
EH-PAS	94.9 ± 24.9	138.8 ± 17.1	165.5 ± 14.5	168.1 ± 17.4	133.1 ± 12.0	133.7 ± 14.6	135.1 ± 18.5
EM	89.6 ± 16.4	123.3 ± 26.6	160.6 ± 13.7	161.9 ± 17.8	159.1 ± 15.5	147.0 ± 12.5	150.7 ± 12.7
EM	85.9 ± 21.1	120.2 ± 24.7	152.9 ± 13.5	150.4 ± 21.1	154.9 ± 15.2	144.7 ± 19.9	141.5 ± 22.0

Table 7. Physiological measurements for 30-minute increments across all interventions as mean and ± SD with significant differences.

	PAS		**EH-PAS**	
Duration (minutes)	**0-30**$^{\mathrm{a}}$	**30-60**$^{\mathrm{b}}$	**0-30**$^{\mathrm{c}}$	**30-60**$^{\mathrm{d}}$
Core Temperature (°C) ◊				
Baseline	37.3 ± 0.3 $^{d, f, h}$	37.4 ± 0.4 $^{\mathrm{d, f, h};\ d, h}$	37.3 ± 0.3 $^{d, f}$	38.2 ± 0.2 $^{\mathrm{b};\ a, b, c, e, g}$
Average	37.3 ± 0.4 $^{\mathrm{c, e};\ b, d, f, h}$	37.8 ± 0.4 $^{\mathrm{d, f};\ a, d}$	37.6 ± 0.2 $^{\mathrm{a};\ d, f}$	38.6 ± 0.4 $^{\mathrm{b, h};\ a, b, c, e, g, h}$
Increment	0.1 ± 0.1 $^{\mathrm{c, e, g};\ b, c, e}$	0.7 ± 0.2 $^{\mathrm{h};\ a}$	1.0 ± 0.2 $^{\mathrm{a};\ a, h}$	0.7 ± 0.4 $^{\mathrm{h}}$
Peak	37.5 ± 0.4 $^{\mathrm{c, e, g};\ b, c, d, f, h}$	38.1 ± 0.5 $^{\mathrm{d};\ a}$	38.3 ± 0.2 $^{\mathrm{a};\ a}$	38.9 ± 0.5 $^{\mathrm{b, h};\ a, g\ h}$
Heart Rate				
Baseline	73 ± 13.1 $^{b, d, f, h}$	92 ± 16.3 $^{\mathrm{d, f, h};\ a, d, e, f, h}$	86 ± 24.5$^{d, f, h}$	161 ± 20.8 $^{\mathrm{b};\ a, b, c, e, g}$
Average	87 ± 14.0 $^{\mathrm{c, e, g};\ b, c, d, e, g, h}$	110 ± 17.2 $^{\mathrm{d, f, h};\ a, c, d, e, f, h}$	148 ± 14.8 $^{\mathrm{a};\ a, b}$	136 ± 13.2 $^{\mathrm{b, f};\ a, b}$
Increment	39 ± 10.5 $^{\mathrm{c, e, g};\ c, d, e, g}$	41 ± 10.2 $^{\mathrm{d};\ c, d, e, g}$	100 ± 30.1 $^{\mathrm{a};\ a, b, d, f}$	74 ± 6.1 $^{\mathrm{b, f, h};\ a, b, c, e, g}$
Peak	112 ± 13.8 $^{\mathrm{c, e, g};\ b, c, d, e, f, g, h}$	133.5 ± 20.5 $^{\mathrm{d, f, h};\ a, c, d, e, f, h}$	186 ± 10.7 $^{\mathrm{a, e, g};\ a, b, g}$	169 ± 18.7 $^{\mathrm{b};\ a, b}$
Skin Temperature (°C) □				
Baseline	33.5 ± 1.18 ^{b}	39.3 ± 0.48 $^{\mathrm{d, f, h};\ a, c, e, f, g, h}$	35.0 ± 0.90 $^{\mathrm{g};\ b, g}$	37.2 ± 0.46 $^{\mathrm{b, h};\ e, g, h}$
Average	39.0 ± 0.38 $^{\mathrm{c, e, g};\ c, g, h}$	39.1 ± 0.26 $^{\mathrm{f, h};\ c, e, g, h}$	36.9 ± 0.43 $^{\mathrm{a, g};\ a, b, d, g, h}$	38.6 ± 0.77 $^{\mathrm{f, h};\ c, e, f, g, h}$
Increment	6.6 ± 1.46 $^{\mathrm{e, g};\ b, f, h}$	0.5 ± 0.19 $^{\mathrm{d};\ a, c, d, e, g}$	2.7 ± 1.09 $^{\mathrm{g};\ b, f, h}$	2.3 ± 0.64 $^{\mathrm{b, f, h};\ b, h}$
Peak	40.1 ± 0.58 $^{\mathrm{c, e, g};\ b, f, h}$	39.8 ± 0.45 $^{\mathrm{f, h};\ a, c, d, e, g}$	37.7 ± 0.42 $^{\mathrm{a, g};\ b, f, h}$	40.1 ± 0.71 $^{\mathrm{f, h};\ b, h}$
Energy Expenditure (kcal)	98 ± 39 $^{\mathrm{c, e, g};\ b, c, d, e, f, g, h}$	131 ± 47 $^{\mathrm{d, f, h};\ a, c, d, e, f, g, h}$	262 ± 74 $^{\mathrm{a};\ a, b, d}$	180 ± 56 $^{\mathrm{b, f, h};\ a, b, c, e, f, h}$

