

**Monitoring the efficacy of a field-based heat acclimatization protocol to
improve performance in elite female soccer players.**

by

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Abstract

The purpose of this study was to investigate the efficacy of a 14-day, two-phase, field-based heat acclimatization (HA) training camp in international female soccer players. Sixteen outfield players engaged in (i) baseline absolute Plasma volume (PV) testing in Vancouver, Canada ($\sim 15^{\circ}\text{C}$; 72.0% relative humidity: RH) 16 days prior to the start of the camp, (ii) Phase 1: 7 days of pre-HA ($22.1 \pm 3.3^{\circ}\text{C}$; $44.8 \pm 9.4\% \text{RH}$), (iii) Phase 2: 6 days of HA ($34.5 \pm 1.2^{\circ}\text{C}$; $53.2 \pm 4.3\% \text{RH}$), and (iv) 11 days of post-HA training ($18.2 \pm 4.6^{\circ}\text{C}$; $51.3 \pm 20.9\% \text{RH}$). Change in PV (%) from baseline was measured at the start of Phase 1, the end of Phase 1, and two days post-Phase 2. Core temperature (T_c), heart rate (HR), rate of perceived exertion (RPE), and Global Positioning System (GPS) derived metrics were recorded during all sessions. The physiological change during 5'-1' submaximal running (12km/h) was observed pre and post Phase 1 and 2, two days post Phase 2 (2dayP), and eleven days post Phase 2 (11dayP) using HR during exercise (HREx) and recovery (HRR), as well as RPE. GPS metrics, HRR, and HREx during a four-a-side soccer game (4v4SSG) were used to observe physical performance in the heat pre and post Phase 2. All data were analyzed using magnitude-based inference statistics. PV increased by $7.4 \pm 3.6\%$ (Standardized effect; $\text{SE}=0.63$) from the start of Phase 1 to the end of Phase 2, and this occurred primarily in Phase 1 ($\text{SE}=0.64$). 5'-1' submaximal running improved over Phase 2 in hot conditions (HREx; $\text{SE}= -0.49$, HRR; $\text{SE}=0.53$). The greatest improvement in submaximal running in temperate conditions was delayed as the largest change from Phase 1 in HREx ($\text{SE}= -0.42$) and HRR ($\text{SE}= 0.37$) occurred 11dayP. The 4v4SSG revealed a moderate reduction in HREx (-3.5bpm), a large increase in HRR (5.7%), and a moderate increase in inertial explosive movements (20%) from pre to post Phase 2. Field-based HA can induce physiological change beneficial to soccer performance in temperate and hot conditions and the 5'-1' submaximal running test may be used to effectively monitor submaximal HR responses that may have been induced by HA up to two-weeks out of the heat.

Lay Summary

Many international level female soccer players compete in hot environments around the world throughout a competitive season. Exercising in the heat induces a large stress on the body's cardiovascular and thermoregulatory system. Previous research has highlighted the adverse effects of heat on players' aerobic capacity and match-running performance. While heat acclimatization has been utilized as a method to combat these effects in mainly sub-elite athletes, there is limited evidence regarding the adaptive response in international level female team sport players. The key goals of the research were to implement current and novel non-invasive monitoring methods during a field-based heat acclimatization training camp of the Canadian National Women's Soccer Team. We aimed to determine the physiological and performance relevant adaptations that occur from heat loading in the camp in order to better understand how to effectively utilize field-based heat acclimatization prior to competition.

Preface

This dissertation is an original, unpublished, independent work by the author; K.Bowman. The fieldwork reported in Chapter 3 was covered by the University of British Columbia Clinical Research Ethics Board [certificate #H15-0003] approved March 31st, 2015. The research was identified by Dr. Cesar Meylan (Canadian Women's National Team Sport Scientist, Strength and Conditioning Coach) and proposed to the National Women's Team in light of the 2015 Fédération Internationale de Football Association (FIFA) Women's World Cup of Soccer. The research was supported by Own the Podium and the Canadian Sport Institute Pacific. The study design as outlined in Chapter 3 was structured around the National Women's Soccer Team training camp in Los Angeles, USA and Cancun, Mexico. All field-testing and monitoring noted in Chapter 3 was performed by Dr. Meylan and I, with the assistance of Wendy Pethick (Canadian Sport Institute Lab Technician) in Los Angeles. Soccer testing within the training sessions was directed by Dr. Meylan. The laboratory tests in Chapter 3 were performed by Wendy Pethick and I.

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List of Abbreviations

4V4SSG: Four versus four-small sided soccer game

2dayP: Two days post heat exposure

11dayP: Eleven days post heat exposure

AUC: Total Heat Load; Area under the core temperature curve.

AUC_{phase2}: Heat Load in Phase 2

AUC_{phase1-2}: Heat Load in Phase 1 and 2

BV: Blood Volume

RH: Relative Humidity

CO: Carbon Monoxide

CL: Confidence level

COD: Change of Direction

FIFA: Fédération Internationale de Football Association

GPS: Global Positioning Monitoring System

HA: Heat Acclimatization

Hb: Haemoglobin

Hct: Hematocrit

HR: Heart Rate

HST: Heat Stress Test

HRex: Exercise Heart Rate

HRR: Recovery Heart Rate

HRmax: Maximal Heart Rate

IMA: Inertial Movement Analysis

LA: Los Angeles

LTHA: Long Term Heat Acclimation

SD: Standard Deviations

SE: Standardized Effect

SR: Sweat Rate

Pre-HA: Pre-Heat Acclimatization Training

Post-HA: Post Heat Acclimatization Training

PV: Plasma Volume

PO: Power Output

*c*Hb: Total haemoglobin concentration

*c*HbCO: Total Carboxyhemoglobin concentration

HbCO: Carboxyhemoglobin

Hb_{Mass}: Haemoglobin Mass

M/min: Meters per minute

MTHA: Medium Term Heat Acclimation

STHA: Short Term Heat Acclimation

TEM: Typical Error of Measurement

TT: Time Trial

T_a: Ambient Temperature

T_{ac}: Auditory Canal Temperature

T_c: Core Body Temperature

T_{cskin}: Skin Temperature

T_{c-start}: Core Body Temperature at the start of exercise

T_{c-end} : Core Body Temperature at the end of exercise

T_{Rectal} : Rectal Temperature

ΔT_c : Total change in core body temperature in a single training session.

RBCV: Red Blood Cell Volume

USG: Urine Specific Gravity

V_E : Minute Ventilation

[Na⁺]: Sweat Sodium Concentration

WNT: Women's National Team

WWC: Women's World Cup

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Chapter 1: Introduction

1.1 Physiological challenge of exercising in the heat

Elite athletes often compete in hot and humid environmental conditions around the world, exposing them to heat stress during high-intensity exercise characterized by a progressive increase in core temperature (T_c). Hyperthermia increases the physiological strain on the body and is highlighted by reductions in exercise capacity and heat exhaustion that commonly result in an early onset of fatigue [34]. The literature has suggested that exercise-based thermal strain is centred on a combination of a rapid incline in exercising heart rate and a critically high T_c [17]. Taken together, this thermal strain directly accelerates fatigue during exercise by impairing both the voluntary action of muscle as well as blood pressure to induce a cardiovascular strain [34]. The extent of thermal stress that may arise when competing in these conditions is assessed via four primary quantities: air temperature, mean radiant temperature, water-vapour pressure, and air velocity, while also taking into consideration the athletes' clothing and exercise type [23]. Thermal balance occurs when the heat that is produced by the body and absorbed from the environment equals the heat dissipated from the body via evaporation [134]. Heat transfer between the body and the environment occurs by heat flow down temperature and humidity gradients through three independent processes: thermal radiation, convection, and evaporation [17].

In highly competitive athletes, heat production is a function of maximal aerobic power and relative exercise intensity [17]. Additionally, heat is retained in the body as a function of its mass and the mean specific heat of the body tissues; therefore, in a high-speed, intermittent running sport such as soccer, athletes often have a large degree of metabolic heat production [17]. Specifically, the heat that is generated by contracting muscles during exercise increases T_c , which in turn, redirects blood flow to the skin for peripheral blood vessel dilation and sweating [27]. Therefore, in order to prevent heat exhaustion and maintain a tolerable core body temperature, there must be a heat transfer from the body to the environment [17]. However, when air temperature is higher than skin temperature (35°C), the temperature gradient favours a physical heat transfer from

the environment and as a result, convection adds to the body's overall metabolic heat load [27].

Previous literature has also demonstrated that even in a more temperate condition ($17\pm 4^{\circ}\text{C}$), elite athletes can experience an extensive amount of metabolic heat production that has the potential to induce early fatigue [7]. This presents a disruption in thermal balance that is problematic for athletes during competition, as metabolic heat cannot be effectively dissipated at the rate it is produced [107]. When this occurs, sweat evaporation becomes the primary means of heat transfer and is dependent on the water vapour pressure gradient between the skin, the environment and the movement of air over the skin [107,165]. A high relative humidity (RH) $\sim 70\%$ indicates that the water vapour pressure in environmental air is high [85]. When evaporative cooling required by the body exceeds the environment's evaporative cooling capacity (ability to hold more water vapour) it can lead to thermoregulation inefficiency and a total body water loss that cannot be regained [37,108,149]. Physiologically, this results in a rapid rise in heart rate (HR) that overwhelms the blood flow required for exercising muscle [128, 130]. Athletes often experience early fatigue when this occurs, as the only way to achieve thermal balance is to reduce metabolic heat production by lowering overall exercise intensity [128]. Furthermore, if sweat loss is not adequately replaced, dehydration from a reduced central blood volume causes a rise in T_c [130, 134]. A body mass loss of 3-4% from dehydration reduces muscular strength by approximately 2%, muscular power by 3%, and high-intensity endurance by approximately 10% [90]. Cardiovascular strain is common during exercise in the heat and is characterized by an accelerated increase in exercising HR and an overall reduction in sweat rate from sustained dehydration [41]. In a study of both untrained and trained subjects exercising in the heat (60-75% $\text{VO}_{2\text{max}}$), findings revealed that cardiovascular strain over thermoregulatory factors was foremost in the cause of fatigue during aerobic exercise in the heat and was characterized by significant declines in stroke volume (15-26%), cardiac output (5-10%), and mean arterial pressure (15-26%) [128].

Aside from cardiovascular stress, there is also metabolic concern for fuel depletion [30]. A previous study highlighted that when subjects exercised in a hot environment (44°C) versus a cold environment (9°C), they utilized 76% more muscle

glycogen in the heat [57]. There was also evidence of a 3.9% loss in body mass that occurred during prolonged cycling exercise in the heat (35°C) that caused a large increase in muscle glycogen use (45%) [73]. As many high-speed intermittent running sports rely on glycogen as a primary fuel source [25], the added effect of a hot environment during competition depletes energy sources at a faster rate than temperate conditions which is likely to contribute to the early onset of fatigue commonly seen in the heat [56]. Soccer in particular requires a large degree of technical skill that may potentially induce more stress on the metabolic system in the heat [30,47]. In addition, thermoregulatory responses associated with exercise in extreme heat including an elevation in core and skin temperature as well as a reduction in sweat rate, cause a greater perception of effort affecting an athlete's ability to pace and strategize appropriately [77]. These adaptations are particularly important for team sport athletes who rely a great deal on technical and tactical components within competition for a successful performance [7].

1.2 Heat adaptation for team sport players

While heat stress is primarily thought of as an issue for endurance athletes, it is also problematic for intermittent, high-speed running sports such as soccer where matches are often played in extreme conditions and ambient temperatures (T_{ambient}) can exceed 30°C with moderate to high relative humidity (40-70%) [30,115,124]. Repeated sprint ability is highly correlated to soccer match performance in top-level players [12,141]. However, when playing in hot conditions, hyperthermia affects various physiological systems and can limit a players' ability to sustain repeated high-intensity running and fast directional movements (acceleration and deceleration) [22,106,108,116]. The adverse effect of heat stress during competition was highlighted in a previous soccer study when players experienced a reduction in total running distance (-7%), high-intensity running (-26%), and running velocity (-3%) during a hot weather match (~43°C) [115]. Additionally, the physiological strain of an added heat load has occurred in more mild conditions as well (~21°C) and there is evidence that this added heat load can negatively affect the distance that players can cover in one game [26], as well as the number of high-speed runs (<14km.h⁻¹) that players can achieve

throughout a game [115]. Soccer performance in terms of the total distance covered in a hot weather match and running speed at the end of a hot weather match were shown to be adversely affected by a rising internal T_c as they were correlated with either T_c ($r=0.53$) or Δ increase in T_c ($r=0.85$) [115]. A 6% reduction in total running distance during a hot weather match ($\sim 43^\circ\text{C}$) was also evident in another soccer study observing the effect of heat related stress on performance [138]. The 2022 Fédération Internationale de Football Association (FIFA) World Cup of Soccer is scheduled to be in Qatar where average high temperatures from May to November are extreme ($37\text{-}28^\circ\text{C}$) [1]. Therefore, it is important to understand how to utilize an effective field-based heat acclimatization protocol in order to preserve soccer performance in extreme heat and to understand how it may have the potential to be ergogenic under more temperate conditions as well ($\sim 20^\circ\text{C}$)[40].

Heat acclimatization minimizes these performance declines and early signs of fatigue [22]. Specifically, these protocols enable physiological changes to occur that promote regulation of cardiovascular strain as well as counter the inefficiencies in thermoregulation [40,77,136]. Naturally occurring field-based heat acclimatization in team sports has demonstrated improvements in exercise tolerance, exercise capacity, and perceptions of thermal load [30]. In a previous analysis of the 2014 FIFA World Cup of Soccer in Brazil, players did not experience a reduction in peak speed during matches but the number of sprints and high intensity running distance was lower in high environmental stress ($>32^\circ\text{C}$) compared to moderate ($28\text{-}30^\circ\text{C}$) or low stress ($22\text{-}28^\circ\text{C}$) [118]. The review also highlighted that players had a tendency to modulate their activity pattern during matches in hot and humid environments as characterized by less high-intensity running [118].

1.3 Relevance and significance

It is evident that the capacity to exercise in hot environments is markedly reduced relative to that in cooler conditions [75]. Recent evidence among team sports has demonstrated that those players who are un-acclimatized to the heat prior to competition are likely to experience a reduction in performance [77,115]. There is a need to develop more team sport specific strategies for international level players

preparing for competition in hot geographical locations [77]. In soccer, the combined effect of an increasing internal T_c and a reduction in the amount of sprinting and high intensity running characterizes heat related fatigue that is detrimental to match performance [116,124]. However, the research regarding the challenges of heat stress and the adaptive response with heat acclimation have focused primarily on untrained individuals or endurance athletes performing constant-work rate exercise over 1-2 week interventions [104,128,140]. There is a gap in heat acclimatization research as controlled protocols are not relevant to the variable environmental conditions of many international level team sport athletes [40]. Additionally, findings from these studies are not easily transferred to highly trained athletes who typically display higher maximal oxygen consumption (VO_{2Max}) than untrained individuals [16,65,66]. At the international level, athletes are often under a time constraint in terms of availability and training time [136]. Field-based heat acclimatization has the potential to confer greater physiological tolerance and transferability of the adaptation to the sport than in the lab without sacrificing training technique [136,161]. It is not practical or time-efficient for elite players to engage in exercise that they would not encounter in competition (e.g. continuous intensity cycling, treadmill exercise over a long duration ~90 minutes) [95,127,131]. It can often be difficult for teams based in temperate regions to devote an equal time to lab-based heat training [30]. Therefore, the use of sport-specific heat acclimatization in the current study will allow players to maintain their typical training schedule as well as their training technique prior to competition.

A second gap in heat acclimatization literature is the limited knowledge available regarding the adaptive response in female sport players as field-based heat interventions have primarily utilized male athletes (soccer, Australian Football League, rugby) [20,22,116]. Findings from Sunderland and colleagues [158] were the first to highlight the physiological adaptations associated with field-based heat acclimatization in female soccer players. Additionally, the use of both T_c monitoring and GPS (Global Positioning System) software have provided novel information regarding the individualized responses to heat stress in male sport players [20]. Previous research has shown GPS to be an effective model of loading parameters which is especially useful for training in a hot condition as there is a need to ensure adequate recovery in order to

prevent overexertion [45,107]. There is no current evidence of this technology being used to monitor female athletes during heat adaptation. This thesis aims to expand on current knowledge regarding heat adaptation in female team sport athletes by utilizing international-level female soccer players in a sport-specific field-based heat training protocol.

Current evidence on heat adaptation in both female and male athletes has suggested that the time course between physiological and performance-based heat acclimation responses may differ [109]. This was highlighted in a recent study observing the effect of heat training alone as well as the combined effect of heat and altitude on running performance and physiological response; immediately post and three weeks post lab-controlled heat exposure [109]. While there was a 3.8% increase in plasma volume (PV) immediately following the heat intervention, the ergogenic response was not seen until three weeks post heat exposure [109]. The current study will expand on this research by providing a greater insight into the induction and decay of physiological and performance based adaptations as well as the extent of recovery that is required following a heat intervention before an ergogenic response may be observed [109]. Therefore, the current study may assist in the development of a more reliable estimate of how long athletes can be free from heat exposure before re-acclimatization is required [30,77].

There is also a need for further research regarding the temperate weather performance benefit of heat acclimatization as current evidence shows conflicting results [104,122,137]. While some studies using either laboratory or uncontrolled field-based heat acclimation have indicated performance improvement in cooler conditions [104,154], others have noted that an ergogenic effect was observed only in the heat [122]. Previous studies have indicated that a PV expansion from heat acclimation is likely to positively impact temperate weather performance [104]. Specifically, an expansion in PV has shown to mitigate the reduction in cardiac output from fluid loss [131], lower cardiac frequency, and promote a reduced thermal strain and more efficient thermoregulation [161]. With continued investigation towards sport-specific heat acclimatization, these adaptations may allow players to achieve a greater maximal performance in both temperate and hot conditions through greater heat dissipation and

an enhanced cardiac efficiency [131]. The use of simple submaximal testing and HR monitoring in the current heat acclimatization study may provide greater insight into the ergogenic potential of adaptation in temperate conditions [20]. Accordingly, the aim of this research was to use a naturally occurring hot environment to induce soccer-specific heat acclimatization while leveraging novel monitoring metrics in order to provide a greater insight into the female adaptive response.

1.4 Purpose, hypothesis, and objectives

Many major team-sport events are scheduled to take place in hot and humid geographical locations including the 2022 FIFA World Cup (Qatar) and the 2020 Olympics in Tokyo, Japan. There has been considerable interest among teams to adopt heat acclimatization to increase physiological stress and induce performance adaptations as opposed to increasing external load (training duration, intensity, volume) in order to avoid overreaching or injury [109]. The purpose of this thesis is to narrow the research gap in sport-specific, field-based heat acclimatization and provide greater insight into the adaptive response in female soccer players during and post two phases of heat training. Non-invasive performance testing and monitoring metrics will be used to investigate the progression of heat adaptation within these two phases.

It was hypothesized that a field-based, sport specific period of HA training would induce a meaningful PV expansion between 5-7% over six days of heat exposure in Phase 2. In addition, submaximal-running performance would reveal a decrease in exercising heart rate (HR_{ex}) and an increase in recovery heart rate (HRR) at least two days following heat exposure. Furthermore, soccer performance as a measure of GPS external load metrics as well as HR_{ex} and HRR during a small-sided-game would improve in the heat pre to post heat acclimatization. Heat acclimatization is abbreviated to HA in the current discussion.

Thus, the major objectives of this project are as follows:

- To provide insight into the physiological response of the female-soccer player to field-based HA by observing the change in PV following heat acclimatization.

- To confirm the effectiveness of traditional HA monitoring by utilizing T_c sensors to assess individual player response to heat stress within both training phases.
- To assess the aerobic response to HA by observing the change in heart rate response (HR_{ex}, HRR) during a submaximal running test on the first day and last day of Phase 1 in a mild condition (Day 1 and 7), the first and last day of Phase 2 (Day 9 and 14) in a hot condition, as well as two days post Phase 2 (Day 16), and eleven days post Phase 2 (Day 25) in a temperate condition.
- To utilize a four-a-side, small-sided soccer game (4V4SSG) in combination with heart rate (HR_{ex}, HRR) and GPS-derived external load metrics to observe the physical changes to soccer performance pre and post HA

Chapter 2: Literature Review

2.1 Physiological relevance of heat acclimatization

Effective heat protocols have the potential to attenuate both cardiovascular and thermoregulatory strain, improve perceptions of thermal stress, and limit early fatigue during exercise in hot environments [115]. Sport-specific HA has been shown to induce adaptations that minimize the impact of environmental heat stress on exercise performance in both temperate and hot conditions [30,136]. These adaptations are underpinned by repeated exposure to a thermal stressor (rise in T_{c}) that disrupts the body's internal environment to induce morphological and chemical changes that reduce physiological strain [160].

2.1.1 Heat adaptation

Heat adaptation is critical for elite athletes competing in hot weather as the effects of thermal stress have been shown to negatively impact an athlete's ability to sustain a high level of performance [108]. The physiological changes induced by HA support homeostasis and increase thermal tolerance by converting a thermally unmanageable state into a more neutral condition [160]. There are two broad classes of heat adaptation that have been researched in athletes including: acclimation and acclimatization.

Heat acclimation refers to repeated bouts of heat exposure in an artificial or laboratory setting whereas acclimatization refers to exposure to a naturally occurring hot environment [30]. The literature has focused primarily on laboratory-administered heat training bouts (cycling or treadmill exercise) where T_{c} and exercise intensity are fixed [67,104]. Controlled hyperthermia is a common method of lab-based acclimation whereby internal and/or external heat load is manipulated providing a sustained adaptation stimulus [140]. Traditional constant work-rate rate protocols differ from controlled hyperthermia as they result in a progressively lower training stimulus throughout acclimation [140]. Controlled hyperthermia protocols range from 60-120 minutes and utilize a fixed exercise intensity that is set at a T_{c} stimulus of 38.5°C to

stimulate adaptation [68]. These protocols have proven to be effective in demonstrating improvements in endurance performance [68]. In a study of competitive cyclists, controlled hyperthermia over ten days increased PV (~6.5%), maximal cardiac output (~4.5%), lactate threshold (~5%), and maximal O₂ (~8%) in a hot condition (38°C, 30% RH) [104]. These findings are consistent with a five-day rowing acclimation study also utilizing controlled hyperthermia which demonstrated a 1.5% increase in rowing performance [69]. Physiologically, controlled hyperthermia over eight to ten days has also shown to be effective in inducing a plasma volume expansion and greater cardiovascular stability [104,126].

There is also a passive-controlled form of HA that uses exogenous heat to raise body temperatures while minimizing metabolic contribution [154]. However, passive HA has been noted to confer only slight heat strain and is often a less effective adaptation stimulus than exercise-induced HA [161]. In the case of Scoon et al. [154] and Shido et al. [156], passive heat adaptation is gained only if the cumulative thermal impulse is sufficiently large to induce physiological changes which has previously been achieved through hot-water bathing [161]. Therefore, the inclusion of high-intensity exercise has been noted in the literature to induce a more profound adaptation with a smaller rate of decay, as the metabolic heat load from exercise acts as an additional stimulus [127,154].

However, for international level team sport athletes, passive HA and controlled hyperthermia utilizing lactate measurements and TT performance tests are less ecologically valid for teams competing in unpredictable environments [127,136]. Additionally, the artificial conditions in laboratory heat protocols have shown to result in non-sport specific adaptations for team sport athletes with limited transference and practicality towards the athlete's sport performance [140,161]. While TT and time to exhaustion performance tests (TTE) may provide important feedback for endurance athletes, they do not always mimic the demands of high-intensity team sports that combine both aerobic and anaerobic systems during repeated sprinting [47].

Alternatively, heat acclimatization (natural environmental setting) has shown promise for both endurance [92,140] and team sports [22,137,138]. In a previous cycle-based HA study for example, time trial (TT) performance in the heat (34°C, 18%RH)

improved from the first TT to the second TT by 11% following two-weeks of naturally occurring HA [92]. Field-based HA protocols have more recently been observed in team sports such as Australian Football League (AFL) and soccer [22,137]. While acclimatization less controlled than acclimation, the use of environmental heat to induce heat adaptation is a more practical option for team sport players as a sport specific protocol may be utilized [127]. This was supported in a study comparing both T_c and time to heat exhaustion in both lab and field cycle-based protocols when physiological tolerance to heat strain was markedly greater following field (acclimatization) versus lab (acclimation) [148]. Similar to controlled-hyperthermia, these outdoor-based protocols trigger physiological changes and reduce the impact of thermal stress on exercise performance in the heat [77,134]. Evidence has suggested that natural acclimatization gained over 1-2 weeks may confer not only greater overall heat tolerance than acclimation but also allow a greater magnitude of adaptation that is directly related to the competition environment [77]. Complete exercise-induced heat adaptation for both hot-dry and hot-humid environments occurs at different points within HA but most are observed after 7-10 days of exposure [125,134]. Additionally, about 75-80% of the adaptation process is commonly seen in four to seven days with cardiovascular responses occurring first [30,131].

2.1.2 Cardiovascular, sudomotor, and thermoregulatory adaptations

The unique cardiovascular challenge during exercise in the heat is the requirement to provide sufficient blood flow to exercising muscle, while simultaneously allowing peripheral blood flow to the skin to ensure evaporative heat loss and a sustained work capacity [131]. With HA, cardiac output increases or is maintained and greatly improves the regulation of blood flow to working muscle [126,131]. The hallmark cardiovascular changes observed within HA literature include an elevation and/or maintenance of stroke volume (SV) that is mediated through gains in PV, which increases total body water and lowers HR_{ex} [126]. While these cardiovascular adaptations are typically the first to appear (75% in the initial three days of heat exposure) they have also been known to be the first to decay following the first two weeks out of the heat [6,67].

Previous literature has suggested that the mechanism of induction for PV expansion is likely a result of a net increase in total intravascular protein content. Therefore, due to an oncotic effect, this increase in protein content causes a movement of fluid from the interstitial space to the intravascular space. [78]. Specifically, there is an increase in blood plasma volume and total body water as a result of the expansion of the extracellular fluid compartment [126]. A more recent review highlighted that a plasma volume expansion is likely attributed to a greater secretion of aldosterone and arginine vasopressin, as well as a conservation of sodium that assists with extracellular fluid retention [131]. These changes enhance cardiac output by increasing end-diastolic volume and venous return to the heart, which allows more blood to be pumped out of the heart each minute [22,119]. The increase in stroke volume is mediated by plasma volume, as a greater total blood volume allows the cardiovascular system to tackle metabolic, cardiovascular, and thermoregulatory demands more efficiently in extreme heat.

A greater cardiac efficiency is practical for a wide variety of high-intensity sports and there is evidence that a PV expansion is relevant to the enhanced aerobic capacity (VO_{2Max}), commonly observed following heat exposure [40,104]. Both Garrett et al. [65] and Racinais et al. [139] demonstrated improvements in sport performance and an increase in resting PV following short-term (five days) heat acclimation and a long-term (fourteen days) acclimatization (+4.5% and +5.2%, respectively). While these studies utilized different methods of heat adaptation (lab-based versus field-based) they both showed a positive ergogenic response in aerobic fitness (-4sec in 2000m rowing TT, +311m in a Yo-Yo Intermittent Recovery Test Level 2 (Yo-Yo-IR2 soccer test). [10]. The mechanism of this enhanced cardiac efficiency has been previously suggested to be a result of the physiological change from heat adaptation and that the change in cardiac hemodynamics may be correlated to an expanded PV [44]. A previous study found a 7% increase in PV that was largely correlated to an improvement in HR_{ex} as noted by the large effect size [-0.52 (-0.64; -0.38) 90% CL] following seven days of soccer-specific HA [22]. A recent HA study has also suggested that a change in PV may be observed through a change in the volume of red blood cells or hematocrit (Hct) and may also provide insight into a player's ability to cope with heat in a game [138].

Specifically, findings from this study revealed that a net decrease in Hct was correlated to an observed increase in total running distance within the post HA game $r=-0.75$; 90%CI [-0.88 to -0.51] [138]. The utilization of Hct as a measure of PV expansion was also utilized by Karlsen et al. [92] who demonstrated a 5.6% reduction in resting Hct that correlated with an improved cycling TT performance ($r=-0.79$) in the first seven days of a two-week, controlled, lab-based HA protocol. However, there are a number of issues related to the assumption that Hct is exclusively representative of a PV expansion from chronic heat induced adaptation. While Hct has been previously used as a marker for PV expansion, heat adaptation is multifactorial and the observed changes in haematology are not the only indicators of physiological change [131]. Specifically, a reduction in Hct is unlikely to be the physiological mechanism improving exercise capacity in the heat given that an artificial increase in PV does not improve thermoregulatory function [138]. Additionally, there are other factors that can represent an acute reduction in Hct aside from a PV expansion including iron deficiency or anemia as well as an acute intake in fluids (over-hydration) [39,138]. Additionally, a recent review highlighted that while the extent of PV expansion after 3-4 days of heat exposure often varies (4-15%), erythrocyte volume and Hct remain unaltered [131].

Aside from the improvement in cardiac hemodynamics, increases in PV have shown to increase thermoregulation efficiency by enhancing fluid balance and skin blood flow as well as sweating efficiency [77]. With acclimatization, eccrine sweat glands increase sodium reabsorption and reduce sweat sodium concentration $[Na^+]$ [131]. Those remaining solutes that are not lost as sweat during exercise, are used to exert an osmotic pressure allowing for more effective redistribution of fluid and a greater blood flow to working muscle [143]. Additionally, a recent review has suggested that thirst sensations occur in response to changes in plasma osmolality [131]. Specifically, a PV expansion allows for a greater maintenance of fluid balance through an earlier onset of thirst that attenuates perceptions of heat related stress as well as cardiovascular strain [146]. The relationship of thirst to body water needs is greatly improved with HA so that voluntary dehydration is markedly reduced (~30%) [127]. Overall, this allows for a greater uptake of fluid during intense exercise and less body water deficit [127,146]. In terms of sweating efficiency, heat-adapted athletes have

shown to have a smaller relative PV reduction for a given body water deficit, a more dilute sweat, and an increased sensitivity to sweating [120,131,161]. Improvements in core to skin heat transfer, sweating T_c threshold, and sweating efficiency commonly develop after a minimum of seven heat exposures [30,164]. Following six days of HA for example, a mean 4% increase in PV was found to largely translate into improved thermoregulation in the heat (+34% increase in sweating and a 18% decrease in sweat sodium concentration ($[Na^+]$) [138]. These findings are consistent with an earlier study highlighting a 26% increase in sweat rate following 8 to 12 days of HA that contributed to a greater regulation of blood volume and cardiovascular stability [120]. In a short-term laboratory based HA study, resting auditory canal temperature (T_{ac}) as well as T_{ac} sweating threshold, significantly decreased ($-0.21^{\circ}C$; $-0.15^{\circ}C$) from day 1 to day 6 of acclimation [42]. Collectively, these cardiovascular and sudomotor adaptations have the potential to negate early fatigue commonly seen in un-acclimatized athletes exercising in the heat both physiologically and psychologically [138].

Thermoregulatory adaptations typically occur after cardiovascular adaptations within the heat adaptation process [131]. The literature has specified that the potential mechanism for an enhanced thermal tolerance with HA, is the result of a greater core to skin heat transfer due to cutaneous vasodilation occurring at a lower T_c threshold [31]. Aside from the improvement in cardiac output, there are a number of temperature sensitive mechanisms that stimulate thermal adaptation to further assist with the reductions in physiological strain during exercising in the heat [131,164]. With improved heat transfer, there is greater potential for evaporative heat loss which reduces T_c and skin temperature (T_{skin}) as a result [31]. In a recent review it was noted that a mitigation of internal body temperature rise during exercise and a reduction in resting internal body temperature of approximately $0.5^{\circ}C$ occurs by day five of HA [134]. This was evident in a HA study with trained rowers when athletes experienced a lower rectal temperature (T_{Rectal} : $-0.3^{\circ}C$) during the heat stress test on the fifth acclimation day of a five-day protocol [64]. Within the same study, athletes also required a greater amount of time (+3 minutes) to reach their target training temperature of $38.5^{\circ}C$ on the final acclimation day as compared to the first day [65]. A lower T_c post heat exposure has the potential to enhance exercise performance as the athlete is not only starting exercise at a

lower T_c , but has a slower rate of rise and can tolerate a higher T_c from continuous exposure within the adaptation period [46,65,119]. Additionally, two separate HA studies noted improvement in thermal tolerance as evident by the reductions in T_c at the end of exercise (T_{c-end}), specifically, -0.5°C (T_{Rectal}) noted by Daanen et al. [46] and -0.43°C (T_{Rectal}) by Houmard et al. [84]. The literature has suggested that a lowered T_c during exercise and at the end of exercise following HA is beneficial to exercise performance in the heat for a number of reasons [30]. A lower T_c increases the body's heat storage capacity to prevent an early onset of heat stress, while also allowing more work to be done before reaching a critical T_c [92]. Particularly for team sport athletes, reductions in T_c have shown to reduce the thermal strain associated with the drive for heat dissipation and allow athletes to better adjust pacing strategies as a result [30,46].

The physiological adaptations of HA decay at different rates and while the rate of decay is slower than that of induction, cardiovascular adaptations tend to decay before thermoregulatory adaptations [140]. Baseline aerobic fitness prior to HA may have an effect on the extent of loss of HA, which highlights the importance of training status as a factor for adaptation retention [125]. In a recent study, minimal to very little HA was lost in terms of both HR and T_c following twenty-six days of exposure when a daily HA regime was maintained [164]. Therefore, the literature has suggested that athletes maintain a training stimulus during the non-heat exposure period to ensure adaptation is maintained [6]. This is particularly important for elite athletes who already have well-adapted aerobic energy systems and require a high training threshold for adaptation to develop [77]. Additionally, during short-term HA protocols, physiological adaptations have shown to persist for one week, but not two as seen in longer protocols [67]. Therefore, intensity, duration, and frequency of heat exposure within HA are important considerations for team sport athletes preparing for hot weather competition in terms of maximizing adaptation potential [127]. Specifically, there may be an adaptation trade-off in terms of longer heat exposure periods and the risks associated with heat stress versus shorter protocols that tend to have a faster rate of decay in HA adaptations [127]. Aside from the cardiovascular, sudomotor, and T_c adaptations, there are also metabolic changes induced by HA which may also play an ergogenic role during competition in the hot weather [43].

2.1.3 Metabolic adaptations

Recent research has demonstrated that the combination of exercise and heat stress have a major impact on metabolic and cellular processes [52]. Specifically, exercise in the heat has shown to induce an augmentation in glycogenolysis [52]. Logan-Sprenger et al. [102] and Sawka et al. [143] noted that a high T_c and extensive fluid loss from dehydration are likely critical factors in the increased reliance on carbohydrate oxidation observed during exercise in the heat. In two separate cycle-exercise trials in the heat (~135 minutes of cycling, 35°C), a 45% increase in muscle glycogen use was only observed in athletes who were dehydrated (3.9% loss in body mass) versus those who maintained hydration (and body mass) throughout the exercise protocol [73]. This was later supported by in a review of muscle metabolism and performance in the heat which outlined that if submaximal exercise in the heat increased T_c by $>0.5^\circ\text{C}$, intramuscular carbohydrate utilization is augmented [52].

An effective HA protocol has the potential to attenuate carbohydrate metabolism, which is beneficial for many sport players given that glycogen is a primary fuel sports for these athletes [25]. Fujui and colleagues [62] previously reported a reduction in aerobic metabolic rate and minute ventilation (V_E) ($-112 \text{ ml} \cdot \text{min}^{-1}$) during a cycle-based exercise heat test (40-75 minutes at 58% $\text{VO}_{2\text{peak}}$, 37°C, 50%RH) following six days of HA training. A recent review also highlighted a reduction in lactate accumulation, respiratory exchange ratio, blood glucose concentration, and less depletion of glycogen from type 1 muscle fibers following short-term HA [30]. After eight days of field-based HA, an alteration in metabolic substrate use was observed as muscle glycogen utilization markedly reduced by 42% during an exercise test in the heat (39.7°C, 6h intermittent submaximal exercise at 50% $\text{VO}_{2\text{Max}}$) [96]. The mechanisms associated with this decreased reliance on muscle glycogen stores may potentially result from a maintenance of total body water from improved thermoregulation [151] and an increased perfusion of blood to the periphery to support exercising muscle [102]. As a result, blood glucose and fatty acids would reduce the dependence on muscle glycogen for energy production [96]. This is a critical adaptation for elite soccer players as large

reductions in glycogen stores have been associated with early fatigue in high-intensity games [96]. Additionally, Chalmers et al. [30] noted that a lower whole body metabolic rate would decrease metabolic heat production, reduce T_c , and put less stress on thermoregulatory and cardiovascular demands. While it remains unclear at what time point carbohydrate attenuation occurs within the heat adaptation process, it has been suggested to be between the fourth and eighth day when there is enhanced regulation of T_c and thermoregulatory demands [30,102,151]. Therefore, the reduction in carbohydrate utilization commonly seen post HA may have the potential to improve heat tolerance and positively influence player performance at the central level through improved perceptions of fatigue and effort [98].

2.1.4 Perceptual adaptations

The literature has suggested that heat adaptation improves performance in the heat as a result of not only physiological changes but also perceptual changes [30,94]. Specifically athletes have reported feeling more thermally comfortable in higher temperatures following a period of HA, which has translated into reduced heat-related fatigue in competition [34]. Heat stress in un-acclimatized athletes has been shown to cause psychological limitation through impairments in pacing strategy relating directly to the effect of a high T_c and an increase in heat storage from impaired thermoregulation [34]. Hyperthermia also has the potential to affect the functioning of the brain through alterations in cerebral blood flow [30,33]. Specifically, hyperthermia decreases the level of cognitive or neuromuscular drive in the brain, which causes a reduction in maximal voluntary contraction and increases RPE [34]. This impairment in central nervous system function during exercise in the heat is especially problematic in a skill and tactic-based sport such as soccer, which places a large emphasis on strategic decision making within a match [8,32]. Additionally, the literature has consistently reported a loss in coordination with extreme hyperthermia, which adversely affects team sport players likely to a greater extent than endurance athletes [34]. While the literature has indicated a positive psychological response with heat familiarization in endurance athletes [153], the perceptual benefit of HA independent of physiological adaptation is likely also very important for team sports including soccer. Specifically with heat adaptation, the

limitations of heat stress on neuromuscular drive are improved to enhance overall perception of effort in the heat and negate limitations in pacing strategy [34]. However, intermittent team sports in particular may require more tactical and strategic decision making versus endurance sports making heat adaptation a crucial component of mental performance [91].

A secondary consideration regarding the perceptual advantage in team sport relates to the impact of group performance. In individual sports an athlete's result is solely dependent upon individual physical and mental ability while in team sports, performance outcome is highly dependent upon teammate performance as well [91]. Loss of motivation, coordination, and decision making capability within hot weather competitive matches have consistently been observed in team sport with large reductions seen mainly in the last half of competitive matches [71,116]. Therefore, the challenges of heat stress in terms of tactical and technical skill loss may have a compound effect on team performance versus a single endurance athlete. It is possible that with heat adaptation, those athletes who experience a greater degree of heat stress may be less likely to experience a reduction in motivation and intensity that may otherwise impact other players on the field and group performance altogether [71].

Sunderland and colleagues [158] for example, observed a lower T_c following four high-intensity, soccer-specific HA sessions at the same relative intensity in the heat (30°C, 27%RH). Athletes in this study not only felt cooler during exercise but also had a lower T_c early on in exercise and were able to reach a higher T_c target (39°C) post-acclimatization than those who were un-acclimatized. This was later supported in a laboratory-based HA study when a reduction in both thermal sensation (Day 1 to Day 9: 2.3±0.5 to 1.6±0.5) and thermal comfort (Day 1 to Day 9: 2.4±0.8 to 1.9±0.6) following HA [46]. Additionally, the rate of T_{Rectal} rise during exercise in this study decreased by ~0.8°C from Day 1 to three days post HA [46]. Reductions in both thermal sensation (-1.3±0.8) and thermal comfort (-0.8 ± 0.6) were also observed following four days of a lab-based cycling protocol (30-45 minutes, high-intensity cycling in 30°C, 60%RH) [132]. Evidence of improvements in RPE following short-term HA was highlighted by Castle et al. [29] in the initial three days of a ten-day cycle ergometer-based HA protocol. Attenuation in RPE was also evident after eight days of soccer-specific

training in the heat ($\sim 35^{\circ}\text{C}$) [20]. The later study was the first to observe the relationship between the internal cost of exercise defined as RPE and the average external work (running intensity, total distance/min) throughout each session (change in RPE:m/min ratio over eight HA training days) was observed [20]. After an initial two-day coping period to adjust to the increased thermal strain, there was a reduction in the RPE:m/min ratio trend from the second training session onwards [20].

Conversely, there are some studies that have failed to observe a change in perceived exertion with heat adaptation [42,62,158]. It is likely that those studies lacking perceptual adaptation were limited by the subjective nature of the maximal exercise test in which RPE was collected [30]. Additionally, heat protocols limited by a non-sport-specific exercise modality or that utilize too short of a protocol (four-days) are likely to obtain only a partial improvement in thermal sensation and thermal comfort regardless whether the exercise is in temperate ($\sim 20^{\circ}\text{C}$) or hot ($\sim 30^{\circ}\text{C}$) conditions [132]. This was evident following four days of HA in male cricketers when RPE did not change likely due to the use of lab-based cycle ergometer exercise within HA versus the outdoor training specific to the sport of cricket [132]. An important consideration however is that while cricket and soccer are both generalized as team sports, physiologically speaking these two sports are not comparable and the performance demands within a match are completely different [91]. Overall, while many studies have noted an improved perception of effort and higher psychological heat tolerance with HA, perceptual adaptation is largely individual and more evidence is required to understand this adaptive response in team sport players [30].

2.2 The ergogenic potential of heat adaptation under temperate conditions

Current literature has provided strong evidence that repeated training exposures in a hot environment can elicit adaptations that offset the impedance of cardiovascular strain during exercise in the heat and may also have the potential to improve sport performance in more temperate conditions ($\sim 14\text{-}20^{\circ}\text{C}$) [40].