	EH		EM		P1	P2	P3
Duration (minutes)	**0-30^{e}**	**30-60^{f}**	**0-30^{g}**	**30-60^{h}**	0-30	30-60	All Interventions
Core Temperature (°C) ◊							
Baseline	37.2 ± 0.4 $^{d, f}$	38.1 ± 0.5 $^{\mathrm{b};\, a, c, e, g}$	37.0 ± 0.5 $^{d, f}$	37.9 ± 0.4 $^{\mathrm{b};\, a, b}$	0.37 †	<0.001	<0.001
Average	37.4 ± 0.6 $^{\mathrm{a};\, d, f}$	38.5 ± 0.4 $^{\mathrm{b};\, a, c, e, g}$	37.5 ± 0.4 $^{d, f}$	38.1 ± 0.4 $^{\mathrm{d};\, a, d}$	0.01	<0.001	<0.001
Increment	0.9 ± 0.4 $^{\mathrm{a};\, a, h}$	0.7 ± 0.4 $^{\mathrm{h}}$	1.0 ± 0.5 $^{\mathrm{a}}$	0.4 ± 0.3 $^{\mathrm{b, d, h};\, c, e}$	<0.001	0.001	<0.001
Peak	38.1 ± 0.5 $^{\mathrm{a}}$	38.8 ± 0.3 $^{\mathrm{h};\, a, g}$	38.0 ± 0.4 $^{\mathrm{a};\, d, f}$	38.3 ± 0.4 $^{\mathrm{d, f};\, a, d}$	<0.001	<0.001	<0.001
Heart Rate							
Baseline	79 ± 15.6 $^{b, d, f, h}$	154 ± 19.7 $^{\mathrm{b};\, a, b, c, e, g}$	75 ± 20.9 $^{d, f, h}$	143 ± 24.4 $^{\mathrm{b};\, a, b, c, e, g}$	0.29	<0.001	<0.001
Average	142 ± 14.5 $^{\mathrm{a};\, a, b}$	151 ± 10.2 $^{\mathrm{b, d};\, a, b}$	135 ± 13.8 $^{\mathrm{a};\, a}$	149 ± 16.0 $^{\mathrm{b};\, a, b}$	<0.001	<0.001	<0.001
Increment	98 ± 12.5 $^{\mathrm{a};\, a, b, d, f, h}$	23 ± 15.1 $^{\mathrm{d};\, c, e, g}$	89 ± 20.9 $^{\mathrm{a};\, a, b, d, g, h}$	30 ± 21.1 $^{\mathrm{d};\, c, e, g}$	<0.001	<0.001	<0.001
Peak	177 ± 13 $^{\mathrm{a, c};\, a, b}$	177 ± 12.3 $^{\mathrm{b};\, a, b}$	168.9 ± 11.9 $^{\mathrm{a, c};\, a, c}$	174 ± 13.5 $^{\mathrm{b};\, a, b}$	<0.001	<0.001	<0.001
Skin Temperature (°C) □							
Baseline	34.4 ± 0.88 $^{\mathrm{g};\, b, d, f}$	36.7 ± 0.58 $^{\mathrm{b, h};\, b, e, g}$	32.7 ± 0.67 $^{\mathrm{c, e};\, b, c, d, f, h}$	34.1 ± 0.52 $^{\mathrm{b, d, f};\, b, d, g}$	0.004	<0.001	<0.001
Average	36.6 ± 0.70 $^{\mathrm{a, g};\, a, b, d, g, h}$	36.7 ± 0.76 $^{\mathrm{b, d, h};\, d, g, h}$	33.4 ± 0.67 $^{\mathrm{a, c, e};\, a, b, c, d, e, f, g}$	34.1 ± 0.57 $^{\mathrm{b, d, f};\, a, b, c, d, e, f}$	<0.001	<0.001	<0.001
Increment	3.2 ± 0.70 $^{\mathrm{a, g};\, b, f, g, h}$	0.70 ± 0.36 $^{\mathrm{d};\, a, c, e}$	1.8 ± 0.62 $^{\mathrm{a, c, e};\, b, e}$	0.5 ± 0.35 $^{\mathrm{d};\, a, c, d, e}$	<0.001	<0.001	<0.001
Peak	37.6 ± 0.34 $^{\mathrm{a, g};\, b, f, g, h}$	37.5 ± 0.80 $^{\mathrm{b, d, h};\, a, c, e}$	34.5 ± 0.52 $^{\mathrm{a, c, e};\, b, e}$	34.8 ± 0.72 $^{\mathrm{b, d, f};\, a, c, d, e}$	<0.001	<0.001	<0.001
Energy Expenditure (kcal)	254 ± 61 $^{\mathrm{a};\, a, b, d}$	256 ± 64 $^{\mathrm{b, d};\, a, b, d}$	235 ± 55 $^{\mathrm{a};\, a, b}$	257 ± 54 $^{\mathrm{b, d};\, a, b, d}$	<0.001	<0.001	<0.001

Sample size ***n*=10.** ◊Core Temperature ***n*=7.** □Skin Temperature ***n*=5.** P-value set at 0.05
Superscript: Differences between categories are indicated with superscripts (Repeated Measure ANOVA between corresponding portion of interventions; p<0.05, with post-hoc Bonferroni correction)
Superscript: Differences between all treatments indicated with subscript (Repeated Measures ANOVA across all interventions; P<0.05, with post-hoc Bonferroni correction).

First 30 minutes of the heating protocol

Table 3 shows outcomes for variables split with first 30 minutes of heating protocol and second 30 minutes of heating protocols. No differences were calculated for the first 30 minutes between interventions for baseline T_c or HR (P =0.30; P =0.29, respectively). EH-PAS, EH, and EM interventions produced similar results in terms of average, increment, and peak T_c that were significantly different from PAS in terms of average (EH-PAS: P=0.04, EH:, EM: P=0.05), increment (EH-PAS: P<0.001, EH: P=0.001, EM: P=0.02), and peak T_c (EH-PAS: P=0.01, EH: P=0.01, EM: P=0.01) for the first 30-minutes of the interventions.

An EH-PAS and EH interventions produced comparable results in terms of average and increment HR. The EH-PAS method resulted in the highest peak HR of 186 ± 10.7 among the interventions and significant when compared to PAS (P<0.001), EH (P=0.02) and EM (P=0.01). Meanwhile, a peak HR for an EH intervention only produced significant results when compared to PAS (P<0.001).

Second 30 minutes of the heating protocol

An EH-PAS and EH method produced comparable results in terms of average, increment, and peak T_c though no significant differences noted between the two interventions (P >0.05). An EH-PAS method resulted in a slightly higher peak T_c of 38.9 ± 0.5 °C in comparison to EH of 38.8 ± 0.3°C, though no statistical significance noted between the two interventions (P >0.05).

However, peak T_c in an EH-PAS method did produce more statistically significant results when compared to PAS and EM (P=0.03; P=0.02, respectively) than EH vs EM (P=0.02)
EH resulted in the highest peak HR amongst all interventions with 177 ± 12.3 followed by EM 174 ± 13.5, EH-PAS 169 ± 18.7, and lastly, PAS 133.5 ± 20.5. An EH-PAS method produced the highest increment HR amongst all interventions of 74 ± 6.

CHAPTER 5

CONCLUSION AND DISCUSSION

Both EH and EH-PAS interventions produced a body core temperature exceeding 38.5°C, whereas participants during the other methods, i.e., PAS alone or while exercising at room temperature, did not meet the targeted core temperature, while some differences were noted among the interventions in terms of fluid consumed, sweat rate, absolute body weight change, and estimated energy expenditure especially in the interventions that were conducted in a hot environment. No differences noted in terms of hydration status pre- and post-interventions.

The goal was for participants to reach the threshold of 38.5°C within these four interventions. EH and EH-PAS method resulted in similar outcomes. Both interventions produced a body core temperature exceeding 38.5°C, whereas participants during the other methods, i.e., PAS alone or while exercising at room temperature, did not meet the targeted core temperature that should prompt heat acclimatization (Périard et al., 2015). This is of relevance as college athletes may have limited time availability to acclimatize while starting practice, i.e., college athletes are to participate in a 5-day acclimatization training program in which single-day practices do not exceed a 3-hour timeframe (Belval, 2017), and PAS could be a reasonable option to further optimize heat acclimatization status without burdening the maximal countable hours within collegiate athletics. It is important to consider that not all participants were able to always meet the targeted core temperature of 38.5° C. As such, seven participants were able to achieve this in the EH intervention while eight participants reached goal core temperature in the EH-PAS intervention (n=10). Only three participants were able to reach goal temperature in the EM (n=10). For PAS, only two participants achieved goal (n=7).

Previous literature has been conducted looking at the effects of passive heat acclimation alone and its impact on exercise performance. These studies focused on having participants resting in a heat chamber (Henane and Valatx, 1973; Beaudin et al., 2009), sauna (Leppaluoto et al., 1986; Scoon et al., 2007; Stanley et al., 2015), or hot water immersion (Allan and Wilson, 1971; Brazaitis and Skurvydas, 2010; Zurawlew et al., 2016). While still offering many benefits and similar outcomes than active heating methods, passive heating methods require more frequent and longer durations of 6-21 days with 30-60 minutes bouts to reap benefits (Fox et al., 1964; Allan and Wilson, 1971; Henane and Valatx, 1973; Leppaluoto et al., 1986; Shido et al., 1999; Beaudin et al., 2009; Brazaitis and Skurvydas, 2010; Kanikowska et al., 2012). Passive heating alone may not be beneficial for athletes and other populations that are required to adjust to hotter climates in short notice. Our study provided insight that only two out of the seven participants were able to achieve the 38.5°C target core temperature for heat adaptation when following passive heating as only intervention for a duration of 60 minutes. Among the participants, the mean peak core temperature for this intervention was 38.1 ± 0.5 °C.