The ergogenic effect of heat adaptation under more temperate conditions was evident following various lab protocols as noted by the observed increases in VO_{2Max} and lactate threshold [104,117,150]. However, an ergogenic benefit of HA is particularly important for soccer players and other team sport athletes who often compete in variable environmental conditions [136]. Field-based protocols such as those utilized by Buchheit et al. [21,22] and Racinias et al. [138] have demonstrated temperate and hot weather improvements to team sport performance. Petersen et al. [132] also highlighted the ergogenic potential of HA for sport players when four treadmill HA sessions of (30-45 minutes) resulted in a reduction (-11bpm) in HRex and also improved the perception of thermal strain in the heat.

While there is increasing evidence to support an ergogenic effect of HA, the majority of the literature is restricted to non-sport-specific acclimation, which limits the transferability to a real-world team sport setting. While a few studies have specifically reported improved temperate weather performance immediately post-HA [104], there is a need for further investigation in order to isolate the effect of HA adaptations in a cool weather performance test [40]. Additionally, the adverse effect of heat stress on aerobic performance is evident even under relatively cool conditions just beyond 10°C [40]. Therefore, the improved cardiovascular and thermoregulatory capability commonly observed post HA in hot conditions, may also have the potential be ergogenic in temperate conditions as well.

2.2.1 Heat adaptation and endurance performance in temperate conditions

A recent review indicated that there is extensive evidence to show that there is likely an ergogenic benefit of the physiological changes resulting from HA in temperate conditions for endurance athletes [20,40,137]. In two different cycle-based HA studies for example, there were observed increases in thermal comfort, submaximal exercise performance, and maximal aerobic capacity (VO_{2Max}) following exercise-based heat training [104,139]. Lorenzo et al. [104] demonstrated this improvement in cardiac efficiency in a study of trained cyclists who had a PV expansion of 6.5% after ten days of HA ($\sim 50\%VO_{2Max}$, 40°C), as well as an 8% improvement in hot weather time trial (TT) performance (38°C, 30%RH), and a 6% improvement in cool weather TT

performance (13°C, 30%RH). Additionally, cyclists improved their power output (PO) at lactate threshold in both hot (5%) and temperate conditions (5%) whereas the control group showed no change in either physiological or performance parameters [104]. The ergogenic effect of HA was also supported in a recent study during three separate cycling TTs (43.4km) over two weeks of outdoor-based HA [140]. Findings revealed an increase in mean PO that increased from the first to the final TT in a hot condition (37°C) [139]. This study also noted that final TT performance in the hot condition was similar to what was achieved in the cool condition (8°C) [139]. In an earlier cycling study, athletes underwent seven days of HA (37°C, ~90-minutes/day, self-selected training) and only experienced a modest increase in 40km TT performance in cool condition (20°C) [117]. However, the authors of this study suggested that a more marked training stimulus at a specific intensity or T_c threshold similar to that utilized by Lorenzo et al. [104] would likely have resulted in a more substantial ergogenic effect [117]. Passive HA in the form of post-exercise sauna bathing has also shown to be performance enhancing in a temperate conditions when male distance runners demonstrated a 32% increase in running time to exhaustion [154]. However, heat exposure combined with exercise likely allows for a greater magnitude of performance improvement over passive HA but it remains unclear as to whether this is also the case for optimizing endurance performance in temperate conditions [40].

2.2.2 Heat adaptation and team sport performance in temperate conditions

International level team sport players have also shown improved performance in temperate conditions following a period of field-based HA [40]. Following two-weeks of HA in AFL players consisting of one 60-minute session each day of AFL-specific skills, there was substantial improvement in team running performance (44%) during an intermittent running performance test level 2 (Yo-Yo IR2) in a temperate condition (23°C) [137]. Similarly, a mean 44% improvement was observed in the Yo-Yo IR2 in a temperate condition (22°C) following two weeks of sport-specific HA (32°C), in addition to a large change in PV (+5.6%) [21]. These findings are consistent with an earlier study highlighting a 7% improvement in running distance in a temperate condition (22°C) during an intermittent running test (YOYIR1) post eleven-days of in-

season field-based HA (34.6°C, training drills and weekly competitive game) [22]. However, the improvement in running performance in both hot and temperate weather has been noted to be highly individual and dependent upon on the magnitude of HA achieved (partial or complete) as well as the type of heat protocol utilized [136,138]. The ergogenic potential of these adaptations might also be a result of the timing of the protocol within the training plan [21]. Periodization of HA likely has an effect on the performance enhancing potential of these adaptations [21]. Significant improvements were noted when HA was completed immediately following the off-season versus in-season [22,138].

The literature has also shown improvements in submaximal HR-response with field-based HA as evident by the observed reductions in HR_{ex} during submaximal exercise in temperate conditions post-HA [20,21]. A recent study highlighted an improvement in cardiovascular fitness and neuromuscular running efficiency within six days of HA [20]. Findings indicated a decrease in HR-response (~3%) to a 4-min submaximal run performed under temperate conditions (22°C) three days after an eight-day training camp in the heat [20]. These findings are also consistent with an earlier study noting a reduction in HR_{ex} during a 4-min submaximal run [ES= -5.5; 90% CL - 7.0, -3.9] pre to post HA [21].

2.2.3 Mechanisms of heat adaptation and temperate weather performance

The literature has suggested that there are a number of physiological mechanisms that underpin the observed improvements in cool weather performance [127]. Specifically, the ergogenic response commonly seen in temperate conditions following HA is a combination of cardiovascular, thermoregulatory, and cellular based components [127]. The enhanced sweat and skin blood flow responses, as well as PV expansion, provide greater cardiovascular stability during exercise in the heat (better ability to sustain cardiac output) contributing to the ergogenic response [104]. The maintenance of cardiac output is underpinned by an increase in vascular filling as a result of PV expansion, which reduces blood flow to the skin and increases SV to improve cardiac efficiency (reduction in HR) [127]. The summative result of these changes with heat adaptation is a lower T_c which allows a greater distribution of blood

volume to exercising muscle [134]. As heat adaptation increases PV, blood pressure is regulated more effectively as there is less competition between blood flow to the periphery for evaporative cooling and blood flow to the muscles during exercise [131]. Blood flow regulation is a critical factor in allowing athletes the ability to sustain a high intensity of exercise in the heat when they are likely to experience excessive fluid loss as a consequence of a greater sweat output [161]. The ergogenic potential of PV expansion (6.5%) was evident in a recent HA protocol noting an improvement in maximal SV, cardiac output, and VO_{2Max} during cool weather performance (18°C) [104]. An improvement in VO_{2Max} as a result of PV expansion could theoretically improve high intensity sport performance in either cool or hot conditions [40]. However, the potential for PV to increase VO_{2Max} also depends on the balance between the increased maximal cardiac output compared to haemoglobin concentration (*ctHb*) and arterial O_2 content which has shown to reduce in some studies following HA [104]. Findings from Lorenzo et al. [104] support the improvement in VO_{2Max} as a moderate PV expansion induced a small hemodilution (3.3%) but largely increased cardiac output (9%) resulting in an overall increase in VO_{2Max} (5%).

Furthermore, studies have observed correlations between PV expansion and cardiac efficiency (reduction in HR_{ex}) [40]. During a week long, field-based HA study, YOYOIR1 performance was observed pre and post HA and was found to be correlated to the changes in submaximal HR_{ex} but not HRR [22]. Therefore, it is possible that the observed reductions in HR_{ex} may be related to maximal cardiorespiratory function and could potentially be used to track the extent of adaptation and decay during and post HA [22]. In a separate study, there was an observed correlation between PV expansion and sport performance post-HA which suggested that an increase in BV permitted the performance enhancement [105].

Aside from the reduction in cardiovascular strain, the glycogen-sparing effect induced by HA has been suggested to play a role in the improved performance in temperate conditions as the lowered rate of glycogenolysis improves both exercise economy and fatigue perception [127]. Specifically, it has been suggested that the overall increase in total body water would likely delay lactate accumulation and enhance removal as a result of an increase in cardiac output and a decrease in metabolic rate [40].

The physiological adaptations from HA are well documented and have been observed alongside improvements in submaximal exercise [20] and soccer relevant performance testing [22]. There is a great deal of evidence to suggest that an effective HA protocol has the potential to improve both endurance and high-intensity team sport performance capabilities in cool conditions [22,40,104]. However, there are large inter-individual differences in the magnitude of these improvements and there have been some studies that have shown no relationship between a cardiovascular physiological response and an increase in sport performance [21]. The relationship between HA mechanisms and their effect on temperate weather performance needs further investigation. Effective protocols have the potential to provide an in-season aerobic boost that may benefit team sport athletes who are often focused primarily on the technical and tactical components of training prior to competition [40].

2.3 Sex-based differences in heat adaptations

Those studies that have observed sex-related discrepancies in physiological response to heat stress during HA have shown conflicting results [102]. Some studies have noted similar physiological responses in terms of T_c and exercise HR as outlined by Sawka et al. [144] and Wyndham et al. [166], while others have noted discrepancies in sudomotor activity [111]. Heat dissipation between men and women may differ due to anatomical variation, as women tend to have a larger surface area-to-mass ratio than men [30,54]. Therefore, those studies showing no sex-based difference in heat stress response may have been limited by confounding variables including discrepancies between men and women in their body-surface to mass ratio and fitness level (VO_{2Max}) [3,102]. On the contrary, the literature has also suggested that there tends to be no sex-based difference in physiological response when adjusting for fat-free-body mass, body surface to mass ratio, or aerobic power [4]. However, as highlighted in a previous cycle-based hyper-thermic training protocol (120 minutes, 32°C), this adjustment is not relevant for analytical heat stress standards, which are predicted in absolute values [111]. Therefore, regardless of anatomical variation, a sex-based sudomotor discrepancy during exercise in the heat may be characterized by females having a lower SR versus males even when accounting for body surface and surface area-to-mass ratio [111].

The discrepancy in sudomotor response is likely underpinned by the threshold for the onset of sweating as well as SR [121]. Once sweating begins in women (often at a higher T_c than men) their SR is likely to be lower than it would be in men [121]. Therefore, a higher T_c in women during exercise in the heat compared with males may potentially be the result of less efficient thermoregulation capability or a delay in the onset of sweating in females compared to men [121]. This discrepancy was evident following ten days of outdoor-based HA ($\sim 36^\circ\text{C}$) in humid heat when the onset of sweating was delayed in women as compared to men both before and after HA [9]. Within this study, fitness level was eliminated as a potential heat tolerance discrepancy as all female and male participants were matched on both $\text{VO}_{2\text{Max}}$ and surface area-to-mass ratio [9].

While a variety of studies have observed the sex-based differences in thermal response to heat stress and adaptation in untrained participants [9,101], there is limited evidence in team sport players [30]. The thermoregulatory inefficiencies observed in women during exercise in the heat may be especially problematic for team sport athletes as less heat dissipation results in a higher overall exercise T_c and a greater utilization of glycogen stores [9,54]. Only Sunderland et al. [158] has examined the heat adaptive response in female team sport players. Findings within this study noted an increase in intermittent sprint performance (33%) following four sessions within a short term HA protocol [158]. However, there were no differences in resting T_{Rectal} , and SR between the heat acclimated group and the control group suggesting that heat dissipation did not improve post acclimation [158]. The lack of a physiological response may have been a result of utilizing a non-consecutive, short-term HA protocol as it was recently suggested that women may need a longer protocol (10-days) to induce both cardiovascular and thermoregulatory adaptation [110]. A recent study also highlighted the discrepancies in protocol length and heat adaptation between men and women when comparing both short-term HA (<8 days) and long-term HA (>10 days) protocols (30-min fixed-running, 9km/h, 2% gradient, 39.8°C , 39.5%RH) [110]. While accounting for body mass, both males and females responded to short-term HA (5-days), however, females required an additional five days (10 days total) to induce similar thermoregulatory and cardiovascular stability compared to men [110]. Additionally, SR

relative to body surface area increased in females ($\sim 428 \text{ g/h/m}^2$) but not in males (-11 g/h/m^2) [110].

Aside from the sex-based discrepancies in HA protocol length, there is also the potential effect of hormones and the menstrual cycle on heat related stress and T_c [110]. It has been suggested that women are more likely to experience a higher T_c in identical heat stress and at similar work capacities to men by $\sim 0.2^\circ\text{C}$ [111]. This was supported in a later study which specified that women tend to have less efficient thermoregulation capabilities than men and are more likely to experience a greater degree of heat stress while exercising at similar hot conditions [102].

Lei et al. [101], recently added to this observation noting that hormones and the stage of menstruation (early-follicular or mid-luteal phase) in women may play a role in the development of a higher T_c during exercise in the heat. Specifically, when women performed cycle-based acclimation trials, during either rest or at a fixed-intensity exercise, T_{Rectal} was $\sim 0.2^\circ\text{C}$ higher in the mid-luteal phase than the early-follicular phase of menstruation [101]. The effect of menstrual phase on heat stress in women was supported in an earlier study when females who performed a heat stress test (incremental test to exhaustion, 32°C , 60%RH) experienced a higher resting and submaximal T_c and a higher rate of increase in T_c within the mid-luteal phase of menstruation during exercise to exhaustion in the heat (32°C , 60%RH) [88]. Women experienced a $\sim 6\%$ decrease in endurance time within heat stress testing during the mid-luteal phase compared to the early-follicular phase [88] which is consistent with an earlier study ($\sim 11\%$ reduction) [9]. Conversely, a recent study utilizing cycle-based, self-paced exercise instead of time to exhaustion, found that autonomic heat loss responses at rest and during fixed-intensity exercise was not affected by menstrual cycle phase [101]. Currently, there is limited evidence available observing the physiological sex-based discrepancies in international-level sport players [64].

2.4 Optimization of heat adaptation

The underlying principle of a HA training protocol is to increase and maintain an elevated T_c and skin temperature (T_{skin}) to induce sweating and increase skin blood flow [136]. The extent of adaptation largely depends on the exercise intensity, duration and

total of exposures, frequency as well as the environmental conditions (e.g. humid or dry heat) [131]. Trained athletes have been shown to develop partial adaptation over four to five days of HA training [68,95] compared with untrained individuals [5] but require up to ten days to achieve full cardiovascular and sudomotor adaptation [22]. Additionally, heat exposure status prior to a period of HA has an impact on the extent of adaptation that may be achieved and likely has an effect on the resulting change in PV [69]. A non-training period of heat exposure prior to HA may induce a transient increase in PV that can adversely impact an athlete's ability to achieve a PV expansion within the planned heat-training period [69]. It has also been suggested that endurance-trained athletes who exhibit high aerobic fitness might already have a large baseline PV from aerobic training and may be limited in their capacity to achieve a notable change in PV as a result [127]. Therefore, it has been recommended that coaches determine the HA response of each player by utilizing a heat-response test to observe the inter-team differences in post-heat Hct concentration and PV [138]. Aside from pre-acclimatization status and baseline testing, the magnitude of thermoregulatory adaptation achieved within HA is closely related to the amount of heat strain to which the athlete is exposed [108]. In a hot humid environment, the critical factors that will influence the magnitude of physiological change induced by a thermal stimulus include the extent of the rise in T_c , as well as sweating response [107,160]. Therefore, optimization of HA in terms of induction and decay depends on several factors relating to environment type, T_c , as well as training protocol length and intensity [136].

2.4.1 Core temperature, protocol, and environment

The consensus holds that the critical factor for a sustained physiological response to HA is the increase in T_c to a threshold sufficient enough to induce adaptation [160]. It has been recommended that a minimum of 25-30 minutes per HA session at a T_c of approximately 38.5°C is required to induce adaptation [161]. However, recent evidence has suggested that a T_c threshold of 38.5°C may be too low for elite athletes who have reached core temperatures greater than 40°C in the heat without any symptoms relating to heat exhaustion [7]. Therefore, a target T_c higher than 38.5°C during HA training [159] may have the potential to induce more sport-specific

adaptation in elite athletes [7]. In lab-based HA protocols for example, the percentage increase in sweating using controlled hyperthermia has been shown to be greater if the subject's T_c is higher (approaching 39.5°C) and if this temperature is maintained throughout the training session [46,120]. A mixed-high-intensity protocol for trained athletes should include at least five sessions with a minimal duration of sixty minutes to improve aerobic-based performance in hot and mild environmental conditions. [30]. Utilizing components of high-intensity or intermittent-sprint exercise within HA (of approximately 15-20 minutes/session) may provide a larger thermal strain than continuous exercise by ensuring that a T_c stimulus is reached and maintained [84]. Studies that have incorporated interval sessions into a HA period have found it easier for athletes to reach a T_c stimulus and in turn, have achieved more thermoregulatory benefits [158]. Daanen and colleagues [46] for example, utilized nine consecutive training sessions consisting of sixty minutes of constant work-rate cycle exercise (80-90rpm, 45% VO_{2Max}) followed by approximately thirty minutes of a progressive load increase (3-Watt increase/minute) [46] and found a reduction in HRex (-25bpm). Similarly, in a two-week outdoor cycle-based HA protocol, athletes completed 2-3 hours of daily training consisting of 30-45 minutes of high-intensity (HI) intervals (>80% maximal HR) followed by a longer period (60-120 minutes) of lower intensity exercise [92]. In this case, high intensity training at the beginning of the sessions was thought to optimize the quality of training and allow more time for acclimatization while also avoiding the negative effect of heat accumulation on mean PO [92]. In team sport athletes, T_c is not as easily maintained and controlled within field-based HA due to variable environmental conditions, but is commonly achieved via integration of sport-specific drills, such as shuttle runs, agility drills, and short sprints at a high-intensity (75% VO_{2Max}) [30]. HA in sport players may be achieved in a minimum of four non-consecutive training sessions over 10-14 days of approximately ninety to one-hundred minutes incorporating high-intensity drills to elevate T_c to threshold (>38.5°C) [134]. Field-based elevation of T_c was utilized in two separate HA studies and consisted of either 30-45 minutes of soccer-specific testing [158], or a combination of field-based skills and tactical training over eleven days to target T_c [22].

Another important consideration in HA protocol development aside from the utilization of a T_c stimulus is, the length of a protocol and the periodization of HA in terms of optimal induction and decay [127]. There is varying evidence for the magnitude of adaptation within short-term HA (STHA) (≤ 7 days), medium-term HA (MTHA) (8-14 days), and long-term (LTHA) protocols (>14 days) [30]. It was previously noted that both STHA and MTHA can elicit changes in physiological parameters including PV expansion, increases in SR, and reductions in exercising HR and T_c as well as sweat concentration $[Na^+]$ [77]. MTHA has shown to offer the most benefit in terms of eliciting PV expansion, inducing sudomotor change, and improving both temperate and hot weather performance [30]. However, in the first few days of heat adaptation there is a transitory period where the thermal stimulus (rise in T_c) required to induce physiological changes will reduce the tolerable training load of an athlete and may have a negative impact on training quality [161]. While LTHA protocols have shown the greatest magnitude of adaptation versus STHA, many athletes do not have the time to engage in 10-day protocols due to the risk of a lowered training intensity from extreme heat [77]. For elite athletes, a shorter adaptation period of five days has been suggested to be more advantageous as this timeframe is less disruptive to routine training [30,110]. It was also recently noted that the magnitude of HA adaptation gained was the same in protocols where athletes engaged in heat training consecutively for ten days versus every third day for twenty-seven days [127]. However, a non-consecutive heat training protocol has been noted to be less time efficient, as adaptation took three times longer to induce which is not practical for elite athletes commonly on strict training schedules [127].

Additionally, each form of adaptation (e.g. cardiovascular or thermoregulatory) has a different time constant within a HA period [77]. Sudomotor changes in SR response have shown to require up to two weeks before adaptation is evident [77]. This was evident following ten days of HA (100 minutes in either hot-dry or hot humid conditions) when only partial HA was revealed with a limited sudomotor response [58]. Therefore, while LTHA (14 days) has shown more complete adaptation than STHA (5-6 sessions), the optimal duration, protocol length and modality highly depends on the athletes' event type and their periodization of competition [30]. Optimal protocol length

is largely influenced by the number of sessions needed to attain appropriate adaptations (cardiovascular or sudomotor) as well as competition preparatory time restrictions [77].

A previous review by Garrett and colleagues [69] also stressed the importance of adaptation decay in the periodization of HA, specifically indicating that those adaptations arising first are also commonly the first to decay once out of the heat. This was previously observed during eight days of MTHA allowed adaptation to last sixteen days before returning to baseline values, however, the decay process began immediately and PV expansion returned to baseline after seven days out of the heat [69]. Therefore, the cardiovascular adaptations gained with short-term HA including a reduced exercise HR and PV expansion are not sustainable once out of the heat while the sudomotor adaptations that take longer to develop will have a slower rate of decay [125]. The review also summarized that for every two days spent without exercising in the heat following HA, one day of acclimatization is lost and further suggested that full adaptation will decay to baseline values after three weeks post heat exposure [69]. However, it has been suggested that re-acclimation following a period of heat training may be regained in a temperate condition through maintenance of a high-intensity training stimulus [69].

A re-acclimation timeframe and rate of HA loss (signified by a 3-day plateau in T_{Rectal}) was recently observed via HR and T_{Rectal} adaptation decay over a six week period and re-acclimation time after two and four weeks of de-acclimation [6]. Findings concluded that HR and T_{rectal} increased with increasing time away from the heat and a re-acclimation period of four days for two-weeks out of the heat, five days for four weeks out of the heat, and six days for six weeks or more out of the heat was recommended [6]. However, this study did not control for participant baseline HA status and aerobic fitness as these factors have previously been shown to affect the magnitude of adaptation and extent of decay [6,69].

Aside from the number of days spent out of heat following HA, the literature has also suggested that environmental conditions (dry-heat versus humid-heat) might play a role in the rate of HA decay [127]. Early findings noted that the effect of HA was retained to a greater extent following exposure to dry versus humid heat [125]. A recent review suggested that dry HA allows for a greater sweating capacity versus humid heat

by allowing more sweat evaporation and a greater thermal adaptation as a result [127]. Specifically, for sudomotor adaptation to occur, sweat must evaporate at a rate sufficient enough to achieve thermal balance and this requires a higher vapour pressure at the skin in humid conditions as compared to what would be necessary in a dry environment [127]. Therefore, dry heat has commonly been used during HA [58,92,95] and has been suggested to be the preferred method for inducing sudomotor and circulatory adaptation due to the limitations of a high ambient water vapour pressure and evaporative cooling commonly seen in a humid environment [127]. Furthermore, in a study of lab-based HA, SV responses observed during exercise, both before and after HA over 9-12 days (45–50%VO₂max) in dry (40 °C, 10% RH) or 8-13 days in humid (35 °C, 87%RH) conditions [120]. It was found that dry HA increased SV (~21 mL/beat) and cardiac output (~1.8 L/min)[119], whereas humid HA did not influence either response [120].

The utilization of using either a high T_{ambient} or high humidity (%RH) for physiological benefit has also been assessed in the literature [136]. In a soccer-specific HA study, the difference in heat adaptation was observed in groups who trained in; a hot condition with low humidity (30°C, 24%RH) or a mild condition with moderate humidity (18°C, 41%RH) [158]. The only group to experience a lower exercise T_{c} following acclimation were those who exercised in low humidity, which provides support for the importance of utilizing high T_{ambient} to drive thermoregulatory responses versus high humidity [158]. Conversely, both Patterson et al. [126] and Griefahn et al. [76] noted that HA in humid heat would minimize circulatory strain but allow for greater circulatory adaption to support skin blood flow than dry heat. This was also supported in a review by Racinas and colleagues [127] who suggested that humid HA induces a higher T_{skin} than dry-HA and increases the maximum rate of evaporative heat loss from the skin as a result. Additionally, humid heat might induce adaptation at a faster rate with less physiological strain than dry heat which typically causes a higher T_{c} , HR, and SR [76]. Patterson et al. [126] further suggested that while humid HA does not elicit preferential sweat redistribution towards the limbs for whole-body cooling, it does allow more efficient use of the skin as an evaporating surface. This was also evident in a study by Sawka et al. [144] who found a similar reduction of T_{Rectal} , T_{skin} , and HR post-HA in subjects matched for aerobic power and who exercised in both hot-dry (49°C,

20%RH) and hot-humid (35°C, 79%RH) conditions. It was also suggested that utilizing a combination of conditions, specifically, dry heat in the initial stage of HA followed by humid heat towards the end of HA may allow for a greater stress on the cardiovascular system and sustained adaptation versus dry heat alone [136].

A recent study by Hayes and colleagues [79] was the first to observe the effect of dry heat (~40.2°C, 33%RH) versus humid heat (~34°C, 78%RH) during a cycle-based, intermittent-sprint protocol in team sport players, and found only slightly greater physiological strain in a humid environment. However, most athletes in the study did not attain a T_{rectal} of 38.5°C in either dry or humid trials and likely explains the lack of an observed difference in peak PO between the conditions [79]. It is clear that the evidence directly comparing the induction and decay of matched groups after both humid and dry HA needs further evaluation, particularly for team sport players competing in variable environmental conditions [131]. While optimal HA depends on a combination of training and T_c stimulus as well as environmental conditions (T_{ambient} , RH), some recent evidence has suggested that a secondary stimulus utilized within heat training may allow for an even greater magnitude of adaptation than heat stress alone [68].

2.4.2 Dehydrated and euhydrated heat protocols

Elite athletes preparing for competition in the heat are often advised to maintain hydration to prevent excessive fluid loss and dehydration from heat stress [165]. The adverse effects of dehydration while exercising in a hot environment are well established, as high a SR has the potential to cause large reductions in total body water, increase cardiovascular strain, and impair aerobic performance [27]. However, some recent studies have suggested there is a benefit to manipulating hydration status within HA [68,69]. This is based on the notion that dehydration stresses fluid regulatory hormones and induces a state of fluid regulatory strain [43]. Therefore, a secondary dehydration stimulus utilized within HA was thought to generate a larger response of fluid regulatory hormones through osmolality and volume effects of hypo-hydration [69]. As a result, this would induce a greater cardiovascular response and PV expansion than just heat stress (clamping of T_c) alone [68,69].

The effect of fluid regulatory strain and dehydration was observed in a study by

Garrett et al. [68] during five days of HA when athletes were restricted fluid intake prior to training (only 100ml of water before exercise). Findings highlighted that PV expansion post-HA was slightly higher in the dehydrated group than the hydrated group (8+/-3%, 4+/-3%) [68]. However, of the two methods used to measure PV following this HA protocol; the Dill and Costill equation [49] and the carbon monoxide (CO) rebreathe protocol [152], only the Dill and Costill method noted a difference in PV response between dehydrated and hydrated groups [68]. There was no statistical difference between groups when the CO rebreathe method was used to measure PV, which is known to be a more reliable and consistent method of PV measure over the Dill and Costill [49] as it is based on measures of Hct and Hbmass [74]. The study also found physiological adaptations in both dehydrated and hydrated groups along with an increase in work capacity to a similar extent post-HA (dehydrated; 19%, hydrated groups; 14%) [68]. Furthermore, both groups experienced expanded protein content in the vascular space that contributed to hypervolemia, irrespective of whether participants were hydrated or dehydrated [68]. During highly intense exercise in the heat, it is not uncommon for athletes to moderately dehydrate due to *ad libitum* fluid intake [69] and some recent studies have noted dehydration up to 4-6% body mass loss during exercise in the heat [43]. Therefore, it has also been suggested that moderate permissive dehydration during HA may facilitate adaptation while also avoiding the risks associated with a lowered training intensity from excessive fluid loss [35,161]

Regardless, there still remain a number of studies that have highlighted the severe reduction in exercise performance that results from dehydration [72,123]. While there is a possibility that dehydration could increase extracellular fluid and PV via electrolyte and water retention, it may also limit PV by impairing plasma protein production if electrolytes and carbohydrates are not available post-exercise [68]. A recent HA study noted a reduction in BV of 6% and a lowered VO_{2Max} by 6% when T_{skin} was at 31°C and this was accelerated to a reduction of 16% when T_{skin} reached 36°C with a 4% loss in BM [43]. There are a number of HA studies that utilize full fluid replacement during exercise in the heat as part of the protocol and have shown substantial physiological adaptation [22,104,138]. Fluid replacement ensures that athletes can maintain a strong training intensity within each training session [32]. A

positive effect of doubling water intake over a seven-day HA protocol was also observed when athletes who increased their intake by 50% were able to complete a 15km TT 10% faster than the control group who kept their normal water-intake regime [97]. Additionally, hydration maintenance throughout HA has shown to be more practical for elite athletes as it reduces the relative exercise intensity and promotes better fluid retention for enhanced perceptual effort [165].

Overall, the optimal frequency and extent of dehydration required to induce benefits remains uncertain [43] and T_c and whole body heat stress are the primary drivers for PV expansion [165]. There is not enough evidence to suggest that dehydration during exercise in the heat is beneficial unless it provides adaptations that are ergogenic or aid fluid retention [43]. Therefore, the use of permissive dehydration within HA warrants further investigation as the risks associated with dehydration are severe including reductions in blood pressure, cardiac output, and skeletal muscle blood flow [35,36]. Additionally, there is minimal evidence of valid statistical differences for a greater percent change in PV utilizing a dehydrated over a full-hydrated HA protocol [69,136].

Chapter 3 Methods

3.1 Experimental overview

A field-based HA study occurred over twenty-five days in association with the Canadian National Women's Soccer team training camp, one month prior to the start of the 2015 FIFA Women's World Cup (WWC - Canada-June 6-July 4). The initial fourteen days consisted of two phases of heat acclimatization (mild and high heat) and the final eleven days made up the post-training camp period (Figure 1). Thermoregulatory, cardiovascular, soccer-specific performance, and field metrics were monitored during rest and exercise throughout the training camp. Specifically, external and internal load measures were tracked and used in combination with a 5'-1'-submaximal running test as well as a small-sided soccer game to observe the overall effect of extreme heat on aerobic performance and soccer physical performance pre and post-HA, two days post-HA (2dayP) and up to eleven days post-HA (11dayP).

3.2 Ethical approval

The experimental protocol was approved by the University of British Columbia Clinical Research Ethics Board (UBC CREB number H15-00031). All players who participated in the study provided written informed consent. Parental approval was obtained for one player who was under the age of nineteen.

3.3 Participants

From an initial sample of nineteen competitive female soccer players who participated in this study, three players missed at least one training session due to injury and/or did not perform the post-HA performance test and were excluded from the final analysis (n=16 analyzed). All participants were assumed to be non-heat acclimatized as initial testing took place at the end of April in Vancouver, British Columbia, Canada, where the mean T_{ambient} in April is $\sim 13^{\circ}\text{C}$ and 72.0%RH. Table 1 outlines baseline physical characteristics of the Canadian Women's National Soccer Team (WNT).

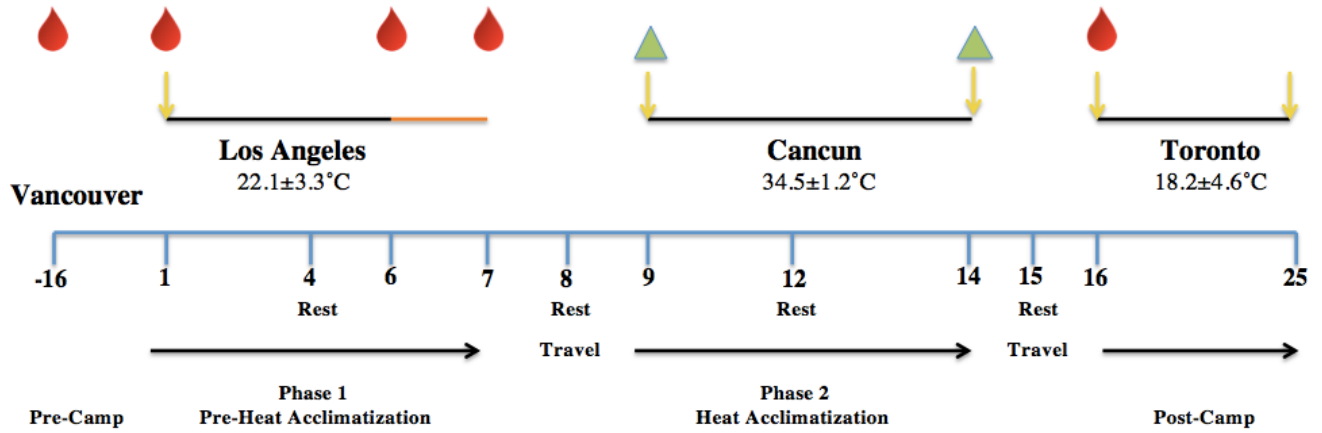
Table 1. Baseline physical characteristics (Mean \pm SD) of WNT

Age (years)	27.0 \pm 5.0
Body Mass (kg)	65.7 \pm 5.3
Height (cm)	170 \pm 6.0
Sum of 8 (mm)	85.2 \pm 12.4
VO _{2Max} (ml.kg ⁻¹ .min ⁻¹)	53.1 \pm 3.1

Key: WNT: Women’s National Team; Sum of 8: The total sum of eight sites of skinfolds; VO2Max: Maximal oxygen consumption was determined via calculation specific the 30-15 Intermittent Running Test (Adapted from Buchheit et al. [18], $r=0.96$)

3.4 Heat exposure, training, and performance metrics

Pre-camp testing consisted of one day of baseline PV assessment in Vancouver ($\sim 13^{\circ}\text{C}$; 72%RH) (Day -16) (Figure 1). Phase 1 pre-acclimatization (pre-HA) (7 d in Los Angeles, USA, $22.1 \pm 3.3^{\circ}\text{C}$; $44.8 \pm 9.4\%$ RH), consisted of four training sessions ~ 100 min/day of soccer-specific training drills, one rest day, and two international match days ~ 90 min/day. Phase 2 heat acclimatization (HA) (6d in Cancun, Mexico, $34.5 \pm 1.2^{\circ}\text{C}$; $53.2 \pm 4.3\%$ RH), consisted of five training sessions ~ 97 min/day, one rest day, as well as two 4x2min, 4v4 small-sided soccer games (4v4SSG) on the first and last day of the 6-day period (Figure 1). Following Phase 2, players continued training in a post-HA period consisting of eleven days in Toronto ($18.2 \pm 4.6^{\circ}\text{C}$; $51.3 \pm 20.9\%$ RH). During this post-HA period, players engaged in a final PV assessment (Day 16) as well as two additional 5’-1’ submaximal running tests occurring 2dayP HA (Day 16) and 11dayP HA (Day 25) (Figure 1). Players were exposed daily to the heat for approximately five minutes when walking to/from the training facilities, 120 minutes during all outdoor recreation time, and 90 minutes during all skill sessions. Athletes were housed in air-conditioned rooms ($\sim 21^{\circ}\text{C}$) and on rest days, players were allotted approximately two hours of time outdoors.



Legend: Training Period: — Match Days: — 5'-1' Submax: ↓ Plasma Volume: ● 4V4 Small-Sided Game: ▲

Figure 1. Overview of the two-phase WNT heat acclimatization training camp. Pre-camp: Vancouver (Day -16); Pre-Heat Acclimatization: Los Angeles, U.S.A (Phase 1, Day 1-7); Heat Acclimatization: Cancun, Mexico (Phase 2, Day 9-14); and Post-Camp: Toronto, Canada (Day 16-25). No training sessions occurred on rest days (Day 4 and 12) as well as travel days (Day 8 and 15).

3.5 Plasma volume measurement

PV was measured on five different occasions during the study period (Figure 1). The CO Rebreathe protocol as outlined by Schmidt and Prommer [152] was used as a baseline measure of both absolute and relative PV (Day -16) at the Canadian Sport Institute Pacific Laboratory (Vancouver, British Columbia, Canada) (Appendix A). The Dill and Costill [49] method was used to determine percentage change in PV from baseline during all follow up measurements (Day 1, 6 or 7, 16). The third PV measurement was separated into two days (either Day 6 or 7) and athletes were measured on the non-playing match day (Figure 1). Prior to all visits, athletes were asked to refrain from alcoholic and caffeinated drinks 12 hours before testing and to be well hydrated on testing day. During baseline testing, athletes were asked not to engage in strenuous exercise the day prior to testing to avoid fluctuation in PV, however, this could not be replicated in the ensuing testing windows due to the nature of the pre-competition training camp. Athletes were assessed in groups of four with a 30-minute break period between groups for blood analysis. Athletes rested in a seated position for 15 minutes before blood measurement in order to stabilize any shift in PV and to receive verbal instruction specific to each measurement. Body mass, height, and hydration status using urine specific gravity (USG) were determined and recorded immediately upon arrival to each PV assessment.

The first PV measurement (absolute baseline, ml) was performed using the CO-rebreathing technique (Appendix A). The sampling procedure consisted of three stages; i) baseline venous blood sample ii) two minutes of CO-rebreathing and iii) post CO-rebreathing venous blood sample to obtain the total concentration of carboxyhemoglobin (*ctHbCO*) [152]. This method is based on the principle that CO has a strong affinity with haemoglobin (Hb), yielding carboxyhemoglobin (HbCO), which allows hemoglobin mass (Hbmass) to be determined from the extent of increase in *ctHbCO* due to rebreathing a known mass of CO [152]. Body mass was used to calculate individual dosage of 99.5% pure CO (0.8ml/kg body mass for females). Approximately 1.0ml of venous blood was drawn from the antecubital fossa using a sterile syringe and needle technique performed by a certified Canadian Sport Institute Pacific lab technician.

Immediately following each blood draw, two capillary tubes of blood (200 μ L of the venous sample) were analyzed using a hemostat centrifuge for one minute with the mean of the two values recorded as the Hct value. The remainder of the blood sample was then analyzed immediately for *ctHb* in an ABL80 FLEX CO-OX Hematological analyzer. The blood sample was analyzed approximately five times for *ctHb* or until there was <5% difference between values. The CV and SD for the hematological analyzer was previously reported to be 1.0%, 0.07 for *ctHb* (g/dL) and 0.4%, 0.09 for HbCO (%) (SenDx Medical, Inc. FDA Decision Summary). After the baseline blood draw, athletes engaged in a two-minute period of rebreathing of a known dose of CO, followed by five minutes of rest in a seated position, and a post-rebreathing blood draw using the same blood analysis and measurement procedure previously noted (Appendix A). Hb mass was used to calculate BV, red blood cell volume (RBCV), and PV for each athlete using mean values obtained for Hb (g/dL) and Hct (%). The values were utilized in the equations of Schmidt and Prommer [152] in the software provided by SPICO Blood Tec obtained for each player. Average Hb mass, Hct, and *ctHb* values were used to determine BV in the software provided SPICO Blood Tec, which utilizes the equations of Schmidt and Prommer [142] (Equation 1).

$$BV \text{ (mL)} = (\text{Hbmass}) \text{ (g)} / (\text{ctHb}) \text{ (g/dL)} \times 100$$

$$RBCV \text{ (mL)} = BV \text{ (mL)} \times (\text{Hct}/100)$$

$$PV \text{ (mL)} = BV - RBCV$$

Equation 1. Schmidt and Prommer [152] equation used during the pre-camp Vancouver PV assessment (Day -16) to determine absolute baseline PV (ml) and relative baseline PV (ml/kg) (See Appendix A). **Key:** BV: blood volume, Hbmass: haemoglobin mass; *ctHb*: haemoglobin concentration; Hct: Hematocrit; RBCV: red blood cell volume; PV: Plasma Volume.

The Dill and Costill equation [49] was used to indirectly assess PV from pre to post Phase 1 (Day 1, 6 or 7) and post Phase 2 training (Day 16) (Figure 1; Equation 3.2). The relative change from baseline (assumed to be 100%) in BV, RBCV, and PV were estimated from a single venous blood sample from the antecubital vein (~1.0ml) utilizing the same needle and syringe technique as the CO-rebreathe method (Appendix

A). Hct was measured in quadruplicate by centrifugation and ctHb was measured in the same ABL80 Co-Ox auto-analyzer that was utilized in the baseline measurement. The change in the concentration of these values ctHb (g/100ml), Hct (%), and measured baseline values of BV and RCV were used to calculate the %PV change as described by Dill and Costill [49] (Appendix A). Measurements were performed in the morning and athletes were encouraged to hydrate appropriately the night before (~250-500ml water).

$$PV (\%) = 100 \times (ctHb \text{ pre} / ctHb \text{ post}) \times (100 - Hct \text{ post}) / (100 - Hct \text{ pre}) - 1$$

Hct is in % and ctHb is in g/100ml

Equation 3.2 The Dill and Costill [49] equation used in Phase 1 and Phase 2 to measure the change in plasma volume (PV) from pre-camp absolute baseline (ml). Pre and post values of total haemoglobin concentration (ctHb) and hematocrit (Hct) were used in the calculation (See Appendix A).

Hydration status at the time of PV measurement has a large impact on the measured PV value, as dehydration or hyper-hydration can modify the vascular fluid compartment [61,145,149]. Therefore, all players provided a urine sample every morning upon waking, which was used to determine hydration status via USG-Atago PAL10-S refractometer. Hydration status was based on the National Athletic Training Association (NATA) guidelines as: <1.010, well hydrated; 1.010-1.20, minimum dehydration; 1.021-1.030, significant dehydration; >1.030, serious dehydration [28]. To account for limitations associated with USG and variability in body composition [36], the individual USG mean and SD values were calculated on average of 26 samples of each player's pre-camp and were used as individual standardized USG values. Observing standardized values in relation to measured values provided validation for the variation in athlete body composition and muscle mass throughout the training camp [44,147]. Z-score represented the number of standard deviations (+1; above, or -1; below) that the measured USG value was on PV test day relative to the individual's normative USG distribution (Appendix B) [142].

3.6 Environmental, physiological, and training load monitoring during heat acclimatization

3.6.1 Environmental factors

Temperature (°C) and humidity (RH%; Davis, Perception II weather meter) and wind (kph; Kestrel 1000 Pocket Wind Meter, USA) were recorded at the start of each training session and at 15-minute intervals throughout the training period with the average of these intervals recorded. All training and games in Phase 1 were performed on an artificial turf (70x100-m) and on a natural grass pitch in Phase 2 (i.e. 70x100-m). Training occurred in the afternoon (14 00 h) on Days 1, 2, 3, 5, 6, and 7 (Phase 1) and the morning (10 30 h) on Day 9, 14 and late afternoon (1600h) on Days 10, 12, 13 (Phase 2). Friendly international matches were played under FIFA rules on Day 6 and 7 (Figure 1). Body mass was measured at the start and end of each training session using a calibrated electronic scale (Seca, Germany). Players were allowed to drink *ad libitum* during each training session.