Research that has utilized a mixed method of exercise followed by passive heating to achieved higher peak temperatures, which theoretically should result in higher impact on heat acclimatization than passive heating alone. Our study resulted in elevated peak T_c in both an EH-PAS method and EH (39.1 ± 0.4°C, 38.9 ± 0.3°C, respectively). This information along with previous research adds to the existing knowledge why combined methods of active exercise and passive heating methods have produced positive results (Stanley et al., 2015; Zurawlew et al., 2016). Stanley et al, reported a mean tympanic temperature of 39.2 ± 1.1°C during the post-exercise sauna exposure and observed an increase in plasma volume (17%) although its relation to heart rate measures is still unclear (Stanley et al., 2015). Other researchers, such as Zurawlew et al., noticed a hot water immersion following exercise showed a decrease in resting T_{re} (mean -0.27°C, $P<0.01$) and final T_{re} during submaximal exercises performed a moderate (-0.28°C, $P <0.01$) and warmer conditions (-0.36°C, $P<0.01$) (Zurawlew et al., 2016). Peak T_{re} was not reported during time of exercise or hot water immersion intervention.

Our study is the first to utilize a HIIT 30-minute exercise protocol in a hot environment followed by a 30-minute passive heating for a combined intervention. Previous research did not control for exercise prior to a passive heating intervention (Scoon et al., 2007; Stanley et al., 2015). While one research team had participants perform a submaximal exercise for 40-minutes before entering a sauna, the environment during exercise was moderate (18 °C, 40% RH). The environmental conditions for EH averaged around 42.8 ± 1.4°C with actual average vapor pressure noted of 0.8 ± 0.1 kPa. Vapor pressure was calculated as it does not change with ambient temperature; RH changes with temperature (Davis et al., 2016). EM produced average ambient temperature of 22.4 ± 0.4°C and actual average vapor pressure of 2.7 ± 0.1 kPa. Our moderate conditions were slightly warmer than that of submaximal exercise environment in one research study (18 °C), though core temperature was not measured during exercise (Zurawlew et al., 2016). It should be important to note that three out of ten participants were able to reach goal core temperature in an EM intervention compared to that of EH in which seven participants achieved a core temperature of 38.5°C.

Some research utilized impermeable suits (Fox et al., 1964; Beaudin et al., 2009) to reduce sweat evaporation and encourage a rise in core temperature. Sauna suits have also been used to replicate similar conditions (Mee et al., 2017). Our study also utilized coverall suits for a EH-PAS method (first 30-minutes), EH, and EM. The purpose of the coverall suits in this study was to minimize the cooling properties of sweating while participants exercised as our goal was to test the raise in core temperature efficacy of each heating intervention. As sweat evaporates, it cools the skin which is one modality to cool the body in hotter environments (Cisneros & Goins, 2009). Despite potential sweat differences between athletes, the microclimate for each participant was fairly stable while executing exercise interventions with the suit on. The coverall suit reduced the airflow of the fan in the outdoor environment which was needed in a few instances to ensure the ambient temperature was stable. In a practical application, this also mimics athletes wearing protective gear such as padding, which hinders the evaporation of sweat when worn by athletes.

Overall, this study T_{sk} results were higher than when compared to previous research that has been conducted in this field in similar conditions. As T_{sk} is a balance of radiative, evaporative, convective, and conductive exchanges as the body thermoregulates, it remains largely independent of T_c in a wide range of ambient temperatures of 5-30°C (Nielsen and Nielsen, 1965). In one research looking at skin temperature and hydration on plasma volume during exercise, Kenefick et al. noticed an increase in overall skin temperature before and 30-minutes after a submaximal exercise test in various environments ranging from 10°C to 40°C in euhydrated and hypohydrated participants (Kenefick et al., 2014). Skin temperature for all participants was monitored continuously with a mean weight utilizing four sites which included the left chest, arm, thigh, and leg (Ramanathan, 1964). After performing a submaximal ergometry exercise test, participants sat in various environmental conditions ranging from 10°C to 40°C (Kenefick et al., 2014). In euhydrated participants, a mean T_{sk} of 35.6 ± 0.4°C and 35.9 ± 0.4°C directly after exercise and 30 minutes of rest, respectively. In the hypohydrated participants, mean Tsk at baseline and 30 minutes were slightly higher (36.1 ± 0.3°C and 36.3 ± 0.6°C, respectively). Researchers observed a core-to-skin temperature gradient, resulting in progressively higher skin blood flow response as the ambient temperature increased (Kenefick et al., 2014). However, utilizing a skin temperature site of the thigh may not be representative in conditions where the environment may rapidly change such as exercise, sweating, or moving from varying climates of hot to cold (Ramanathan, 1964).

In a second study, researchers investigated the role of skin temperature in the acclimation process during two heat stress tests (HST) when compared to temperate conditions during exercise (Regan et al., 1996). Skin temperature was measured from eight sites with a layer of waterproof tape to the forehead, right scapula, left upper chest, right upper arm, left lower arm, left hand, right anterior thigh and left calf (Regan et al., 1996). Upon the onset of sweating, T_{sk} was recorded for the forehead (HST 1: 36.93 ± 0.36, HST 2: 36.64 ± 0.11) as well as the forearm (HST 1: 36.82 ± 0.34, HST 2: 36.37 ± 0.14). While not quantifiable, researchers noticed a difference of 4.2°C in T_{sk} between acclimated regimens within the study subjects (Regan et al., 2016). While this is a relatively small difference, Regan et al. hypothesized that an elevation in skin temperatures may be just as critical of a component in heat adaptation. They observed that an elevation in skin temperature may be critical towards peripheral adaptation as well as central adaptations (Regan et al., 1996). Our results can partially be explained by exercise and heat induced thermal adaptation in the body when exposed to hot environments (Périard et al., 2015)

Our results measured skin temperature with relatively less sites (neck, right shoulder, left hand, and right shin) and various sides of the body, yet produced similar results: as the ambient temperature was elevated in conditions, skin temperature followed. The environmental conditions of this study (22.9 – 58.4 °C) are within the temperature range that is associated for the best accuracy for the device of ±0.5°C (0 – 70°C). While T_{sk} for EH-PAS, EH, and EM were encapsulated with a coverall suit, the device on the left hand was not encapsulated though only weighted with a smaller coefficient (0.16) compared to the three other sites (0.28). Participants did not wear a coverall suit for the PAS, though the environment itself, a small changing tent, did create its own capsule in that regard.

Exercise intensity was similar between treatments in a EH-PAS method, EH, and EM as evidenced by average HR in all three interventions. Very few studies have measured HR data for workload status. Two studies have measured end-exercise heart rate values (Zurawlew et al., 2016, 2018) while one measured heart rate during submaximal exercise (Stanley et al., 2015). Our study provided insight that HR outcomes (average, increment, and peak) were comparable in a combined EH-PAS intervention to that of EH. When looking at the first 30-minutes of both interventions (**Table 7**), average, increment, and peak HR were still similar. When constant work rate exercise is performed in warm environments (~40°C), there is a proportional relationship between the rise in core temperature and increase in HR (Gonzalez-Alonso et al., 1999). As thermal strain develops, HR increases as a response to the effect of temperature on the sinoatrial node and to vagal withdrawal and sympathetic activation (Périard et al., 2021). Travers et al. recently demonstrated this relationship as maintaining a HR equivalent to 65% VO_{2max} for 10 days in a heat acclimatization protocol resulted in a progressive increase in work rate (Travers et al., 2020). Participants in this study were healthy, adult males (ages 18-30) who trained on average 5-10 hours per week in various sports. Our results may not be representative of other populations. As our participants consisted of healthy individuals, it does not automatically carry over to populations such as the elderly and construction workers. As humans age, the ability to dissipate heat properly decreases. This is primarily due to the fact that the mechanistic routes the body utilizes to thermoregulate is affected by metabolic heat production, reduced skin blood flow, smaller increases in cardiac output, reduced sweat gland output, and less redistribution of blood flow from renal and splanchnic circulation (Kenney & Munce, 2003). The elderly population also utilizes polypharmacy, including diuretics, beta-blockers, and anticholinergics which can block the increased cardiac output and sweating needed to cool the body (Gaudio & Grissom, 2016). Seniors are unable to adapt or acclimate as quickly compared to young adults. They can also suffer from side effects from prescription drugs which can alter the body's ability to cool properly. Another population that this data does not represent are construction workers. Construction workers are subjected to various environmental conditions such as hot climates and humidity which require sufficient scheduled breaks to properly cool down the body and prevent EHI. This population can quickly become at risk if safe working conditions, protocols, and education is not set in place. According to the CDC, Department of Human Health Services, and National Institute for Occupational Safety and Health (NIOSH), continuous work in hotter environments is not advisable.