3.6.2 Core temperature

Core body temperature (T_c) was monitored during each session of the training camp using a VitalSense Telemetric Monitoring System (Mini Mitter Philips Respironics) and thermal sensor (Jonah™ Ingestible Core Temperature Capsule). The sensor was activated using the VitalSense telemetric monitor and ingested ~5 h prior to each training session (See Appendix B). All incoming sensor data were displayed in real-time on the telemetric monitor and recorded for each athlete periodically throughout the training session during each water-break (~4-6 times, approx. every 15-minutes). Verbal T_c feedback was provided to the players and coaches in Phase 1 but there was no specific target T_c . In Phase 2, T_c feedback was provided to players and coaches with the goal to maintain a targeted T_c threshold (i.e. 38.5°C) during training session, however, it was not a requirement. Area under the T_c curve or total heat load (AUC) was calculated as the sum of the average time between two consecutive T_c measurements (T_{c1} , T_{c2}) divided by T_{c1} for all given T_c measurements in a single training session. This value (total AUC) was then corrected for initial baseline (T_{c1} * final session time) so that only

the net change in T_c over the session was accounted for and is further referred as relative AUC. As a measure of T_c response, average ΔT_c was determined for both Phase 1 and 2 acclimatization phases for each athlete and was calculated within each session as $T_{cFinal} - T_{cBaseline}$.

3.6.3 Training load

HR response (R-R series data) was extracted during each training session using a Polar Team 2 System (1.4.1, Polar Electro Oy, Kempele, Finland). Three months prior to training camp, each player performed a maximal aerobic test, the 30-15 Intermittent Fitness Test, to determine maximal HR (HRmax) [18]. RPE (Borg Scale 1-10) was recorded immediately post session. Session RPE was calculated as a measure of session duration (minutes) multiplied by the RPE value (1-10) [87]. HR data and session RPE were used as a measure of internal training load as they have been shown to be highly correlated in the literature [87]. The hardest training days of Phase 1 and 2 training were determined from the highest session RPE over three days. Additionally, ratings of thermal perception; thermal comfort (1-5) and thermal sensation (0-9), were collected [167]. These values provided insight into the psychological impact of thermal stress.

A GPS was used to observe daily external training load during each session [162]. The GPS was activated 15 minutes before the start of each training session and each player wore a special harness that enabled this device to be fitted to the upper part of her back (Appendix B). All athletes wore the same GPS device in each training session throughout the study period to reduce inter-unit variability [155]. GPS was sampled at 10Hz incorporating a 100-Hz triaxial accelerometer. 10-Hz GPS units have been shown to have the best inter-unit reliability for distance and velocities measures across linear, change of direction, and team sport circuits [155]. Catapult Sprint 5.1 software was used to analyze and track the data collected from each GPS device [86]. Player movement was categorized as total distance (TD) and distance within the following speed bands when efforts lasted more than 0.5 s (i.e. dwell time): high-speed (HSR): 16.5-19.9 km.h⁻¹ and sprinting (TSD): >20 km.h⁻¹ [114]. The bands were chosen in relation to physiological capacity thresholds, with 16.5 km.h⁻¹ representing an average of maximal aerobic speed and 75% of maximal sprinting speed [114]. Meters/minute

(m/min) and frequency of high-intensity efforts (HIE), that encompassed both high-speed and sprint running, were also measured. High Inertial Movement Analysis (High IMA) was categorized as accelerations, decelerations, or changes of direction (COD) that exceeded the threshold of $2.5 \text{ m}\cdot\text{s}^{-2}$ [114]. All variables of interest were reported in absolute terms and also relative to minutes played, to minimize the variability of reporting absolute values with varying training session durations [114]. GPS MinimaxX S4 units have been shown to have a CV (%) of 3.1 for instantaneous velocity, <4 for intra-unit total distance, 1.3 for inter-unit total distance and a typical error of measurement (TEM) of 1.3-11.5% for inter-unit total distance [155].

3.7. Cardiovascular and functional performance test

3.7.1 5'1' Submaximal running test

A 5-minute running/1-minute recovery (5'-1') submaximal test was performed as a part of the team's warm-up on five different testing occasions throughout Phase 1, Phase 2, and post-camp in order to monitor the team's change in aerobic fitness pre to post HA (Figure 1). The test had been incorporated into the WNT training schedule for over a year by the time of HA. All players were tested simultaneously for convenience with the intensity of the running bout fixed at 12km/h over a 40-m shuttle (adapted from Buchheit et al. [22]). At the end of the running period, players stopped their effort on the whistle and immediately sat on the grass for one minute, avoiding any movement or talking. RPE was collected for each athlete immediately following this submaximal test as a subjective measure of fatigue (Borg CR-10 scale). HR response was measured during each submaximal test in the camp using the same Polar Team 2 software used in each training session. Mean HR_{ex} during the last 30 seconds of the 5-minute running period was recorded [19]. Change in HR_{ex} was calculated as the change in the raw units (bpm) between baseline assessment (Day 1) and each successive assessment throughout the training camp period thereafter (Day 9, 14, 16, 25) (Figure 1). HRR was calculated by taking the absolute difference between the HR_{ex} and the HR recorded in the final five seconds of the one-minute recovery period as a percent of HR_{ex}. The change in HRR (%) was used to track the improvement in recovery ability while the change in HR_{ex} (bpm) was used as a marker of within-athlete relative exercise intensity as it is

related to O₂ uptake during continuous exercise (Appendix C). The reliability of the 5'-1' submaximal running test was assessed on thirteen members of the WNT team prior to the HA period on the same participants across five occasions. Typical error (TEM) was 2.5bpm for HRex (90% CL: [2.03, 3.29]), 8% for HRR (90% CL: [7%, 10%]), and 0.44AU for RPE (90% CL: [0.37, 0.56]).

3.7.2 *Four-a-side soccer game*

A soccer-specific performance test in the form of a small-sided soccer game (4v4SSG) was performed immediately following warm-up (including the submaximal test) on the first (Day 9) and last training day (Day 14) of Phase 2 (Figure 1). Players were separated into groups of four (4-aside) and played a total of four-two-minute soccer games, with an equivalent two-minute rest period between each game (4x2 minute-4v4 SSG). The 4v4SSG was used to assess functional internal response (HR metrics) and external physical soccer performance (i.e. GPS metrics) to quantify the impact of heat stress using the same technology and analysis methods previously described. GPS data were only collected when the players were playing in the SSG's to offer a true representation of the game intensity. Each of the four games were part of a competition over the duration of Phase 2 training in order to create a highly competitive environment and to ensure players were aiming for a high level of performance. The games were performed on the same grass pitch (40-m wide by 35-m length) on both testing days and a goalkeeper was used in both games. Athletes were familiar with the 4v4SSG as it had been regularly incorporated into the team's daily training environment prior to the training camp. Similar to the submaximal test, both HRex and HRR were determined in the 4v4SSG pre and post Phase 2 HA to observe the effect of heat stress on soccer-specific performance. HRex in the SSG was calculated as the average HR during each of the four SSG (excluding the 2-minute rest period) and was expressed in raw units (bpm) for both pre and post 4v4SSGs. HRR in the SSG was calculated by taking the absolute difference between; (1) Average HRex during each SSG and (2) Average HR 30-seconds prior to the next SSG during the two-minute recovery period. This value was then divided by the average HRex from each SSG and multiplied by 100 to be expressed as a percentage. External load indicators were normalized to the 2-

minute SSG duration and averaged from game one to four to obtain an average game intensity as well as to functionally characterize the effect of pre to post-HA on physical output in a live soccer game setting. These metrics included high IMA/min (constitutes all explosive accelerations, decelerations, and changes in direction) as well as M/min.

3.8 Statistical analysis

All data except HR data were log-transformed for analysis to reduce bias arising from non-uniformity error and then analyzed using magnitude based inference statistics [13]. Uncertainty in the estimates of effects on laboratory and performance metrics was expressed as 90% confidence limits. Threshold values for assessing magnitudes of standardized effects (changes as a fraction or multiple of baseline SD) were 0.20, 0.60, 1.20, and 2.00 for small, moderate, large, and very large, respectively [80-82]. These probabilities are not presented quantitatively but were analyzed using a post-crossover spreadsheet that was used to make a qualitative probabilistic clinical inference about the effect in preference to a statistical inference based on a null hypothesis test [82]. The spreadsheet adapted from Hopkins [77] estimated the chance that the true value of the effect was greater than the smallest worthwhile value [80]. The effect was deemed unclear when the chance of benefit (a standardized improvement in performance of >0.20) was sufficiently high to warrant use of the intervention, but the risk of impairment was unacceptable [82]. Such unclear effects were identified as those with an odds ratio of benefit to impairment of <66, a ratio that corresponds to an effect that is borderline possibly beneficial (25.0% chance of benefit) and borderline most unlikely detrimental (0.5% risk of harm) [113]. The effect was otherwise clear and reported as the magnitude of the observed value, with the qualitative probability that the true value was at least of this magnitude

Pearson's Product-moment correlation (r) was used to assess the relationship between the change in internal load HR-rate derived indices, PV, T_c metrics and GPS-accelerometer external load metrics. The following criteria were adopted to interpret the magnitude of the correlation (r); ≤ 0.1 , trivial; >0.1-0.3, small; >0.3-0.5, moderate; >0.5-0.7, large; >0.7-0.9, very large; and >0.9-1.0, nearly perfect [80]. Pearson's correlation results were obtained using the correlation spreadsheet developed by Hopkins [82].

3.9 Sample size calculation

PV was our primary outcome of HA, with the goal to induce a PV expansion of 5 to 10%, which would be comparable to relevant studies [30]. In a previous laboratory study (n=18) with fifteen of the same athletes utilized in the current study, a $9.5\pm 6.0\%$ increase in PV (5.8 to 13.4, 90% CL) was found with a corresponding $1.5\pm 1.2\%$ increase in Soccer Canada's standardized 30-15 Intermittent Fitness Testing [133]. All athletes demonstrated a positive increase in PV within this intervention and the smallest increase was 1.05%. Based on this previous work, our sample size calculation indicated that we required 16 subjects with an error rate of 5% with 95% power to show a significant difference in PV.

Chapter 4 Results

4.1 Description of environmental, physiological, and training load factors during heat acclimatization

Data in text and figures are presented as means (\pm SD) with (90% confidence limits (CL) respectively. Figure 2 outlines the mean daily variation in heat load, meters/min, high IMA, ΔT_c , Session RPE, Thermal Comfort, and Thermal Sensation. Dry-bulb temperature throughout Phase 1 was mild: $22.5 \pm 2.7^\circ\text{C}$. Dry-bulb temperature throughout Phase 2 was hot: $34.5 \pm 1.1^\circ\text{C}$. Dry-bulb temperature post-camp in Toronto was temperate: $18.2 \pm 4.6^\circ\text{C}$. RH in Los Angeles, Cancun, and Toronto were 45.1 ± 9.8 , 53.3 ± 4.2 , and $51.3 \pm 20.9\%$ RH, respectively. The hardest training days solely based on session RPE were in Phase 2 on Day 8, 9, and 10 (911 ± 235), while the lightest training days were on 2, 4, and 11 (359 ± 14) (Figure 2). Mean playing T_c during the first international match (Day 6, Phase 1) was $38.6 \pm 0.8^\circ\text{C}$ and 39.0 ± 1.0 during the second international match (Day 7, Phase 1). In the first international match (25.1°C , 37.2% RH), four athletes had a $T_c \geq 39.5^\circ\text{C}$ and in the second international match (16.9°C , 71.8% RH), one athlete had a $T_c \geq 39.5^\circ\text{C}$ (40.1°C).

Table 2. Descriptive training data (Mean±SD) for Phase 1: Los Angeles and Phase 2: Cancun. Data is specific to T_c , external and internal load monitoring, and perceived exertion.

Training Phase	1	2
Location	Los Angeles	Cancun
Parameter (units)		
Mean Duration/Type (min)	96.0 ± 11.9	97.4 ± 13.6
Dry Bulb Temp (°C)	22.5 ± 2.7	34.5 ± 1.1
Wind Speed (kph)	5.2 ± 1.9	5.0 ± 1.3
Mean T_c (°C)	38.3 ± 0.6	38.2 ± 0.6
Mean ΔT_c (°C)	1.3 ± 0.6	1.4 ± 0.3
Mean AUC Heat Load (AU)	82.6 ± 38.4	106.5 ± 32.8
Mean Time in HR Zone 4 (min)	18.6 ± 8.2	23.6 ± 5.6
Mean Time in HR Zone 5 (min)	12.3 ± 12.9	23.9 ± 5.6
Mean Time $\Delta T_c > 1^\circ\text{C}$ (min)	54.2 ± 13.0	49.6 ± 29.5
Mean End-Exercise T_c ($T_{c\text{-end}}$; °C)	38.5 ± 0.5	38.6 ± 0.4
Mean Total Distance (m)	5793 ± 564	5081.9 ± 313
Mean High Intensity Efforts (count)	32 ± 8.0	28 ± 6.0
Mean High IMA ($\text{m}\cdot\text{s}^{-2}$)	103 ± 44.7	80.2 ± 25.1
Mean Meters/min (M/min)	76.3 ± 24.1	73.1 ± 6.72
Session RPE (AU)	458 ± 163	721 ± 295.
Thermal Comfort	2.0 ± 1.0	3.0 ± 1.0
Thermal Sensation	6.0 ± 1.0	7.0 ± 1
RPE	5.0 ± 1.0	7.0 ± 1.0

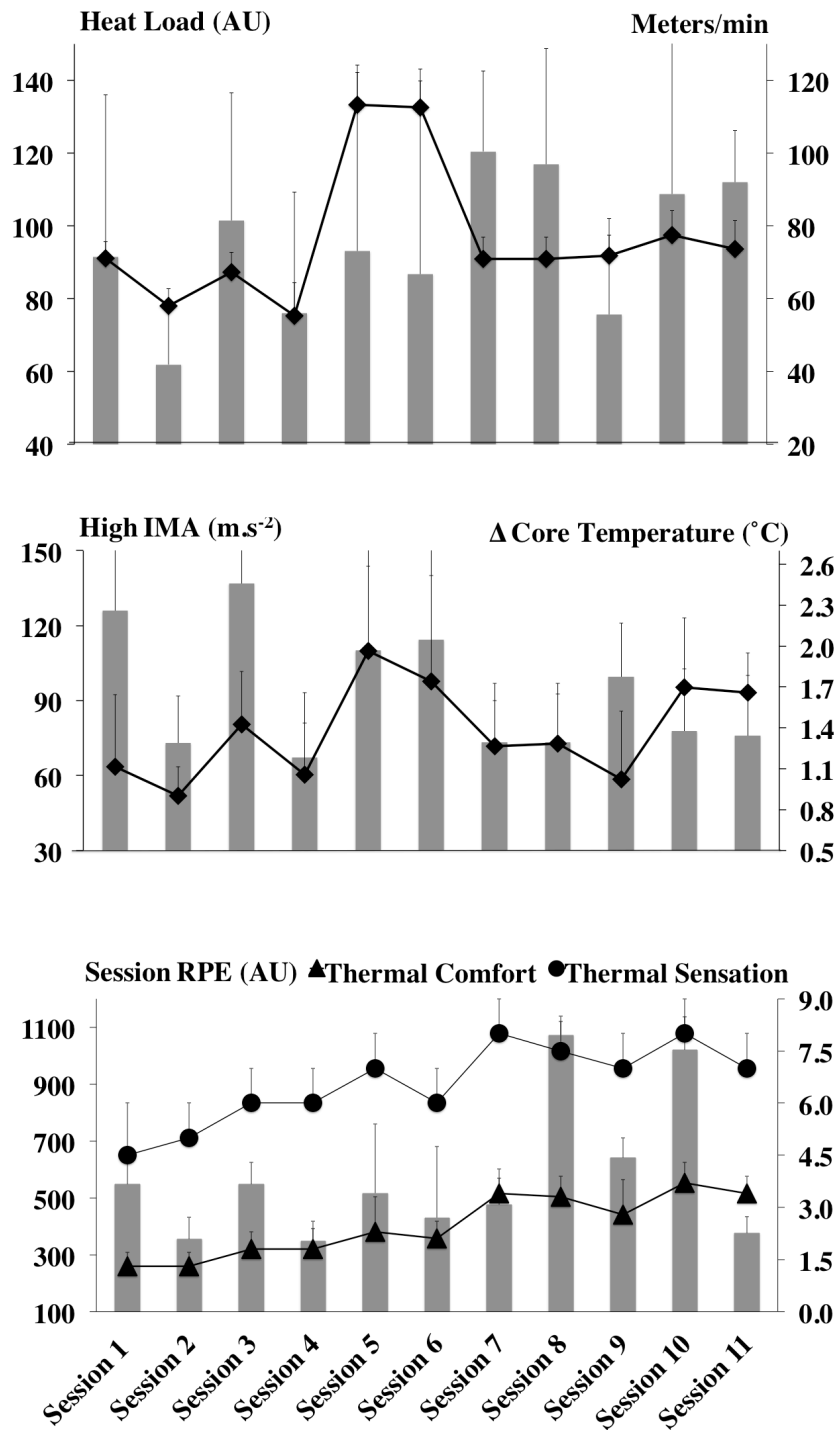


Figure 2. Daily variations in training load during heat acclimatization. Training load data are presented as Means±SD (error bars) with Days 1 through 7 in LA and Days 9 through 14 in Cancun. *Top:* Heat load (AUC: area under the core temperature curve, baseline subtracted) and meters/min from Day 1 through 14; *Middle:* High inertial movement analysis (high IMA) and the total change in core temperature in a single training session (ΔT_c) from Day 1 through 14; *Bottom:* Session RPE (Session Length*RPE value (Borg Scale 1-10), Thermal Comfort (1-5), and Thermal Sensation (0-9) from Day 1 through 14. *Not shown:* Data does not include the non-training days (Day 4, 8, and 12).

4.2 The effect of acclimatization on plasma volume

Mean team USG pre-HA on baseline PV testing (Day -16) was 1.020 ± 0.005 (team z-score: $+1.5 \pm 2.0$). Mean USG on PV testing days pre and post Phase 1 (Day 1, Day 6 or 7) were 1.014 ± 0.005 (mean z-score: $+0.1 \pm 1.2$) and 1.011 ± 0.005 (mean z-score: -0.2 ± 0.7), respectively. Mean USG post-HA (Day 16) was 1.013 ± 0.005 (mean z-score: $+0.1 \pm 0.8$). Mean team change in body mass (kg) from baseline (Day -16) to end Phase 2 (Day 16) was $+1.4\%$. Figure 3 outlines the change in PV (%) from an absolute average Vancouver baseline 3935.5 ± 440.3 ml (Figure 3, Day -16). With reference to Figures 3 regarding the standardized effect (SE), there was a small decrease in PV from Vancouver baseline (Day -16) to pre-Phase 1 (Day 1) (SE= -0.43 ; 90% CL: $[-0.66, 0.20]$). From pre-Phase 1 (Day 1) to post-Phase 1 (Day 6 or 7) there was a large increase in PV (SE= 0.64 ; 90% CL: $[0.99, 0.29]$). From pre-Phase 1 (Day 1) to post-Phase 2 (Day 16) there was also a large positive increase in PV (SE= 0.63 ; 90% CL: $[0.34, 0.93]$) and this expansion occurred primarily in the pre-acclimatization Phase 1 (Los Angeles) as the change in PV from post-Phase 1 to post-Phase 2 was trivial. The correlation between percentage change in PV (%) from Vancouver baseline to post-Phase 2 (Figure 3, Day -16 to Day 16) and absolute PV (ml) at Vancouver baseline was $r=0.07$, [90% CL $-0.38; 0.50$].

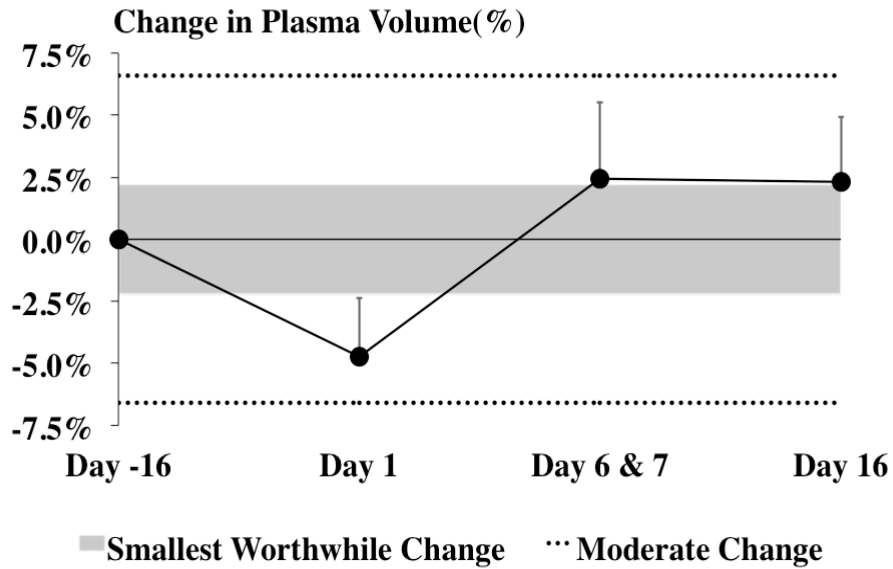


Figure 3. Values show the change in plasma volume (PV) from baseline testing in Vancouver (Day -16) to each follow up test occasion (Day 1, 6 and 7, 16). The shaded area represents the smallest worthwhile change in PV while the dotted line represents a moderate change in PV. *X-axis* represents the PV measurement days within the training camp period: Day 16: Vancouver Baseline; Day 1: Pre-HA, Phase 1; Day 6 and 7: Post-Phase 1; Day 16: Post-Phase 2. Measurement of PV Post-Phase 1 occurred over two days (Day 6 and 7) and was performed on the opposite day of the international match for each player. *Y-axis* represents the percentage change in PV (%).