As a combined active and passive heating protocol to induce heat adaptation will cost less energy on an individual, this could be beneficial towards other populations such as military or athletes. These populations train for similar timeframes and are expected to perform in peak condition in various climates with little notice to acclimate in a new environment. Both athletes and military personnel are considered to be in peak physical conditions to perform at their best. Members of the military branch much be able to adjust to new environments within a condensed time while also being mentally and physically prepared for any scenario (Parsons et al., 2019). They must perform numerous tasks in extreme conditions while in uniform which can add to physical strain on the body. Like the military, athletes are also subjected to traveling to unfamiliar climates for competitions with high relative humidity, which ultimately puts a strain on the body's ability to thermoregulate properly (Coris et al., 2004). High school athletes in particular are treated the most in the United Stated for EHI usually around pre-season football (Yard et al., 2010). The addition of various clothing, uniforms, helmets, and protective gear that is worn during practice and games can also hinder the body's ability to thermoregulate. Athletes are more likely to become dehydrated quickly which increases the risk to develop EHI.

Strengths and limitations

Strengths of this study include the use of a hot environment for an active heating protocol and a mixed protocol of active and passive heating in a hot environment. Other studies have had some combined efforts of exercise and passive heating protocols (Zurawlew et al., 2016), though the environmental conditions in which exercise was performed in was considered temperate (18 °C, 40 % RH) or slightly warmer (33 °C, 40 % RH). Our study performed an exercise protocol in a hotter environment for both EH-PAS and EH with an average ambient temperature of 43.3 ± 1.1 °C (Tent 1) and 42.8 ± 1.4 °C, respectively. Our EM conditions were slightly warmer than that of Zurawlew et al.'s study with an average ambient temperature of 22.4 ± 1.1 °C compared to 18 °C (Zurawlew et al., 2016). Our study also measured humidity for these environments (average relative humidity EH-PAS: 18.3 ± 6.6%; EH: 10 ± 1.8%) but also calculated vapor pressure as it does not change with ambient temperature and relative humidity changes with temperature (Davis et al., 2016).

Another strength to this study is the measurement of HR and T_c throughout each protocol. Previous literature that utilized a post-exercise passive heating method to achieve adaptation did not measure for these parameters during exercise (Scoon et al., 2007; Stanley et al, 2015; Zurawlew et al., 2016). One research team measured tympanic temperature during the post-exercise sauna immersion (Stanley et al., 2015) while another team did not record core temperature (Scoon et al., 2007).

One study did collect rectal temperature (T_{re}), skin temperature, and heart rate during exercises tests performed by the participants before, during, and after the daily interventions, however, the research team only reported resting T_{re}, end T_{re}, changes in T_{re}, T_{re} at sweating onset, and end T_{sk} (Zurawlew et al., 2016). Our study reported baseline, average, increment, and peak T_c, HR, and T_{sk} outcomes for all interventions.

This research does present with some limitations. First, this study did not measure actual bike output in terms of wattage for the exercise portions of interventions, but previous literature has utilized HR as an indicator to define workload for heat acclimatization programs (Taylor et al., 2020). In one study, a stable and sustained elevation in RPE and T_c were also attained and was similar to that observed with isothermal/controlled hyperthermia approaches (Travers et al., 2020). As discussed previously, when a constant work rate is performed in a hot environment (40 °C), there is a proportional relationship as HR increases and a rise in core temperature (Périard et al., 2021). Our findings are in line with those reported using an active method of heat acclimation approach.

Another limitation to this study is that some participants did not reach the goal core temperature of 38.5°C as they were already acclimated to the environment. Several of the participants had been living in the climate for longer than 6 months (n=8) or exercised outdoors (n=3) while only a handful (n=2) of participants had recently moved to the

Phoenix area. One of the reasons why this study was conducted for athletes is that this population may have difficulties achieving an elevated core temperature of 38.5°C needed for heat acclimation. In some interventions, participants were unable to reach an elevated core temperature such as PAS or EM. Depending on the environment that athletes train in and various modalities of training exercises they achieve during practice, various athletes may respond better to one intervention than others.

Some research has provided insight as to the loss of physiological benefits from heat acclimatization protocols. Weller et al looked into heat acclimatization during winter months and found that once acclimatization has been attained, an individual can maintain the physiological benefits for up to one month in cooler environments with the need for extensive re-adaptation to a warmer climate (Weller et al., 2007). Therefore, athletes are able to reap the benefits of heat acclimation in cooler environments and time of the year once acclimatization has been achieved.

An additional limitation to this study is that some data on core temperature and skin temperature was lost due to technology errors. This is primarily on Day 1 of data collection but was corrected for all other days of data collection. Core temperature data was only available for seven participants rather than all ten participants as not every participant had their own monitor. This was also corrected for all the remainder of data collection. Additional data from all participants could have potentially lowered the T_{sk}

trends for interventions. See Appendix F for differences in T_{sk} in terms of mean ± SD for all participants vs five participants. It could be suggested that if all data points for all ten participants for each intervention were evaluated, this could have potentially lowered the outcomes for measured T_{sk}, which would be relatively closer to previous literature, though our environments were elevated in comparison in which exercise was performed in.

It is important to note that data was collected from September 2020 – October 2020 with approval from IRB and COVID-19 protocols in place. Due to the nature of COVID-19 protocols and time restraints, only one participant was able to perform exercise interventions at a time.

Conclusion and Applications

Based on the outcome of this study, a EH-PAS and PAS resulted in a similar raise in core temperature above 38.5°C during 60 minutes of duration in healthy, adults (>18 years old) in Phoenix, Arizona than each method individually. The results were comparable to that of 60-minutes of EH. If heat acclimation protocols require one-hour of training, the athlete may not be required to exercise for the entire 60-minute duration. A combined effort of EH-PAS provided sufficient rise in core temperature as an EH method. However, an EH-PAS method achieved the same results with less workload and time (30-minutes of exercise), which may reduce the need for additional training time. If less workload is done by an athlete, the risk of overtraining and injuries could potentially be lowered, ergo making it safer for athletes acclimating to warmer environments.

Considerations for training methods such as moderate and low intensity in heat vs high intensity in a cooler environment to avoid a reduction in training quality. Other considerations include individual responses to training modalities and environmental stress as well as body size, body composition, and history of EHI of an athlete as individual differences that can elicit different responses to heat stress and heat acclimation responses (Casadio et al., 2017).