4.3 The effect of acclimatization on HR response and RPE during the 5'-1' submaximal running test

Figure 4 outlines the change in 5'-1' submaximal running performance throughout HA. Initial submaximal testing occurred in Phase 1, Day 1 (LA $\sim 22.5^{\circ}\text{C}$, $44.8 \pm 9.4\% \text{RH}$) and was used to assess the change in HR_{ex} ($170 \pm 11 \text{bpm}$), HRR ($39.0 \pm 5.9\%$), and RPE ($3.5 \pm 1.3 \text{AU}$). From the first session of pre-HA (Phase 1, Day 1) in mild T_{ambient} (LA $\sim 22.3^{\circ}\text{C}$, $44.8\% \text{RH}$) to the first HA session (Phase 2, Day 9) and exposure to a hot T_{ambient} (Cancun $\sim 34.5^{\circ}\text{C}$, $53.2 \pm 4.3\% \text{RH}$) there was a small increase in HR_{ex} (SE=0.45; 90% CL: [0.32, 0.57]), a moderate decrease in HRR (SE=-1.02; 90% CL: [-1.38, -0.67]), and a moderate increase in RPE (SE=1.00; 90% CL: [0.53, 1.47]). Following five HA sessions over six days in Phase 2 (Day 9 to 14), the standardized effect indicated a small decrease in HR_{ex} (SE= -0.49; 90% CL: [-0.67, -0.31]) and a small increase in HRR (SE=0.53; 90% CL [0.04, 1.02]) while RPE remained consistent (AU= 4.7 ± 0.9 to 4.9 ± 0.9). From the last day of HA in Phase 2 (Day 14) to 2dayP HA (Day 16) in a temperate T_{ambient} (Toronto $\sim 18.2^{\circ}\text{C}$, $51.3 \pm 20.9\% \text{RH}$), there was only a small decrease in RPE (SE=-0.41; 90% CL: [-0.65, -0.18]). The change in HR_{ex} was trivial (SE= -0.07; 90% CL [-0.17, 0.04]) while there was a small increase in HRR (SE= 0.25; 90% CL: [-0.38, 0.87]). There was also a moderate increase in RPE (SE= 0.45; 90% CL: [0.03, 0.88]) from initial testing in LA (Day 1) to 2dayP HA (Day 16) in temperate T_{ambient} (12°C). From initial testing in LA (Phase 1, Day 1), to 11dayP HA (Day 25) in temperate T_{ambient} (13°C) there was a moderate decrease in HR_{ex} (SE= -0.42; 90% CL [-0.52, -0.31]) and a moderate increase in HRR (SE= 0.37; 90% CL: [-0.17, 0.92]) while RPE was similar to what was observed on Day 1 during initial testing (SE= -0.04; 90% CL: [-0.41, 0.33]).

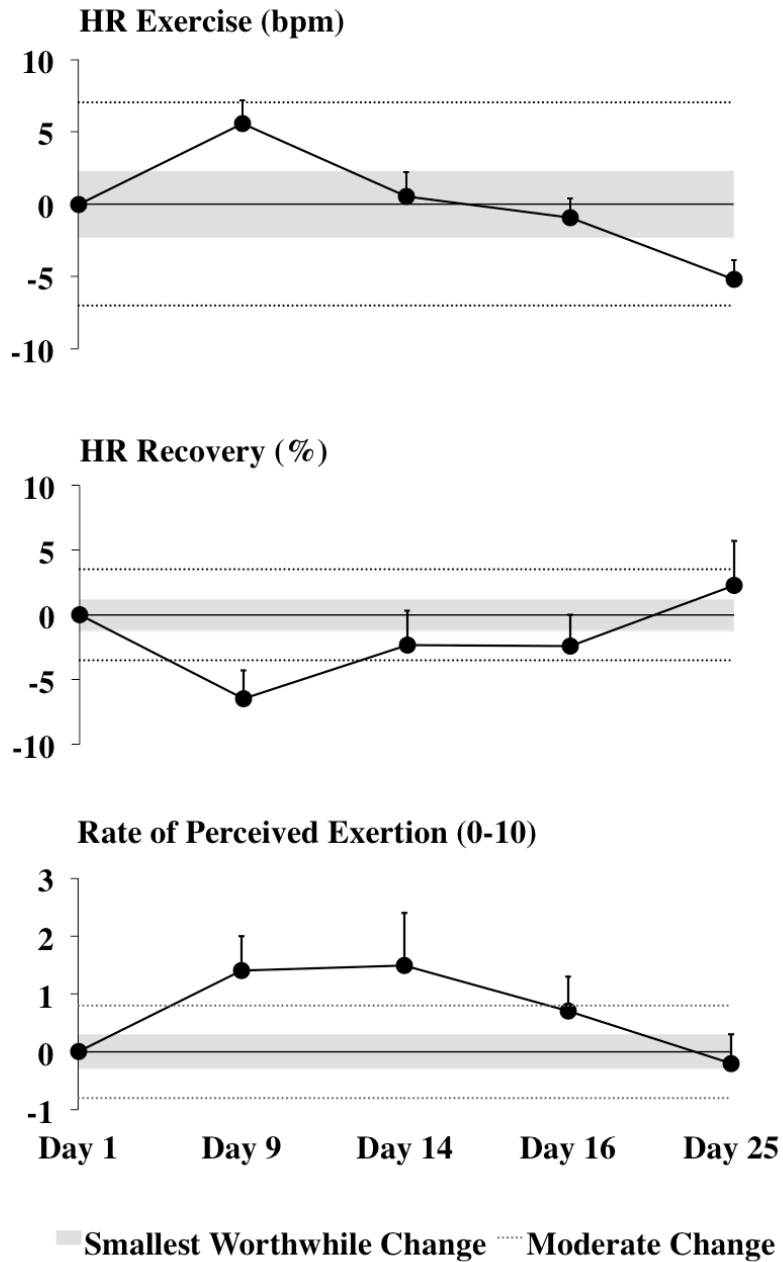


Figure 4. Changes in heart rate (HR) response and rate of perceived exertion (RPE) during the 5'-1' submaximal running test from pre-HA, Phase 1 (Day 1) to 11dayP HA, Phase 2 (Day 25). X-axis: Day 1: First test performed in LA, mild (~22.3°C); Day 9: second test performed in Cancun, hot (~34.5°C); Day 14: third test performed in Cancun, hot (~34.5°C); Day 16: fourth test performed in Toronto, temperate (~23.2°C); Day 25: fifth test performed in Toronto, temperate (~23.2°C). *Top:* exercise HR (HR_{ex}, bpm), *middle:* post-exercise HR recovery (HRR, %), and *bottom:* RPE, (0-10). The shaded grey area represents the smallest worthwhile change in performance (i.e., trivial; 0.20 ES), the dashed line represents the lower limit for moderate changes (<0.20, >0.60) of baseline between-subject standard deviation (SD) averaged from each test day and values are ±SD.

4.4 The effect of acclimatization on small-sided game performance

Figure 5 outlines the standardized differences (90% CL) between the 4v4SSG on the first (pre) and last (post) HA training days in Cancun (Phase 2: Day 9 and Day 14). HRex was moderately lower (-3.5bpm; 90% CL: [-5.5, -1.6]) from pre to post HA while HRR was largely higher ($5.7 \pm 1.6\%$; 90% CL: [4.1, 7.4]) across the four games pre and post HA. There was a small decrease in m/min (-4.7%; 90% CL: [-9.6 to 0.7]) and a small increase in high IMA (20.1%; 90% CL: [6.6, 35.2]).

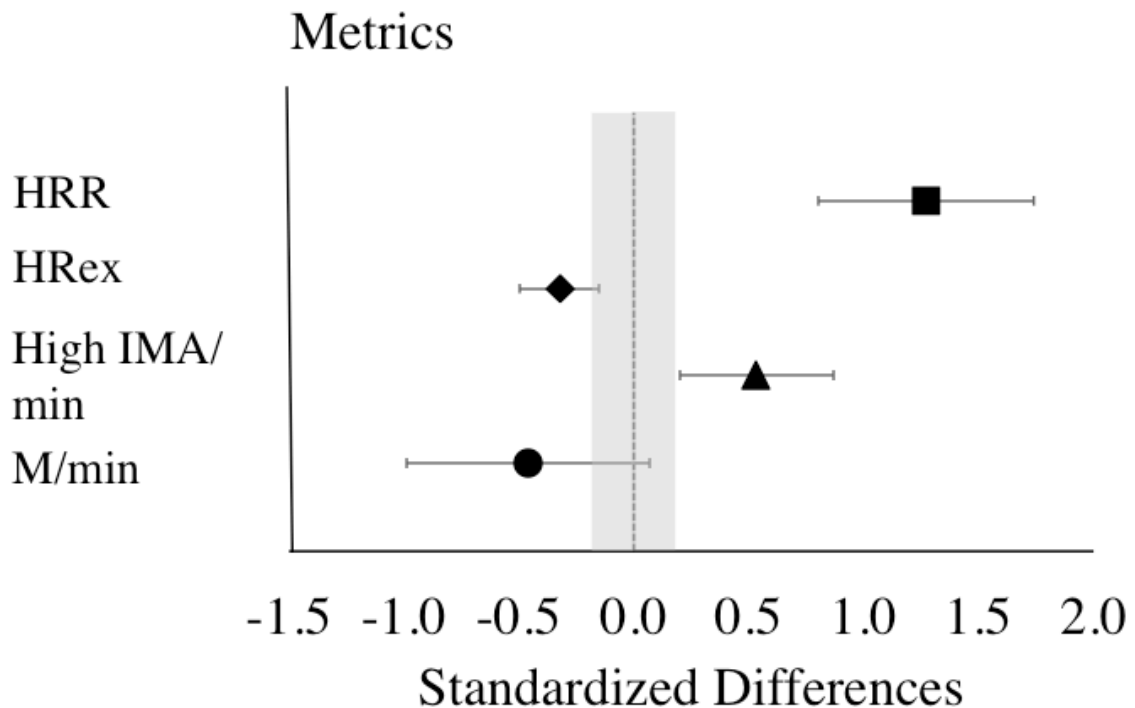


Figure 5. Standardized differences in four-a-side, small-sided soccer game (4v4SSG) performance between the first day of Phase 2 heat acclimatization (HA) on Day 9 and the last day of Phase 2 HA on Day 14. *Y-axis* shows recovery HR (HRR), exercise HR (HRex), high IMA/min and meters/min (M/min). *X-axis* shows the standardized differences in these metrics between Day 9 and 14. The shaded grey area represents trivial differences (i.e., < 0.20). Refer to methods for descriptions of qualitative outcomes. Values are effect sizes \pm 90% confidence intervals (CL).

4.5 Core temperature and external load metric associations.

4.5.1 ΔT_c and high IMA

Values represent the correlation coefficient (r [90%CI]) between the change in T_c and high IMA. There was a small positive relationship ($r=0.23$ [-0.21; 0.60]) between mean ΔT_c and mean high IMA on all training days (Phase 1 and 2) and a small positive relationship ($r=0.20$ [-0.25; 0.58]) for the hardest training days. The sum of the ΔT_c and the sum of high IMA on all training days showed a moderate positive relationship ($r=0.39$ [-0.05; 0.70]).

4.5.2 ΔT_c and meters/min

There was a moderate positive relationship ($r=0.34$ [-0.10; 0.67]) between average ΔT_c and average M/min across all training days. There was also a moderate positive relationship ($r=0.39$ [-0.05; 0.70]) between the sum ΔT_c and the sum of M/min across all training days.

4.5.3 Heat load and high IMA

There was a moderate positive correlation between the sum of relative AUC and the sum of high IMA across all training days ($r=0.32$ [-0.13; 0.68]).

4.5.4 Heat load and meters/min

There was a moderate positive correlation between the average AUC and average M/min across all training days ($r=0.35$ [-0.09; 0.68]) while there was a small correlation between the sum of the relative AUC and M/min across all training days ($r=0.29$ [-0.16; 0.64]).

4.6 Performance, core temperature and associations with plasma volume

4.6.1 Plasma volume and heat load

There was a small positive correlation ($r=0.27$ [-.18; .62]) between average heat load across all training days (relative $AUC_{\text{phase1-2}}$) and average percentage change in PV from the start of Phase 1 (Day 1) to the end of Phase 2 (Day 14). There was a moderate positive correlation ($r=0.42$ [.01; .72]) between average heat-load during Phase 2 (Day 9 to 14) (AUC_{phase2}) and percentage change in PV from the end of Phase 1 (Day 7) to the end of Phase 2 (Day 14) (Figure 1).

4.6.2 Plasma volume and session RPE

There was a large positive correlation during pre-HA ($r=0.57$ [.18, .80]) between the change in PV (%) and the sum of the total session RPE from pre to post Phase 1 (Day 1 to 7).

4.6.3 Plasma volume and external load

There was a small positive correlation ($r=0.23$ [-0.22, 0.60]) between high intensity efforts and the change in PV (%) from pre Phase 1 to post Phase 2 (Day 1 to 16). M/min were moderately correlated with the change in PV (%) ($r=0.32$ [-0.12,0.66]) and relative change in PV (ml/kg) ($r=0.39$ [-0.04,0.70]) from pre-Phase 1 to post-Phase 2 (Day 1 to Day 16).

Chapter 5 Discussion

It is well-established that repeated training exposures in the heat through an artificial (acclimation) or a natural environmental setting (acclimatization) are an effective method to elicit physiological changes that mitigate heat strain [127]. Both laboratory and field-based heat training protocols have been widely used amongst athletes in preparation to perform in hot environments [30,127]. HA laboratory studies have demonstrated improvements in exercise tolerance, perceived exertion, and performance in both temperate and hot conditions [41,58]. However, the highly controlled, isolated nature of these interventions makes it challenging to apply the information to team sport athletes who often compete in variable environmental conditions throughout a competitive season. Additionally, many of the heat protocols utilized in these lab-based interventions were developed and tested primarily using male endurance athletes or untrained participants [22,56]. However, training outdoors and utilizing field-based HA is more relevant to female soccer players as solar radiation and airflow have an effect on overall heat stress as compared to artificial conditions [161]. Therefore, the current study has narrowed the gap pertaining to field-based HA by using international-level female team sport players, novel monitoring metrics, and physiological monitoring to gain a broader perspective on the ergogenic effect of HA in both temperate and hot conditions.

Within the current study, HA induced a meaningful PV expansion from Vancouver baseline to post-Phase 2 HA training (Figure 3) while also improving cardiovascular fitness as highlighted by the reduction in HR_{ex} and increase in HRR during the 5'-1' submaximal test (Figure 4). Additionally, findings from the 4v4SSG indicated an improvement in four-a-side physical performance between the first and last day of HA, specifically a decrease in HR_{ex}, increase in HRR, and an increase in high IMA (Figure 5). A novel finding was that the greatest improvement in cardiovascular fitness was seen one-week post-HA during submaximal testing in a temperate condition (Figure 5). This discussion will focus on five components: (1) the effect of field-based HA on PV expansion (2) the relationship between PV expansion, heat load, perceived

exertion, and GPS-derived indices and their application for monitoring training load (3) the utilization of a 5'-1' submaximal running test to observe both the induction and time-course of aerobic based change throughout HA (4) the utilization of a 4v4SSG in combination with external (GPS) and internal (HR) load monitoring to observe the heat stress response pre and post-HA (5) the T_c response to a competitive match in the heat.

Given that cardiovascular adaptations are typically the first to decay following HA [66], this study utilized two training phases of mild and hot acclimatization (Phase 1; LA, Phase 2; Cancun) to induce adaptation during pre-acclimatization (Phase 1) and sustain this response via maintenance of the exercise-in-the-heat induced stimulus during HA (Phase 2). The justification for utilizing two phases of acclimatization was supported by Periard and colleagues [127] who previously suggested that shorter protocols do not provide a strong enough stimulus for sudomotor adaptation and that two weeks of HA was likely to evoke a greater magnitude of PV change as compared to shorter protocols.

5.1 The effect of field-based heat acclimatization on plasma volume

The current findings support previous literature that demonstrated that heat acclimation or acclimatization can lead to a greater volume of circulating blood via an increase in blood PV [30,40,44,127]. Together, studies utilizing either lab-controlled or outdoor-based HA have demonstrated increases in PV of ~3-27% with a 2-3L increase in total body water [127]. Laboratory studies utilizing controlled hyperthermia protocols have reported PV to increase by 4-15% contributing to the improved cardiovascular stability via maintenance of cardiac output and reductions in HRex [104,119,120,126,131]. A HA-protocol consisting of nine days in moderate heat and three days in extreme heat found that even moderate heat was capable of reducing HRex (-25bpm) [46]. Similarly, a reduction in HRex (-14bpm) was observed over just five days of laboratory-based acclimation [68]. The literature has demonstrated that those protocols that have utilized laboratory heat chambers are often effective at inducing increases in PV, likely the result of athletes exercising at a consistent temperature and humidity at a controlled T_c stimulus [67,104].

However, it could also be that there is not enough evidence in field-based HA that utilizes a higher T_c training stimulus ($>38.5^\circ\text{C}$); as current research has shown that for highly-trained athletes, a larger training stimulus in a sport-specific setting may be required to induce sufficient adaptation [136]. While laboratory HA protocols are more controlled, they are less ecologically valid than field-based HA which is more likely to induce better peripheral adaptations from sport-specific training [77]. This is particularly evident for the sport of soccer as a number of field-based HA studies that have focused on soccer-relevant technical and tactical training in the heat, which translated into an improved sport performance post HA [21,22,158].

Collectively, four field-based HA protocols together with team sport players had a mean PV expansion of 5.4% [20-22,138]. In two different studies of outdoor HA, Buchheit and colleagues [21,22] reported increases in PV following both one week (7%) and two weeks (5.6%) of HA, which translated into substantial improvements in athletic performance with the greater improvement seen the longer HA protocol (+44% vs. 7% in YOYOIR2). It is evident that HA can physiologically benefit athletic performance in the heat through improvements in cardiovascular function and aerobic capacity attributed to a greater circulatory blood flow, SV, and cardiac output resulting from PV expansion [40,44,104,131]. Aside from increases in PV, HA induces other various adaptations that could explain physical performance improvements. HA studies have demonstrated reductions in the respiratory exchange ratio during exercise in the heat as well as muscle glycogen sparing from decreased glycogenolysis [52]. An improvement in anaerobic metabolism from HA allows for a reduction in blood and muscle lactate at a given power output allowing athletes the ability to sustain higher workloads and reduce relative exercise intensity [53]. Improvements in sport performance and $\text{VO}_{2\text{Max}}$ post HA have also been suggested to be a result of an increased end-diastolic volume as a consequence of increased ventricular compliance [40]. HA has consistently been shown to induce more efficient thermoregulation through reductions in the temperature threshold for sweating and cutaneous vasodilation as well as decreases in sweat sodium concentration [22]. While further studies are needed to clearly establish the effects of PV expansion and the ergogenic potential of HA, even minor physiological improvements are likely to benefit athletic performance at the international level [103].

In highly-trained athletes, even a small reduction in oxygen uptake at a given power output or intensity has been shown to make a substantial difference to international performance [83]. Therefore, the current study provides support for previous observations of the ergogenic potential of heat adaptation in aerobic performance, regardless of the ability of an athlete to expand plasma volume.

The present findings indicate a small but meaningful overall change in PV from baseline in Vancouver to post-HA Phase 2 training. While there is evidence that physical training alone induces changes that mimic the acclimatization response (ie. PV expansion) [120], our athletes were at the peak of their aerobic fitness as this camp was performed in-season leading into the World Cup. Therefore, any exercise performed prior to the camp was unlikely to have induced a change in cardiovascular fitness. However, it is possible that because these athletes were at the peak of their cardiovascular fitness at the start of HA training, their ability to expand an already high baseline PV to an even greater extent may have been limited. It is important to consider that PV expansion during an in-season, pre-competition training camp is likely of less magnitude than if the athletes were to engage in HA during pre-season where the effect of aerobic training in combination with HA would be likely to create a greater magnitude of PV expansion [120]. Aerobic performance is underpinned by the interaction of VO_{2Max} and the percentage of VO_{2Max} that can be sustained for a given duration [40]. Therefore, at the elite level, even a slight reduction in oxygen uptake at a given power output or intensity due to improvements in cardiovascular efficiency and plasma volume expansion have the potential to make a substantial difference to elite performance success [40].

As outlined in Figure 3, initially there was a decrease in absolute PV (-4.8%) from the time of measurement during pre-HA assessment in Vancouver (Day -16) to the start of Phase 1 pre-HA training in LA (Day 1). A possible explanation for the observed decrease from baseline to the start of the training camp (Day 1) was that players were not involved in a controlled and scheduled training regime prior to the start of the camp but were instead routinely training on their own time. Therefore, the effect of varied training (time of day, intensity, duration) and lack of hydration monitoring as a result of being out of a team environment likely contributed to this reduction [146]. There was

large change in PV (8.3%) from Day 1 to Day 7 of Phase 1 pre-HA training in mild weather (22.5°C), which resulted in a mean 2.5% increase in PV above Vancouver baseline. This expansion was maintained throughout Phase 2 training (Figure 3), albeit with large variability. The current findings support a previous study also demonstrating a large inter-individual variation in the PV response within field-based HA [138]. Phase 1 showed the greatest PV expansion (SE=0.64) but also had the greatest range (90% CL, [-10.2% to 19.7%]) in PV response likely a result of some athletes having a faster circulatory response than others. Phase 2 also showed a large range in the magnitude of PV expansion but to a lesser degree (90% CL, [-9.3% to 10.4%]). There were likely many factors that allowed certain players to be either responders or non-responders to HA and PV expansion within the current study. It is possible that some athletes may have had a different sweat response due to differences in body composition, specifically, in their surface area-to-mass ratio. A larger body surface area-to-mass ratio allows for greater thermoregulation as a result of a greater area to dissipate heat [70]. Additionally, those athletes who have larger body surface area-to-mass ratios could have had a greater capacity to expand plasma volume, which may have allowed for a greater adaptation than those with smaller ratios. Regardless of body composition, the overall change in PV may have been greater if the direct measure of PV (CO rebreath protocol) was used as the only method of PV measurement throughout the entire study as opposed to using the Dill and Costill method following baseline. Specifically, the Dill and Costill method has been shown to have greater variability than the CO rebreath protocol which provides an absolute measure of haemoglobin mass to obtain PV versus other methods which only provide a concentration of variability of both PV and overall blood volumes [126].

There was no observed limitation in the capacity for those athletes with already large absolute or relative baseline values to expand PV. This was supported by the lack of correlation between mean absolute (ml) PV at Vancouver baseline (Day -16) and the percentage change in PV from Vancouver baseline (Day -16) to post-HA (Day 16) ($r=0.07$, 90% CL: [-0.38; 0.50]). This finding challenges previous literature suggesting that athletes with large baseline blood volumes from endurance training may be limited in their ability to expand PV due to a ceiling effect [69,159]. The efficacy of HA is

likely dependent upon the fitness status of the individual as many endurance-trained athletes physiologically appear as though they are already heat acclimatized [69]. Within the current study, there was an increase in PV of 2.3% from Vancouver baseline (Day -16) to post-HA (Day 16). However, during baseline testing on Day -16, the team was significantly above USG team normative data (USG=1.020, +1.5 standard deviations (SD) at the time of PV measurement indicating that hydration status (i.e. dehydration) likely had a large effect on the PV outcome. Additionally, the Dill and Costill [49] method of analysis was used throughout the intervention (training camp) period and is known to be influenced by hydration status [49]. Therefore, the final change in PV from baseline-post-HA may not be representative of a true physiological change. If an athlete is significantly dehydrated at the time of PV assessment it may have the potential to impact the value of PV obtained [147]. In comparison, mean USG during PV assessment on Day 1 of Phase 1 was only 0.1 SD above mean USG team normative data (USG=1.014, hydrated) and 0.2 SD below the mean during the second PV measurement in Phase 1 (Day 6 or 7).

Aside from the changes in hydration status, slight increases or decreases in average training loads may also have an impact on PV as well [44]. However, a recent study that utilized a three-week period of high-intensity interval training versus in well-trained athletes found no significant changes or observed differences between groups in HBmass, PV, or BV [112]. Therefore, the progressive increase in training load from Phase 1 to 2 as well as session RPE (Phase 1: 458 ± 163 ; Phase 2: 721 ± 295) (Figure 2) was unlikely to have a significant effect on the final change in PV (Figure 3).

5.2 Relationship between plasma volume, heat load, rate of perceived exertion, and external load

In a field-based HA, the lack of a controlled setting can make it difficult for a coach to gauge the amount of heat load that an athlete is experiencing. Athlete fatigue, fitness, and performance monitoring throughout HA training is highly important in order to properly individualize and adjust training load [136]. This is of particular importance when an environmental factor such as the heat is added to training, as the goal of HA is to achieve optimal adaptation while also avoiding heat related stress [77,107]. The GPS has been previously used in team sport players to observe external load in the heat in

combination with subjective measures of workload to avoid reductions in training stimulus [20]. However, there are a limited number of studies in elite team sport players that have utilized GPS external load in combination with T_c monitoring while training in the heat [7,20,50,124] and only one study that has utilized both T_c monitoring and GPS in team sport players during a field-based HA protocol [20].

A previous study utilizing GPS-derived indices and RPE during an eight-day HA protocol in elite male athletes found a large increase in thermal strain on the first HA day, followed by a progressive decrease as evident by a RPE:m/min ratio (-0.4AU in RPE:m/min/day) [20]. The current study adds to these findings [20] through investigation of not only the relationship between training T_c data (ΔT_c , relative AUC), GPS-external load metrics, and subjective workload (session RPE) but the relationship between these as well as the physiological PV response.

The relationship between ΔT_c , relative AUC, and the change in PV was observed to determine whether the total temperature difference (ΔT_c) between baseline T_c (T_{cstart}) and final T_c (T_{cfinal}) in a single HA training session would have a greater impact on cardiovascular change versus duration of time spent at an elevated T_c in a single HA training session. A novel finding was the observed moderate correlation between the mean change in PV from the end of Phase 1 (Day 6 or 7) to the end of Phase 2 (Day 16) (Figure 3) and the mean relative AUC in Phase 2 training ($r=0.42$). Given that relative AUC takes into account the duration of the session while ΔT_c does not, the accumulated duration of total time spent above T_{cstart} within the training session likely had a greater impact on PV adaptation versus the total ΔT_c change which showed a limited correlation ($r=0.15$). Therefore, a greater amount of time spent at an elevated T_c is likely to influence cardiovascular adaptation and PV expansion more than the overall difference (ΔT_c) between pre-session T_c (T_{cstart}) and post-session T_c (T_{cfinal}) or a rapid increase in T_c in a given HA session. This was supported in a previous short-term HA study [95] when twenty-seven minutes of time at an elevated T_c was not sufficient to induce cardiovascular changes in participants as compared to longer training bouts (90-100 minutes) [46]. Thus, the time spent at an elevated T_c may be a beneficial predictor of cardiovascular adaptation [38].

Additionally, we also observed a small positive relationship between the sum of ΔT_c and the sum of relative AUC, with the sum of high IMA (acceleration, deceleration, changes in direction) within each training session ($r=0.20$, $SE=0.32$). It is well known that a T_c stimulus of $\sim 38.5^\circ\text{C}$ is the threshold for inducing adaptation during HA, as long as it is held for a sufficient duration of approximately ~ 60 - 90 -minutes [127]. Soccer in particular is a sport that requires a high percentage of muscle mass and a high metabolic demand due to the amount of explosive actions during a match [157]. Periard et al. [129] highlighted that when players engage in these short bursts or explosive efforts such as fast changes in direction (high IMA) muscle temperature drastically increases. The small correlation between high IMA and T_c ($r=0.23$ 90% CL: [-0.21; 0.60]) in the current study may be explained by the high metabolic demand and high ratio of muscle mass required to perform these explosive movements and changes in direction that define high inertial movements [11,107]. As we were unable to interfere with pre-planned practices in the current study, the relationship between high IMA and T_c may have been more pronounced if T_c was consistently maintained at 38.5°C . With continued research regarding the relationship between these metrics, the accumulated total of explosive actions during maximal sprinting (acceleration, deceleration) and change of direction observed as high IMA in the GPS may be a potential method for increasing and maintaining T_c at a target stimulus (38.5°C).

Aside from the relationship between session T_c and high IMA, a notable finding from the current study was the positive correlation between the percentage change in PV and the total sum of session RPE ($r=0.57$) in Phase 1, while there was no strong relationship between the percentage change in PV and the sum of session RPE in Phase 2 ($r= -0.50$; 90% CL: [-0.76, -0.09]) or the sum of session RPE in Phase 1 and 2 together ($r= -0.31$; 90% CL: [-0.65, 0.13]). This finding suggests that the initial exposure to mild heat in pre-HA Phase 1 induced a sufficient perceived workload and heat stress enough to increase PV in Phase 1 and maintain this expansion throughout Phase 2. While further evidence is warranted, the additional small positive relationship between PV and HIE ($r=0.23$) and the moderate positive relationship between M/min and PV ($r=0.32$) provide evidence that these metrics could potentially be used within

HA to create a training intensity threshold large enough to induce a sustainable cardiovascular adaptation.

This study is the first to observe GPS-external load metrics and their relationship with physiological markers of HA (PV). Therefore, with continued research, coaches may be able to utilize these GPS-derived measures to monitor the intensity of training that is required to reach a T_c of 38.5°C. Therefore, current research supports previous literature suggesting the use of GPS to measure intensity, pace, and workload during field-based HA as an effective asset to monitor pacing strategy in hot environments [7]. Therefore, the real-time data from GPS allows for within-practice adjustments to ensure players are reaching predetermined intensities. In support of Pryor et al. [134], utilizing GPS metrics in combination with HR monitors and if available, internal body measurements (T_c), will not only help athletes train precisely but will also prevent overtraining or overreaching prior to competition. Additionally, these findings support Meylan et al. [114] and Johnston et al. [89], as GPS-derived high IMA may be used as a reliable metric to not only monitor and assess explosive actions during training in temperate conditions but as a method to observe changes in training intensity due to the effects of environmental heat stress.

Ultimately, the goal of understanding the relationship between GPS metrics, physiological markers of acclimatization (PV), and subjective workload (RPE) is to be able to effectively monitor the magnitude of intensity required to reach an optimal T_c via GPS-derived indices. As a result, this would allow heat stress to be controlled within HA while also eliminating the need for T_c monitoring and costly T_c sensors.

5.3 Heart rate response to a 5'-1' submaximal running test in temperate and hot conditions

The literature has consistently demonstrated improvements in submaximal exercise performance following HA characterized by a reduced HR_{ex} and an increased VO_{2max} [131]. However, there are a limited number of studies that have observed improvements in HR response during submaximal testing within elite athletes [22,104,136,137]. In line with previous literature [22], our data show meaningful improvements in HR response (Figure 4; HR_{ex}, HRR) in international level female soccer players during submaximal running pre-post HA. While a previous soccer-based

HA study did note a significant reduction in HRex (~8%) in male soccer players utilizing a 5-minute submaximal running test (9km/h) outdoors, there was no improvement in HRR [22]. This limited response in HRR was suggested to be the result of using a low running speed for the elite level players (9km/h instead of 12km/h) [22]. Therefore, the current study increased the stimulus for adaptation by utilizing a higher running speed (12km/h). The 5'-1' submaximal running test was effective in tracking HRR and HRex to observe the induction and time course of aerobic adaptation throughout and post-HA in both hot and temperate conditions. As outlined in Figure 4, initial submaximal performance during Phase 2 (Day 9 ~34°C) was more stressful than in mild conditions in Phase 1 (Day 1 ~22.1°C), as there was an increase in HRex (+5.6bpm), a reduction in HRR (-6.5%), and an increase in RPE (+1.4 AU). This observed increase in heat strain upon arrival in the hot condition (Day 9) was likely a result of the large increase in temperature from LA to Cancun over one day (+12°C). This was supported by Buchheit et al. [20] who observed an increase in RPE during HA (34.9°C) when athletes underwent a 12°C increase in temperature over two days [20].

An important observation within the current study was the ability of the players to acclimatize aerobically to the heat and perform submaximal running post-HA (Day 14) in Cancun similar to what was observed at baseline pre-HA on Day 1 in LA. Figure 4 shows that from Day 1 to Day 9, submaximal RPE initially increased by 1.4AU, indicating that players perceived initial training in the heat to be harder than in a milder condition. From pre to post HA (Day 9 to Day 14) there was no meaningful change in submaximal RPE, likely due to the effect of training fatigue as players were under a large training load on Day 13 (Figure 2). On Day 16, 2dayP HA following five sessions of HA training, submaximal RPE as well as HRex and HRR returned to baseline values similar to those seen in more mild conditions (Figure 4). Therefore, in support of previous findings both cardiovascular and perceptual adaptation can be observed in as little as five field-based HA sessions [30]. Cardiovascular adaptations are commonly the first to arise within HA [131], therefore, it is likely that the Phase 1 pre-HA training (Figure 1) in a mild condition allowed for these adaptations to occur while the five training sessions in the heat during Phase 2 HA (Day 9 to 14) provided an aerobic boost

that enabled players to achieve submaximal testing in a hot condition that was similar to what was observed in a mild condition.

A novel finding from the submaximal test was the large improvement in HR response observed 11dayP HA in a temperate condition (Figure 4, Day 25). This challenges previous research suggesting that competitive athletes may only retain HA for up to one week [125], and highlights the importance of a submaximal retest up to two-weeks post-HA training given that the overall change in aerobic fitness may not be complete until several days out of the heat. This finding is further supported by Buchheit and colleagues [20] who utilized a 4-min submaximal test (12km/h) before, immediately post, and three days after a HA camp and found that the greatest reduction in HR-response did not occur immediately but three days following the camp (3%). This delayed aerobic adaptation post-HA could be partly attributed to an improvement in neuromuscular efficiency after recovering from HA [20]. Studies have shown that physical fitness typically plateaus at the final stages of in-season training, therefore, the improvement in aerobic capacity cannot be justified as a training adaptation due to the fact that players were at their highest aerobic fitness (two-weeks before FIFA World Cup) but continued to improve with HA [18,22]. A possible explanation for the lack of a HR-response 2dayP HA (Figure 4, Day 16) could be attributed to residual fatigue from a period of physical training, as previous evidence has shown increased sympathetic activity and a reduced vagal activity during periods of intensified training [14]. These effects are typically a result of overreaching and are known to vanish after 3-4 days of recovery [14,107], which is consistent with current findings as HR-response drastically improved after eleven days out of the heat. Additionally, the RPE values during the first 5'-1' submaximal running test 2dayP HA (Day 16) were rated moderately harder (~1.5-1.7AU, Figure 4) as compared to initial testing (Day 1) which provides support for an increased perceived intensity likely having an overall impact on HR-response immediately post-HA.

There is also evidence that mood, cognitive, and physical performance are adversely affected by jet lag or travel fatigue which can persist one day for each time zone crossed [14,59]. A recent review outlined the impact of airline travel on performance and noted that athletes exhibit different rates of resynchronization

following flight as well as motivation [100]. Therefore, the impact of airline travel from Cancun to Toronto on Day 15 may have limited submaximal performance during the first test (Day 16) following arrival in Toronto (Figure 4). The moderate mean decrease in HRex (SE=-0.87) from initial testing in a mild condition (Day 1) to 11dayP HA (Day 25) was comparable to the mean change in three previous HA interventions (SE=-0.57, 90% CL) [20-22]. Secondly, the observed improvement in HRex is substantially greater than the day-to-day variability in the team's standardized submaximal running test (CV 1.5; 1.3, 2.1). It has been suggested that HRex at a set intensity or speed is indicative of an improvement in aerobic fitness while a lowered HR post-exercise (HRR) is indicative of faster recovery from highly intense exercise [19,22]. Aside from HRex, observation of HRR is important to utilize within HA as it provides indication of fitness level and can act as a powerful marker for the positive change in high-intensity exercise performance [19]. The positive response seen in HRR 11dayP HA (Figure 4, Day 25) suggests that a greater running speed may be required (12km/h) during submaximal testing in order to stimulate a change in recovery, particularly for highly trained athletes [22]. In support of Lamberts et al. [99] and Buchheit et al. [18], submaximal HRR can be used as an effective non-invasive tool to assess the responsiveness of an athletes' autonomic nervous system following a training stimulus while also allowing for the observation of recovery capability throughout and post HA. From a mental perspective of workload, the reduction in RPE during submaximal testing from Day 1-25 (Figure 4) is consistent with other studies reporting reductions in RPE and perceptions of thermal stress [30,95,158]. In three different field-based HA protocols, subjective workload perception (RPE) post-HA was improved [7,95,158]. Therefore, it is likely that HA not only has physiological benefit for aerobic fitness but also has psychological benefits in terms of perception of training load which is critical given that pacing strategy is negatively affected under heat stress [7]. Current findings have demonstrated an improvement in temperate weather submaximal performance 11dayP HA (Figure 4, Day 25) providing support for Lorenzo and colleagues [104] who demonstrated a 5% improvement in cold weather cycling performance (13°C) and Buchheit et al. [22] (7% improvement in a soccer-specific Yo-Yo IRT in 22°C. Therefore, a submaximal running test in combination with HR measures (HRex, HRR) may be used as an effective non-

invasive method to track cardiovascular fitness during and post HA in temperate conditions [40,104,127].

Aside from the relationship between HR and RPE, the current study did not observe any meaningful relationship between the change in PV and HR metrics utilized during submaximal testing (HR_{ex}, HRR). This finding contrasts with an earlier study noting an improvement in HR_{ex} within HA in soccer players during 5'-5'-submaximal running with heat adaptation and this response was moderately correlated [$r=-0.52$ (-0.64; -0.38)] to the increase in PV (~6%) [22]. The lack of an observed relationship between PV and HR_{ex} in the current study was likely attributed to the large fluctuation in PV throughout training due to inconsistent timing of measuring PV and submaximal HR_{ex}. Specifically, in the current study PV was measured in the morning while HR_{ex} was observed during submaximal testing prior to each training session. Additionally, PV expansion may not be the only indication of an improvement in HR_{ex}. Reductions in resting internal core body temperature in the heat have been shown to be directly related to exercise intensity and could contribute to the improvements in HR_{ex} aside from a PV expansion [134]. While it is possible that PV expansion played a role in the positive aerobic response in both temperate and hot conditions, further research is needed to justify the use of submaximal HR_{ex} as a non-invasive marker for tracking PV induction and decay.

The optimal time to engage in HA without having to re-acclimate before a competition is still uncertain and requires further investigation. Previous studies have recommended HA be utilized one week prior to competition [125], however, current findings from submaximal testing suggest that HA performed two weeks before competition will ensure athletes have sufficiently gained adaptation while also avoiding heat related fatigue.

5.4 The effect of HA on small-sided soccer match-running performance and heart rate response

Small-sided soccer games have been validated as an effective test for replicating match play as the smaller field dimensions allow for continued ball contact and short intermittent running in order to attain a similar maximal HR (80-90% HR_{max}) as in competitive matches [38,48]. Within the current study, the aim of using the 4v4SSG on

the first and last day of Phase 2 (Day 9 and 14) as a measure of soccer performance was based on its highly replicable demand to competitive match performance specific to movement (acceleration, deceleration), physiological intensity, as well as technical skills [55]. However, even though the SSG is a well-controlled method of replicating the demands of a match, the field dimensions within a 4v4SSG make it challenging for players to attain maximal or near maximal high-speed running.

As outlined in Figure 5, the current study suggests that the (i) 4v4SSG was an effective measure of heat stress on soccer performance and (ii) five days (sessions) of training in a hot condition (Figure 1, Phase 2) induced meaningful heat adaptation that improved soccer-specific GPS-derived metrics and associated HR response and recovery. 4v4SSG performance showed an improvement in both of the HR-indices noted by the large increase in HRR (5.7%) and decrease in HRex (-3.5bpm) across the four games pre and post Phase 2. Average intensity in terms of M/min was lower across the four games on the final day (-4.6%), however, the combined accelerations, decelerations and changes in direction (high IMA) were substantially higher (20.1%). We acknowledge that there was a reduction in M/min in the 4v4SSG from Day 9 to Day 14, however, this was likely due to training fatigue as the final 4v4SSG was performed on the last day of the camp in the heat (Figure 1, Day 14) and players had already been under a significant training load from previous training days as highlighted in Figure 2. Additionally, M/min consists of movement at all speeds, however, when playing on a smaller field, players typically do not cover as much distance at lower speeds, therefore the accumulated total of M/min is lower [8]. In a SSG there is also less opportunity for athletes to cover M/min by nature (small field size), however, it is more likely that they would engage in a larger number of IMA directional events [55,63]. As a result, players in the current study had less ability to cover more M/min but were able to move more explosively and make more directional changes in order to achieve more ball interactions [48]. Therefore, a positive increase in high IMA in the 4v4SSG is more relevant to a positive change in soccer performance as compared to M/min.

Utilizing a SSG with GPS metrics acts as a valid assessment of heat stress within HA-protocols by quantifying a player's ability to complete repeated sprints with limited rest while also measuring the number of acceleration/decelerations events. Current

findings add to previous findings from Aughey et al. [7] by utilizing GPS monitoring in addition to HR outcomes (HR_{ex}, HRR) within field-based HA to interpret the impact of heat stress on soccer performance. Therefore, SSGs provide a valid replication of the physical demands of competitive match play and allow for meaningful interpretation of soccer match performance, especially when an environmental factor such as heat is added to training [55].