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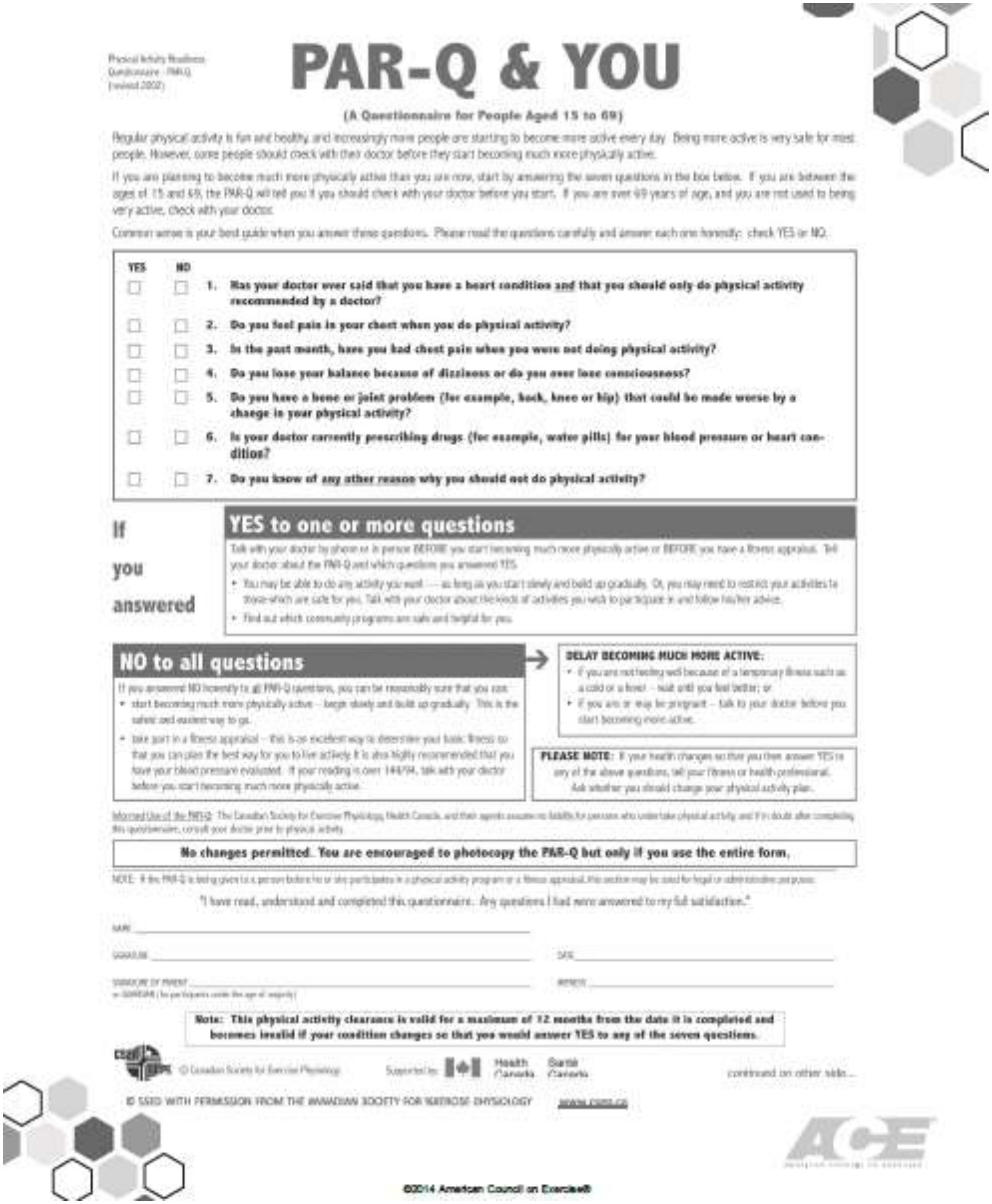
APPENDIX A

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE



Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME ______________________________

SIGNATURE ______________________________ DATE ______________________

SIGNATURE OF PARENT ______________________________ WITNESS ______________________
or GUARDIAN (for participants under the age of majority)

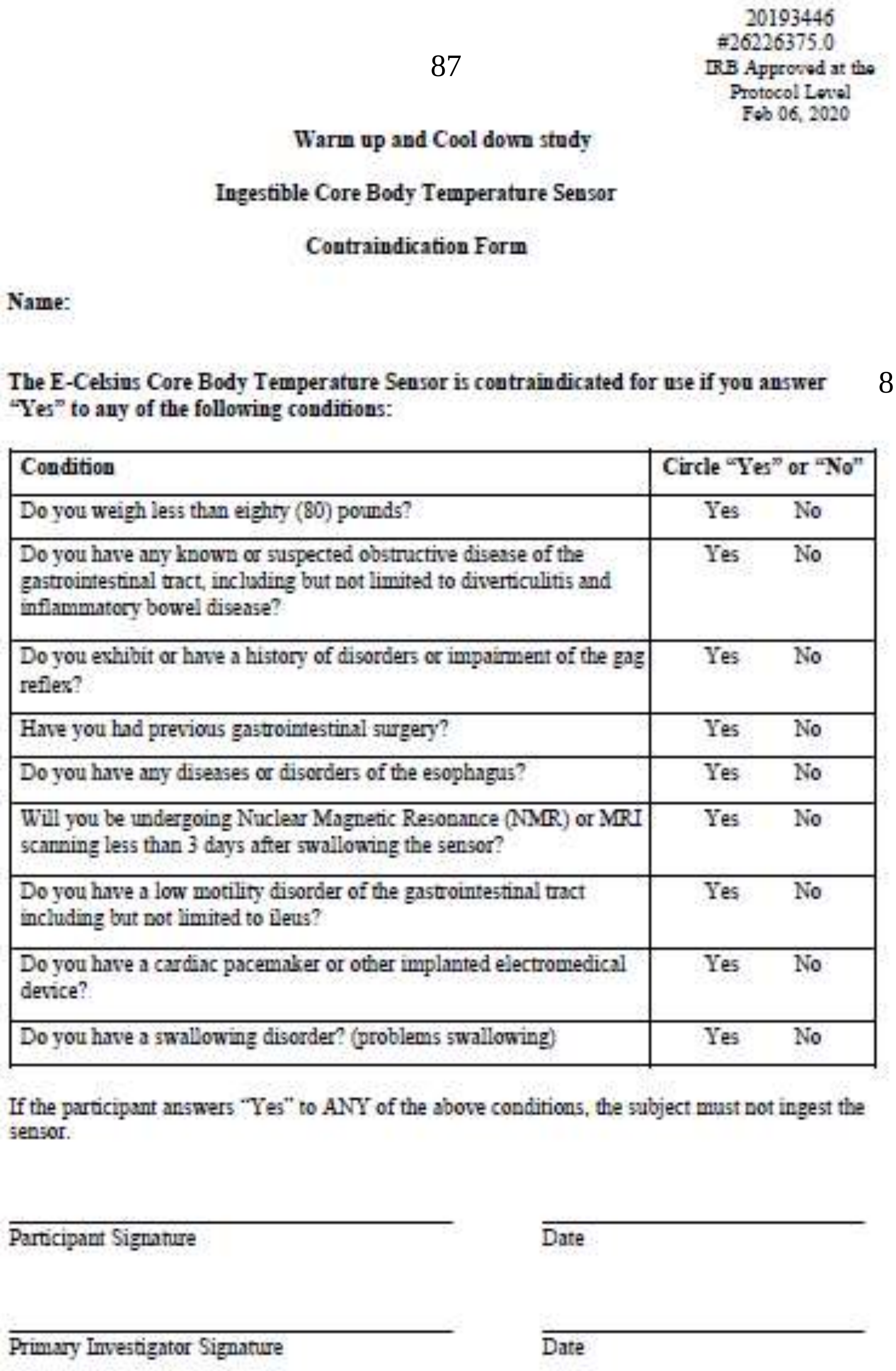
Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada Santé Canada

continued on other side...

APPENDIX B

CONTRAINDICATION FORM



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IRB Approved at the
Protocol Level
Feb 06, 2020

Warm up and Cool down study

Ingestible Core Body Temperature Sensor

Contraindication Form

Name:

The E-Celsius Core Body Temperature Sensor is contraindicated for use if you answer "Yes" to any of the following conditions:

87

Condition	**Circle "Yes" or "No"**
Do you weigh less than eighty (80) pounds?	Yes No
Do you have any known or suspected obstructive disease of the gastrointestinal tract, including but not limited to diverticulitis and inflammatory bowel disease?	Yes No
Do you exhibit or have a history of disorders or impairment of the gag reflex?	Yes No
Have you had previous gastrointestinal surgery?	Yes No
Do you have any diseases or disorders of the esophagus?	Yes No
Will you be undergoing Nuclear Magnetic Resonance (NMR) or MRI scanning less than 3 days after swallowing the sensor?	Yes No
Do you have a low motility disorder of the gastrointestinal tract including but not limited to ileus?	Yes No
Do you have a cardiac pacemaker or other implanted electromedical device?	Yes No
Do you have a swallowing disorder? (problems swallowing)	Yes No

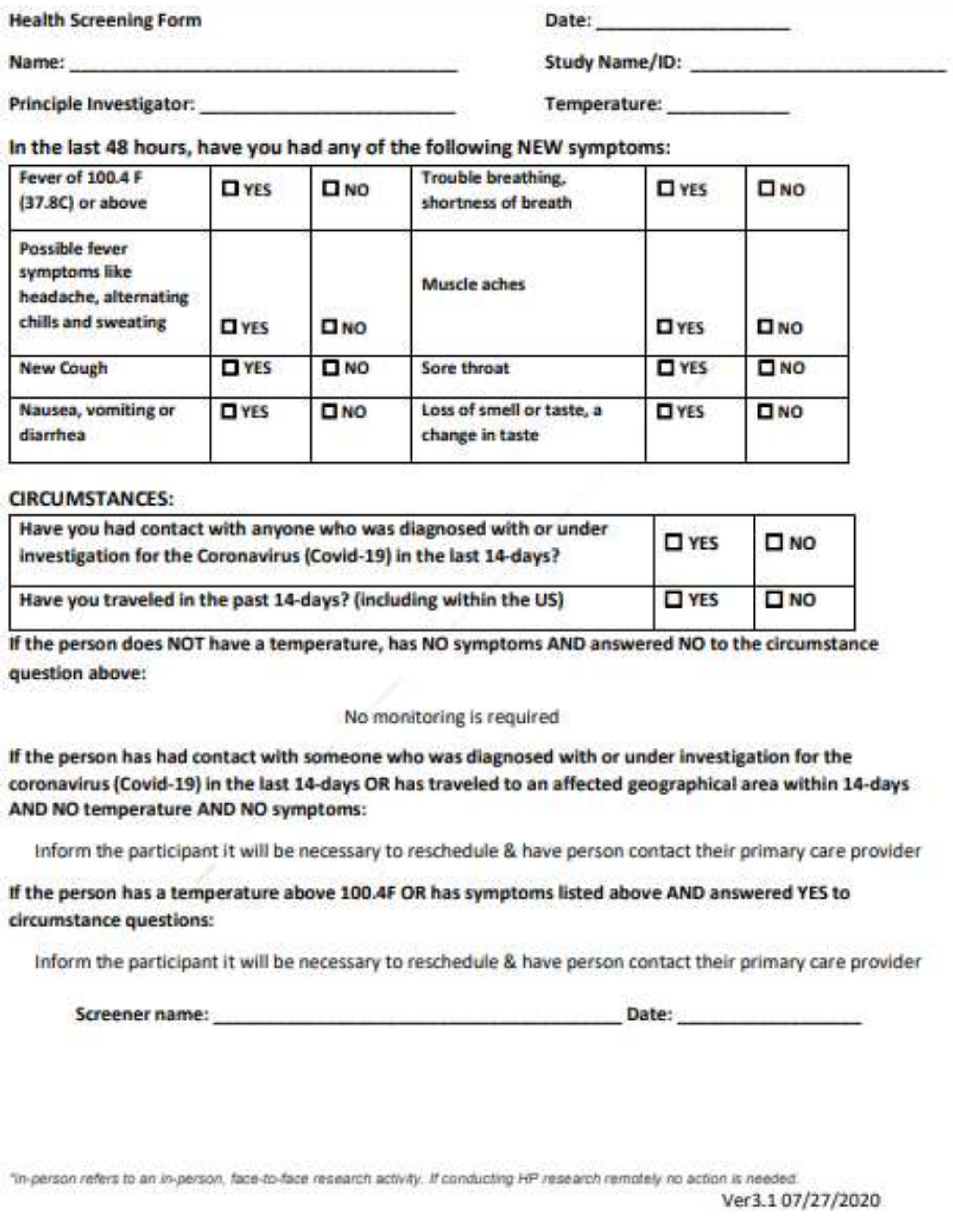
If the participant answers "Yes" to ANY of the above conditions, the subject must not ingest the sensor.

Participant Signature ____________________ Date ____________________

Primary Investigator Signature ____________________ Date ____________________

APPENDIX C

COVID-19 QUESTIONNAIRE



Health Screening Form **Date:** ____________________

Name: ____ **Study Name/ID:** __________________________

Principle Investigator: __________________________ **Temperature:** ____________

In the last 48 hours, have you had any of the following NEW symptoms:

Fever of 100.4 F (37.8C) or above	☐ YES	☐ NO	**Trouble breathing, shortness of breath**	☐ YES	☐ NO
Possible fever symptoms like headache, alternating chills and sweating	☐ YES	☐ NO	**Muscle aches**	☐ YES	☐ NO
New Cough	☐ YES	☐ NO	**Sore throat**	☐ YES	☐ NO
Nausea, vomiting or diarrhea	☐ YES	☐ NO	**Loss of smell or taste, a change in taste**	☐ YES	☐ NO

CIRCUMSTANCES:

Have you had contact with anyone who was diagnosed with or under investigation for the Coronavirus (Covid-19) in the last 14-days?	☐ YES	☐ NO
Have you traveled in the past 14-days? (including within the US)	☐ YES	☐ NO

If the person does NOT have a temperature, has NO symptoms AND answered NO to the circumstance question above:

No monitoring is required

If the person has had contact with someone who was diagnosed with or under investigation for the coronavirus (Covid-19) in the last 14-days OR has traveled to an affected geographical area within 14-days AND NO temperature AND NO symptoms:

Inform the participant it will be necessary to reschedule & have person contact their primary care provider

If the person has a temperature above 100.4F OR has symptoms listed above AND answered YES to circumstance questions:

Inform the participant it will be necessary to reschedule & have person contact their primary care provider

Screener name: ____ **Date:** __________________

in-person refers to an in-person, face-to-face research activity. If conducting HP research remotely no action is needed.

Ver3.1 07/27/2020

APPENDIX D

COVID-19 PROCEDURES

Arizona State University

COVID-19 Protocol

The following guidelines have been established to help protect the safety of our staff and participants. Guidelines are based on CDC and ASU guidelines to help prevent COVID-19 transmission.

Minimizing risk of transmission:

- Participants and personnel will be asked to answer a COVID-19 Questionnaire as they enter the lab for data collection days
- Temperature will be taken at the doorway of the building by designated personnel to ensure absence of fever (defined as >99.6°F). Ear-thermometer will also be available for use, if needed
- All participants and personnel must wash hands when entering the lab and prior to leaving
- All high-touch surfaces must be disinfected minimum twice per day (keyboards, chairs, lab bench, equipment, doorknobs)
- Participants will be required to wear a mask before and after intervention, and during pre- and post- testing. Mask will not be required during interventions as this will influence the results
- Ensure proper disposal bins available for PPE disposal

Social distancing:

- Occupancy will be based on the ASU recommendation of 150 sq ft per person.
- Incoming participants will have a designated chair. There will be at least six feet distance in between chairs. Baseline measurements and cooling protocol will take place at participant's designated chair. Participant will be asked to leave designated chair only as necessary to proceed with testing.
    A. Social distancing during intervention
        1. Maintain minimum of 12ft distance from participant during active exercise or have a screen in place between participant and tester
        2. Measure and designate areas for personnel at proper distances (using tape strips to indicate social distancing measurements as mentioned above)
        3. Contact or in close proximity to participants will require use of PPE
           (i.e., masks, face shield, gloves) when social distancing is not possible.
            a. Masks and face shield: used at all times to reduce aerosolized and droplet transmission

        b. Gloves: used when handling specimens (i.e., urine, etc) or as deemed necessary
        c. Gowns worn if sustained contact >5 minutes