5.5 Observation of core temperature in a competitive soccer match in the heat

Soccer is a multi-directional and dynamic sport that relies on repeated high-intensity actions during a match [114]. The short intermittent bouts of intense activity seen within a match translate into an average work intensity of ~80% of maximum oxygen uptake (VO_{2Max}) at about 80-90% of HR_{max} [50]. Maintenance of this high work-rate during a match in a hot environment has been reflected by a higher exercising T_c [7,50,124] which likely arises due to the added thermal strain from both metabolic and environmental heat [160]. Understanding the T_c response of elite athletes in a competitive setting is critical for coaches as it acts as an important physiological marker for exhaustion from heat strain [134]. While the cut-off for exertional hyperthermia set by the American College of Sports Medicine is reported as a temperature above 40°C, five athletes were able to achieve a T_c of ≥39.5°C during a friendly international match in mild heat (22.5°C) (Figure 1, Day 6 and Day 7) and one athlete reached 40°C without any indication of heat exhaustion [7]. Previous literature has suggested that elite athletes performing at higher intensity exercise may be able to tolerate a higher T_c. [149], however, more evidence within this population is needed. A previous HA study of AFL athletes also observed an exercising T_c greater than 40°C during matches in both cool (~17.5°C) and hot (~27.5°C) conditions with no athletes indicating symptoms of exertional heat stress [7]. Additionally, Pugh and colleagues [135] observed a T_c rectal response between 39.5°C and 40°C in ten runners post marathon and seven runners with T_c >40°C while Racinais and colleagues [140] recently showed that trained cyclists were able to reach a T_c of 40.1-40.2°C at the end of HA during a cycling TT (37°C). While the current study supports Periard et al. [127], more evidence regarding T_c in competitive matches is required to better understand the exertional cut off for heat

exhaustion in elite team sport players. Specifically, if sport players are able to achieve a T_c greater than 40°C in competitive matches, the recommended clamping of a T_c threshold of 38.5°C for HA induction during heat training may not provide enough of a stimulus for elite athletes [7, 127].

Chapter 6 Conclusion

6.1 Summary

The challenge of exercising in extreme heat is thoroughly established [30] and poses significant physiological difficulties for international-level female soccer players preparing for competition in these environments. Many competitions are held in hot climates [124], therefore, a time and cost-efficient heat protocol that enables players to sustain the same level performance as in temperate conditions is extremely beneficial. In support of previous literature, the current study presents results that indicate that two-phases of field based HA, in a mild (6 sessions of pre-HA, 22.5°C) and hot (5 sessions of HA, 34.5°C) condition is beneficial for elite female team sport players. In support of previous findings, it is recommended to engage in HA over two weeks at approx. 90-100 minutes/day to induce complete adaptation. However, shorter-term protocols (~5 days) may be used within congested training schedules, especially when the risk of heat stress before competition is a concern. Additionally, a longer duration training session in the heat combined with high-intensity modality-specific training bouts will be more beneficial for inducing complete HA rather than shorter training sessions, which have been shown to induce a lesser cardiovascular response.

The current findings have added to the previous literature by providing new insight for elite female soccer players with regards to their physiological, performance, and perceptual HA response. The increase in PV from Vancouver baseline (ie. 2.3%) had a positive relationship to heat load suggesting that an expanded PV is likely attributed to a longer duration of training in the heat at an elevated T_c versus overall ΔT_c within a single session.

HA training positively impacted athletic performance not only in a mild condition during the submaximal test but during the 4v4SSG within the hot weather training period in Cancun as well. This was indicated by the reduction in HR_{ex} and increase in HRR during 5'-1' submaximal running in a temperate condition post-HA and an increase in HRR and high IMA during the 4v4SSG on the final training day in the heat. The HR metrics (HR_{ex}, HRR) utilized in the current study are simple, non-

invasive measures that can show sensitivity to HA and track changes in aerobic fitness. The greatest aerobic improvement within the current study was not complete until eleven days post-HA in a temperate condition (Figure 4, Day 25). Therefore, it is worthwhile to monitor these metrics up to two weeks post-HA in order to effectively identify the smallest worthwhile change in performance. These measures are valuable to coaches who may need to adjust exercise intensity based on HR responses to specific workouts in order to create a larger training stimulus as well as to prevent overtraining in the heat.

Additionally, monitoring of GPS-derived metrics (high IMA, M/min, high intensity efforts) throughout HA may allow coaches to quantify and specify the intensity required to individually monitor training intensity for each player. Specifically, longitudinal collection of these external load metrics within HA may be utilized as a method to ensure that players are reaching the training target required to trigger the adaptive response while also avoiding heat stress and overexertion. The current study supports the use of field-based HA amongst international female team sport players. Individualized tracking of internal and external load may be used as a method to improve heat tolerance, thermal comfort, and soccer-specific performance while also reducing the risk of heat illness and injury.

6.2 Limitations

Due to the nature of this training camp occurring only two weeks prior to the FIFA World Cup, we were unable to utilize an appropriate control group. Therefore, the observed ergogenic effect of HA in the current study may be confounded by the effects of training load (intensity x duration) [133], travel fatigue, changes in sleep, recovery, diet, and motivation especially on the final day of training camp when players were more likely to be fatigued. It is also possible that additional physiological metrics including; sweat rate, sweat sodium concentration (sweat [Na⁺]), and plasma protein concentration may have impacted the extent of PV expansion [120]. Additionally, the pre-planned nature of the camp made it challenging to control for the players' living conditions. Players were also housed in air-conditioned rooms throughout day when they were not training. This may have impacted the extent of PV response as certain

athletes may have spent a greater amount of time indoors with air conditioning. Furthermore, it is likely that some athletes may have been partially heat-acclimated prior to the start of the camp, as we could not control the T_{ambient} that the athletes were living in prior to the camp. Another limitation was the inability to control for the effect of the menstrual phase as this has previously shown to affect the T_c response in females [60,102].

In terms of the sudomotor adaptation, we did attempt to observe the SR response, however, we could not accurately account for all fluid consumed during each session as we did not want to disrupt the team's training environment. There are also a number of factors that can impact the magnitude of increase in PV including (i) the acclimatization day when PV is measured; (ii) the type of method used to measure PV; (iii) the hydration state when measured; (iv) T_{skin} at the time of measure and (v) fluctuation in training load [131,147]. While there was a positive increase in PV overall, the lack of consistent elevation of T_c at a target threshold on a daily basis during Phase 2 training likely limited the increase in total body water [131]. Additionally, due to the highly controlled measurement procedure for obtaining PV, it can be difficult to obtain an accurate measure within a field setting. The use of open, ambient conditions, rather than laboratory-controlled heat chambers likely limited PV expansion. Specifically, the literature has recommended not to perform strenuous exercise the night prior to PV measurement as this can cause fluctuation in PV levels [104]. However, in the current study PV measurement was scheduled around the time of training and it was not possible for athletes to adjust their schedules to ensure PV was consistently measured at the same time of day.

It is also possible that athlete hydration status within each training session may have impacted the extent of PV expansion as hydration level can cause fluid shifts between the circulatory system and interstitial spaces during exercise in the heat [147]. While we were able to use a consistent sampling site while measuring PV, we were unable to account for the change in fluid intake throughout both phases of acclimatization. Furthermore, Hct, and Hb are highly responsive to changes in plasma osmolality; therefore, the inability to effectively monitor plasma osmolality via fluid intake may have resulted in a larger variation of Hct during the training camp, which in

turn would have largely impacted the accuracy of blood sampling and the overall change in PV [93,163]. This was observed in a previous analysis of results comparing measurements of Hb and Hct before and after an exercise bout in the heat and found that even a 10 mOsm·kg⁻¹ increase in plasma osmolality resulted in a 0.8-unit reduction in Hct, corresponding to a difference of 1.6% in the calculated change in PV [163].

Measurement variability also has an effect on the PV outcome [2]. There are also several methodological limitations to using the Dill and Costill method [15,49,93,108] as opposed to the CO rebreathe method which has proven to be more effective in reporting consistent and accurate values of PV [152]. Specifically, the Dill and Costill method determines the percentage change in PV from a baseline (assumed to be 100% i.e. $BV = RCV + PV$) [2] and does not provide an absolute measure of PV as seen in the CO rebreathe method [152]. In comparison, the CO rebreathe protocol has shown the greatest validity and reliability in determining BV and PV [21,66,68]. While it would have been preferential to use the CO rebreathe method within both phases of the training camp, this method requires a longer protocol (~30-40 minutes per athlete) and we wanted to limit the time taken out of an athlete's schedule prior to competition [24]. Therefore, the CO rebreathe technique was only utilized to obtain an absolute baseline for which the percentage change in PV was determined throughout the remainder of the camp.

The challenges associated with obtaining an accurate measure of PV highlight the importance of obtaining additional physiological data within HA including SR or sweat sodium concentration [Na⁺] in order to provide a better overall assessment with regards to the magnitude of HA.

6.3 Practical Applications

The present research provides insight into a team's ability to adapt to the heat with regards to both physiological and performance variables. Consistent with findings from a recent review, two-weeks of field-based HA utilizing soccer-specific training has demonstrated ergogenic effects in both temperate and hot conditions [40]. Current findings support using HA at least two-weeks prior to competition in hot weather to induce positive adaptations. Training sessions in the heat should be no less than 90-

minutes and should include some high intensity threshold training (15-20 minutes) where T_c is elevated at or above 38.5°C.

The current findings support the use of daily, on-field training load monitoring of GPS external load (high IMA, M/min), T_c (ΔT_c , T_{c-end}), AUC (baseline subtracted), and Session RPE to monitor intensity within HA. Data specific to an athletes' T_c response in combination with external load metrics (high IMA, M/min) is novel and provides insight into the intensity of a HA training camp in order to avoid heat exhaustion. The 5'-1' submaximal running test in combination with HR monitoring (HR_{ex}, HRR) and perceptual workload (RPE) are effective tools to observe the extent of cardiovascular adaptation throughout and post HA. It is also recommended that athletes perform a specific measure of performance that closely resembles what the athletes would perform in competition both before and after HA training. While the current study utilized a 4v4SSG, it is likely more effective to utilize eight players over a larger field-dimension (8v8SSG) and to use 6-8 minute small-sided games instead of two-minute games. The use of a slightly larger field and a longer game duration would be a more realistic measure of soccer performance especially when observing GPS metrics such as M/min. Coaches are recommended to engage in simultaneous monitoring of the changes in physiological and performance metrics during HA and up to two-weeks post HA. Monitoring the extent of adaptation will provide a better overall picture of the induction and decay of heat adaptation and how physiological changes within the body may impact sport-specific performance.

6.4 Future Directions

Elite female soccer players are under-investigated and there is a need for further research, particularly in the role that hormones may play in the adaptive response to HA [110]. Additionally, studies observing female team sport athletes should consider measuring not only cardiovascular change (e.g. PV) but thermoregulatory adaptation as well (SR, sweat concentration). In terms of performance, there is no current evidence of neuromuscular fatigue monitoring in combination with HR response during HA which could potentially be used as a secondary method to monitor the fatigue induced by environmental heat stress.

While the current study highlights the importance of monitoring HA up to two-weeks out of the heat, the evidence specifying the rate of decay of HA is limited to controlled hyperthermia in untrained participants or endurance-based sports [51,58]. With continued observation of submaximal testing in combination with HR-monitoring, coaches can understand when the optimal time is for athletes to heat acclimate in order to maximize the cardiovascular response in competition. It is recommended that coaches who are using HA prior to competition should collect various haematological, performance, and perceptual data from athletes prior to a heat training camp. This will provide information with regards to an athlete's individualized adaptation potential as well as how they may react to heat stress.

Team sports performing field-based HA should utilize not only internal load monitoring (HR_{ex}, HRR) as markers of adaptation, but GPS-metrics (high IMA, M/min) and session RPE. The combination of internal and external load monitoring within field-based HA is non-invasive and may be used to track a team's change in aerobic fitness and sport-specific performance. These metrics have the potential to be used as a method to fine-tune training intensity within HA. Specifically, it is possible that GPS-derived metrics utilized within field-based HA could potentially be used to set training intensity and monitor heat stress as opposed to the use of costly T_c sensors. However, more evidence with regards to the relationship between these metrics (T_c and high IMA, M/min) is required before justifying their use as a supplement to T_c sensors.

Another area of investigation within HA is the relationship between changes in HR response in submaximal testing (HR_{ex}, HRR) and PV as these metrics could be utilized as effective markers of cardiovascular adaptation. There is also a need to gain more knowledge regarding the sex-based differences in heat stress and physiological adaptation from HA between elite athletes in order develop more individualized and effective protocols [3,121].

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Appendices

Appendix A: Laboratory Procedures

A1: The Carbon Monoxide (CO) Rebreathing Protocol and Venipuncture

Height and weight are measured first upon arrival to the laboratory. After weigh-in, there is a 15-minute period of sitting quietly in a chair to help stabilize shifts in plasma volume. During this time, the participant will receive familiarization with the breathing protocol. Weight is then used to calculate the CO dosage, which is approximately 0.8ml/kg for females. Once the syringe is filled, another lab technician also checks the amount. The filling procedure takes approximately 3-5 minutes. The alarm limits on the portable CO monitor are set at 35ppm (lower limit) and 55ppm higher limit. The following conditions were maintained throughout laboratory testing; ambient temperature 22-25°C, relative humidity 30-50%, average CO₂ content in atmospheric air, and barometric pressure ~755mmHg.

The CO-Rebreathe Protocol is split into three stages:

1. **Baseline Blood Draw:** After 15 minutes of sitting quietly, the site of blood draw is selected (one of the veins in the inner arm at the elbow) and the area is disinfected. Using sterile technique, approximately 1.0ml of blood will be drawn and analyzed.
2. **Breathing Protocol:** Two 3L bags of pure O₂ as well as the CO bolus are attached to the closed- system spirometer. The athlete will wear a nose-clip to prevent any air from escaping out their nose. The athlete must fully exhale the air and completely empty your lungs, before placing lips firmly around the disposable mouthpiece attached to the spirometer ensuring a tight seal. When the athlete gives us the signal that they are ready, the O₂ valve is opened and they should begin a slow maximal inhalation. The CO is injected into the spirometer during this time. At the end of full inhalation, they will signal again, the clock starts while they hold their breath for 10sec, after which time they will be instructed to breathe easy for the final 1min: 50sec. At 1:50 (min:sec), they will be instructed to take one last inhalation & begin a maximal exhalation completely emptying the lungs. The spirometer is closed and they will now be finished the breathing protocol (total time is 2:00 min). The athlete will then remain sitting quietly for 5 more minutes until the post breathing blood draw. The stopwatch keeps running.
3. **Post-Breathing Venipuncture:** At 6:30 (min:sec), the band aid is removed, the site of blood draw is located and disinfected. Using a sterile technique, approximately 1.0mL of blood is drawn and analyzed immediately for Hematocrit (Hct) using the Radiometer ABL80 CO-Oximeter and blood gas analyzer. The wash-out period for CO is 3-4 hours for half removal and up to 12 hours for full removal from the system.

A2: ABL80 FLEX CO-Oximeter and Blood Gas Analyzer

The ABL80 Flex CO-Oximeter is a portable, automated system that measures pH, blood gases, electrolytes, glucose, and oximetry in whole blood. The system is intended for use by trained technologists, nurses, physicians and therapists. It is intended for use in a laboratory environment, near patient or point-of-care setting. Calibration: The ABL80 Flex CO-Oximeter is equipped with the QC3 automatic quality control system. This provides a calibration process that measures three solutions with different analyte concentrations. These three measured values are used in different combinations of two points each to establish three two point calibration lines for each analyte. One calibration line is consistently used to report sample results, with the other two calibration lines used to evaluate system linearity.

A3: Calculations of Dill and Costill Percentage Change in Plasma Volume

In accordance with Dill and Costill [49], the subscripts for “baseline” (b) and “after” (a) heat acclimatization training are shown below. Values specified as (a) “after” represent a test sample following baseline testing on either Day 1 (Pre Phase 1), 6 or 7 (Post Phase 1) or 16 (Post Phase 2).

i) Blood volume (BV): $BV_b = [100\% \text{ (assumed)}]$; $BV_a = [BV_b * (Hb_b / Hb_a)]$

ii) Red cell volume (RBCV): $RBCV_b = [BV_b * (Hct_b / 100)]$; $RBCV_a = [BV_a * (Hct_a / 100)]$

iii) Plasma volume (PV): $PV_b = [BV_b - CV_b]$; $PV_a = [BV_a - CV_a]$

Percent changes

$$BV (\%) = [100 * (BV_a - BV_b) / BV_b]$$

$$CV (\%) = [100 * (CV_a - CV_b) / CV_b]$$

$$PV (\%) = [100 * (PV_a - PV_b) / PV_b]$$

A4: Calculation of Z-Score

Z-score represents the number of standard deviations (+1; above, or -1; below) an individual's normative distribution. A Z-score for Hydration (USG) was determined for each PV measurement day within both phases of heat acclimatization training as follows:

Player Z Score = [PlayerX USG on day x] – [PlayerX USGmean] / [PlayerX SD]

Team Z Score= (Mean of all Player Z scores on day x)

The mean USG and standard deviation (SD) of each player for each PV test day (day x) was determined from previous USG data collected on all team members from October 2014-February 2015. Team Average USG Z-score for a PV test day was calculated as mean of all Z-scores from all players for that particular day.

Appendix B Field Monitoring

B1 Core Temperature Sensor and Telemetric Monitoring System

The VitalSense[®] telemetric physiological monitoring system (Mini Mitter Co., Inc., Bend, OR, USA) consists of a receiver and a thermistor (thermometer) based ingestible capsule that is used for core body temperature measurement. The single-use intestinal thermistor is biocompatible and less than 1 cm thick and 2.3 cm long contains a crystal quartz oscillator that transmits a low frequency radio wave to an external data logger (I.e. VitalSense Monitor). Specifically, in the VitalSense telemetric monitoring system, each thermistor sensor has an identification number to avoid noise among different sensors in the same participant or among those in close proximity. The sensor is activated prior to ingestion and requires four to eight hours of gastric transit time before the start of a training session to obtain accurate measurement. The pill stays in the gastrointestinal tract on average for 40 hours (range 12-93 hours, before being excreted).

B2 Catapult Global Position Monitoring System

All GPS-external load metrics collected from the Catapult GPS System as shown below, (10-Hz, S4 minimaxX, Catapult Innovations, Australia), and were downloaded and analyzed using a 5.1 Catapult Sprint software program following each training session.



Catapult Minimax S4 GPS Receiving Tower.



Catapult Minimax Device (Via Catapult Sprint 5.1 Manual). This device was worn on the player's back in a Catapult training harness underneath the uniform. The S4-GPS device can monitor; total distance covered, meters per minute (m/min), number of high-speed runs (>16.5km/h), and high IMA or accelerations/ deceleration/change in direction (>2.5m/s²).

Appendix C Aerobic and Functional Performance Testing

C1 5'-1' Submaximal Running Test and Heart Rate Response

The 5'-1' Submaximal running test consists of a 40m shuttle at 12km/h for 5 minutes with a 1-minute recovery sitting post-test. This is approximately 1 km or 25 shuttle runs. This test is effective at creating a steady heart rate response in the last 30 seconds of the 5-minute running period and has been incorporated into the Women's National Team weekly training regime as a method to monitor the changes in fitness levels and fatigue/ readiness. Heart Rate Response to Exercise (HRex) and post-exercise Heart Rate Recovery (HRR) are assessed using a Polar Team 2 Heart Rate system (1.4.1, Polar Electro Oy, Kempele, Finland) as well as player load using Catapult GPS system and rate of perceived exertion using a 10 point Borg scale. The two measures of HR response:

Exercising HR (HRex): Mean HR during the last 30 s of the 5 min running period. Post-exercise HR recovery (HRR): The absolute difference between HRex and the HR recorded after the 1 minute of recovery (mean HR of the last 5 sec of recovery). These metrics were determined through post-training analysis using the Polar Team 2 Software.

C2 4 x 2-minute, 4-a-side, small-sided soccer game (4V4SSG)

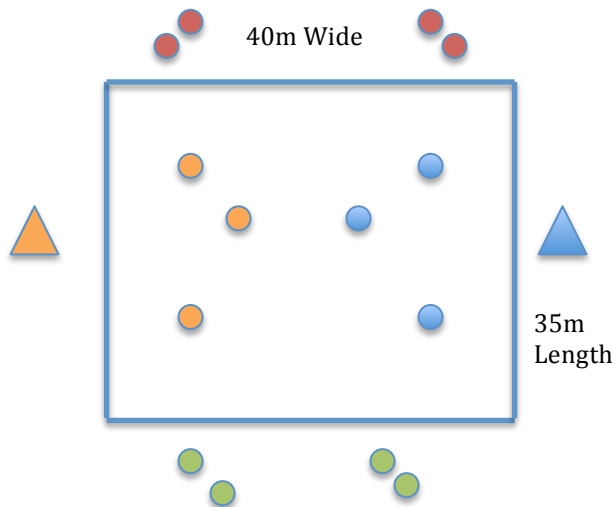


Diagram of the 4x2 minute, four-a-side soccer game. Circles represent Groups 1-4; players separated into four groups of four with two groups playing at a time. Triangles represent the goalkeeper utilized in each game. All players completed a total of four two-minute small-sided games.

HRex in SSG (bpm)= Average HR from each of the four SSG (not including the 2-minute rest period). HRex was used in the calculation of HRR below.

$$\text{HRR in SSG (\%)} = \frac{[\text{Average HRex in the SSG}] - [\text{Average HR of 90s post each SSG}]}{[\text{Average HRex in SSG}]} * 100$$

External load indicators were normalized to the 2-minute SSG duration and averaged from game one to four to obtain an average game intensity as well as to functionally characterize the effect of pre to post-heat acclimatization on physical output in a live soccer game setting. These metrics included high IMA/min (constitutes all explosive accelerations, decelerations, and changes in direction) as well as meters/min.