Specific precautions based on intervention:

- Exercise Tent:
    A. Minimizing risk of transmission
        1. Disinfect training bike prior to and after training using ASU-approved disinfectants
        2. Allow 15-minutes between testing to allow proper air circulation
    B. Social distancing
        1. One participant allowed during intervention

- Passive heating:
    A. Minimizing risk of transmission
        1. Allow 15-minutes between testing to allow proper air circulation and reduce aerosolized and droplet transmission
        2. Disinfect high-touch areas prior to and after intervention using ASU-approved disinfectants
    B. Social distancing
        1. One participant allowed per tent during intervention

- Ice water bath
    A. Minimizing risk of transmission
        1. Disinfect high-touch areas prior and after intervention using ASU-approved disinfectants.
    B. Social distancing
        1. One participant allowed in room during intervention
        2. Maintain a minimum of 12ft between each participant

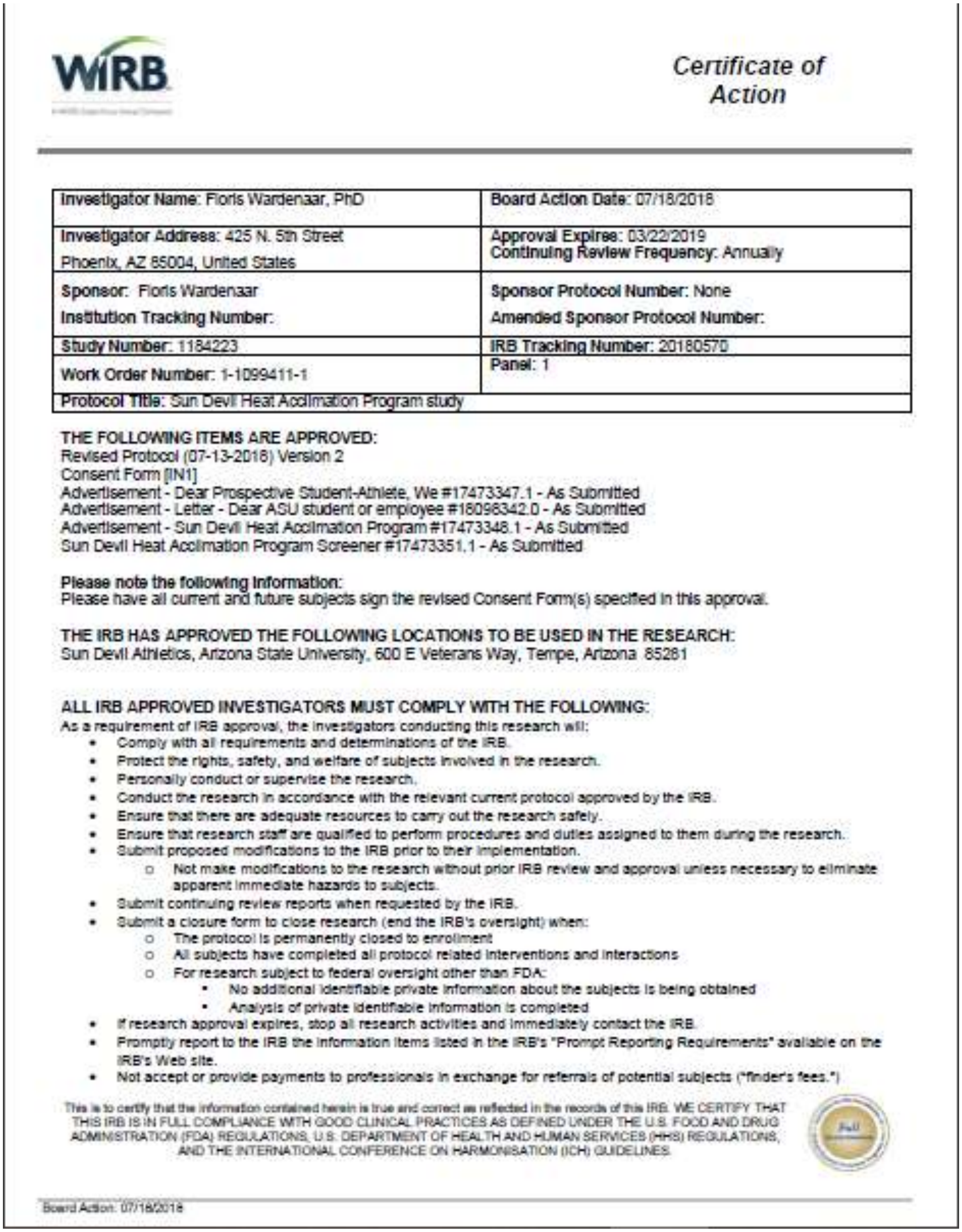
- Icy towels
    A. Minimize risk of transmission
        1. Disinfect chairs prior and after intervention using ASU-approved disinfectants

        2. Placed used towels in dirty laundry bin to be properly laundered
    B. Social distancing
        1. One participant allowed in room during intervention
        2. Maintain chairs at a minimum of 12ft between each participant

- Ice vest
    A. Minimize risk of transmission
        1. Disinfect ice vests, ice packs, and chairs prior and after intervention using ASU-approved disinfectants
    B. Social distancing
        1. One participant allowed in area during intervention
        2. Maintain chairs at a minimum of 12ft between each participant

APPENDIX E

IRB APPROVAL



WIRB

Certificate of
Action

Investigator Name: Floris Wardenaar, PhD	**Board Action Date:** 07/18/2018
Investigator Address: 425 N. 5th Street Phoenix, AZ 85004, United States	**Approval Expires:** 03/22/2019 **Continuing Review Frequency:** Annually
Sponsor: Floris Wardenaar **Institution Tracking Number:**	**Sponsor Protocol Number:** None **Amended Sponsor Protocol Number:**
Study Number: 1184223	**IRB Tracking Number:** 20180570
Work Order Number: 1-1099411-1	**Panel:** 1
Protocol Title: Sun Devil Heat Acclimation Program study	

THE FOLLOWING ITEMS ARE APPROVED:
Revised Protocol (07-13-2018) Version 2
Consent Form [IN1]
Advertisement - Dear Prospective Student-Athlete, We #17473347.1 - As Submitted
Advertisement - Letter - Dear ASU student or employee #18098342.0 - As Submitted
Advertisement - Sun Devil Heat Acclimation Program #17473348.1 - As Submitted
Sun Devil Heat Acclimation Program Screener #17473351.1 - As Submitted

Please note the following information:
Please have all current and future subjects sign the revised Consent Form(s) specified in this approval.

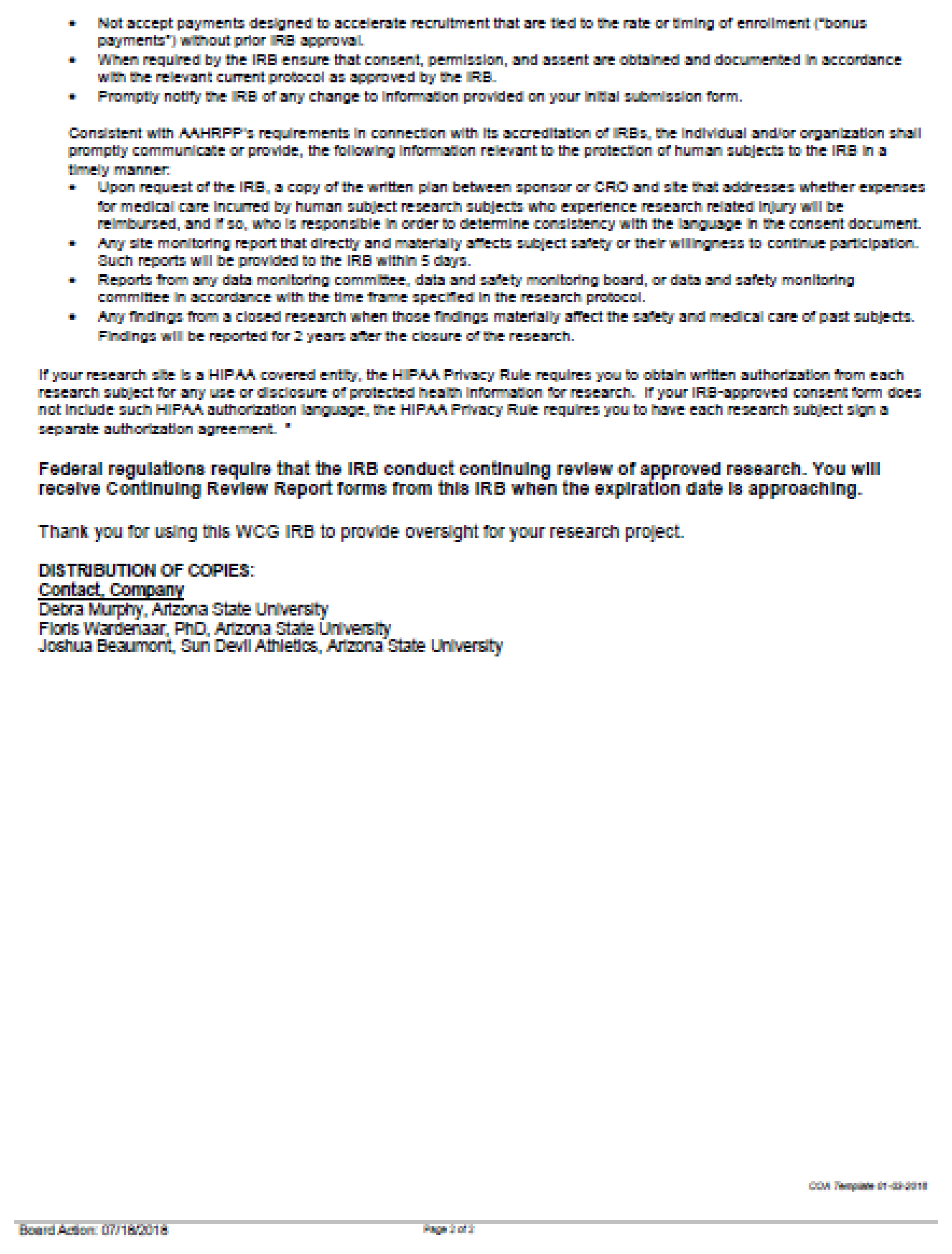
THE IRB HAS APPROVED THE FOLLOWING LOCATIONS TO BE USED IN THE RESEARCH:
Sun Devil Athletics, Arizona State University, 600 E Veterans Way, Tempe, Arizona 85281

ALL IRB APPROVED INVESTIGATORS MUST COMPLY WITH THE FOLLOWING:
As a requirement of IRB approval, the investigators conducting this research will:

- Comply with all requirements and determinations of the IRB.
- Protect the rights, safety, and welfare of subjects involved in the research.
- Personally conduct or supervise the research.
- Conduct the research in accordance with the relevant current protocol approved by the IRB.
- Ensure that there are adequate resources to carry out the research safely.
- Ensure that research staff are qualified to perform procedures and duties assigned to them during the research.
- Submit proposed modifications to the IRB prior to their implementation.
    - Not make modifications to the research without prior IRB review and approval unless necessary to eliminate apparent immediate hazards to subjects.
- Submit continuing review reports when requested by the IRB.
- Submit a closure form to close research (end the IRB's oversight) when:
    - The protocol is permanently closed to enrollment
    - All subjects have completed all protocol related interventions and interactions
    - For research subject to federal oversight other than FDA:
        - No additional identifiable private information about the subjects is being obtained
        - Analysis of private identifiable information is completed
- If research approval expires, stop all research activities and immediately contact the IRB.
- Promptly report to the IRB the information items listed in the IRB's "Prompt Reporting Requirements" available on the IRB's Web site.
- Not accept or provide payments to professionals in exchange for referrals of potential subjects ("finder's fees.")

This is to certify that the information contained herein is true and correct as reflected in the records of this IRB. WE CERTIFY THAT THIS IRB IS IN FULL COMPLIANCE WITH GOOD CLINICAL PRACTICES AS DEFINED UNDER THE U.S. FOOD AND DRUG ADMINISTRATION (FDA) REGULATIONS, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES (HHS) REGULATIONS, AND THE INTERNATIONAL CONFERENCE ON HARMONISATION (ICH) GUIDELINES.

Board Action: 07/18/2018



- Not accept payments designed to accelerate recruitment that are tied to the rate or timing of enrollment ("bonus payments") without prior IRB approval.
- When required by the IRB ensure that consent, permission, and assent are obtained and documented in accordance with the relevant current protocol as approved by the IRB.
- Promptly notify the IRB of any change to information provided on your initial submission form.

Consistent with AAHRPP's requirements in connection with its accreditation of IRBs, the individual and/or organization shall promptly communicate or provide, the following information relevant to the protection of human subjects to the IRB in a timely manner:

- Upon request of the IRB, a copy of the written plan between sponsor or CRO and site that addresses whether expenses for medical care incurred by human subject research subjects who experience research related injury will be reimbursed, and if so, who is responsible in order to determine consistency with the language in the consent document.
- Any site monitoring report that directly and materially affects subject safety or their willingness to continue participation. Such reports will be provided to the IRB within 5 days.
- Reports from any data monitoring committee, data and safety monitoring board, or data and safety monitoring committee in accordance with the time frame specified in the research protocol.
- Any findings from a closed research when those findings materially affect the safety and medical care of past subjects. Findings will be reported for 2 years after the closure of the research.

If your research site is a HIPAA covered entity, the HIPAA Privacy Rule requires you to obtain written authorization from each research subject for any use or disclosure of protected health information for research. If your IRB-approved consent form does not include such HIPAA authorization language, the HIPAA Privacy Rule requires you to have each research subject sign a separate authorization agreement. *

Federal regulations require that the IRB conduct continuing review of approved research. You will receive Continuing Review Report forms from this IRB when the expiration date is approaching.

Thank you for using this WCG IRB to provide oversight for your research project.

DISTRIBUTION OF COPIES:
<u>Contact, Company</u>
Debra Murphy, Arizona State University
Floris Wardenaar, PhD, Arizona State University
Joshua Beaumont, Sun Devil Athletics, Arizona State University

COA Template 01-02-2018

Board Action: 07/18/2018 Page 3 of 3

APPENDIX F

SKIN TEMPERATURE OUTCOME COMPARISON

Skin Temperature (°C) outcomes represented in mean ± SD (n=5)

	PAS	**EH-PAS**	**EH**	**EM**
Baseline	33.5 ± 1.18	34.7 ± 0.56	34.4 ± 0.56	32.7 ± 0.73
Average	39.1 ± 0.28	38.2 ± 0.45	36.8 ± 0.49	33.8 ± 0.51
Increment	6.7 ± 1.50	5.4 ± 0.90	3.6 ± 0.58	2.17 ± 0.58
Peak	40.3 ± 0.58	40.1 ± 0.71	38.0 ± 0.43	34.9 ± 0.65

Skin Temperature (°C) outcomes represented in mean ± SD (n=10)

	PAS	**EH-PAS**	**EH**	**EM**
Baseline	n/a	35.0 ± 0.90	34.4 ± 0.88	32.7 ± 0.67
Average	n/a	37.8 ± 0.56	36.7 ± 0.67	33.7 ± 0.47
Increment	n/a	4.3 ± 1.48	3.2 ± 0.72	1.8 ± 0.66
Peak	n/a	39.3 ± 0.91	37.5 ± 0.47	34.6 ± 0.61