HEAT ACCLIMATION FOR FEMALE ENDURANCE PERFORMANCE IN HOT AND COOL CONDITIONS

by

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Abstract

This study compared the effects of heat acclimation (HA) on female endurance performance following short-term (4-5 days; STHA) plus long-term (9-10 days; LTHA) heat acclimation, as well as examined HA's ergogenic potential in females. Seven female recreational endurance athletes completed 10-days isothermic HA (40°C, 30%RH), and a 15-minute self-paced time trial (TT) in hot (HTT; 35°C, 30%RH) and cool conditions (CTT; 15°C, 30%RH), before (1) and after STHA (2) plus LTHA (3). Following LTHA, distance cycled (hot: +260±150 m (3.3%), P=0.017; cool: +210±150 m, (2.4%), P=0.038) and mean power output (hot: +10.4±7.4 W (5.5%) P=0.015; cool: +10.7±7.7 W (6.8%) P= 0.040) were increased. Area under the curve (AUC) differences were observed in power output across CTT1 vs. CTT3 (P=0.034) and HTT1 vs. HTT3 (P=0.016). Body mass loss (+2.6±0.5% to 3.2±0.5%; P=0.034), sweat rate relative to body surface area (+613±105 g/h/m^2 to 772±114 g/h/m^2; P=0.018) and active sweat glands/sq. inch (395±135 to 494±157; P=0.016) increased following LTHA. T_{re} was lower (AUC; P=0.036) during CTT3 vs. CTT1. Other thermoregulatory, cardiovascular, and blood lactate measures were not different between TTs (P>0.05). No significant performance or physiological improvements were observed following STHA (P>0.05). The lack of physiological or performance effect following STHA indicates that females require LTHA to augment performance in the heat. Meaningful performance improvements in cool conditions further support HA's ergogenic potential.

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ANOVA	Analysis of variance
AUC	Area under the curve
BM	Body mass
HA	Heat acclimation
Hb	Haemoglobin
Hct	Haematocrit
HR	Heart rate
HTT	Hot time trial
LTHA	Long-term heat acclimation
CTT	Cool time trial
Osm_u	Urine osmolality
RH	Relative humidity
RPE	Rating of perceived exertion
SD	Standard deviation
SR_{BSA}	Sweat rate relative to body surface area
STHA	Short-term heat acclimation
T_{rec}	Rectal temperature
T_{sk}	Skin temperature
TT	Time trial
VO_{2max}	Maximal oxygen consumption

1. Introduction

Since sport's beginning, and as it gained popularity around the world, there has always been an imbalance in the way that men's and women's sports are viewed. For example, throughout the nineteenth century, German men were encouraged to partake in sport and exercise for reasons of military preparedness and as a show of patriotism, while female 'sport' was limited to dances and exercises that would have an aesthetic benefit, as well as better prepare them for healthy childbearing (Hartmann-Tews & Pfister, 2003). Into the 20th century, the passing of the 1972 U.S. legislation called *Title IX* became an event of unforeseen significance for women's sport. *Title IX* banned discrimination on the basis of sex in any federally funded education program or activity. While this legislation was not originally intended to cover sport and athletics, it was interpreted to cover all facets of education and it soon became apparent that discrimination in the areas of sport and athletics would also be banned (Ware, 2013). This modification was a springboard for women's sport, with the USA seeing a 600% increase in girls' sport participation in the six years following the addition of the *Title IX* legislature (Kaestner & Xu, 2010). This trend of female participation has continued upwards, as reflected in reports of the Women's Sports Foundation showing that 41.2% of high school-aged females in the USA were participating in athletics and sport in 2011 (Ware, 2013). The rise in participation has been a factor in driving a similar increase in the success of female athletes, both at the university level, where 46% of intercollegiate scholarships are being awarded to women (Acosta & Carpenter, 2012), and on the world stage. Indeed, 45% of all competitors at the 2016 Summer Olympics in Rio de Janeiro were women; a substantial increase from the high of 29% reached in 1992 in Barcelona (International Olympic Committee, 2016). Although there were slightly fewer female competitors, many countries actually saw women taking home more medals from their events

in Rio than their male counterparts. American women won 61 total medals (compared to 55 won by males), as well as the majority of gold medals (27 of total 46). China, Canada, Netherlands, Russia, Jamaica and New Zealand also had more female medal winners than male. It is evident from examples like these Olympic results that women's sport has now reached a level that warrants an equal amount of attention, yet as more women begin not only to participate, but excel in the most elite level of sport, we are still left with a great discrepancy in funding, salary, leadership positions, facilities, and media coverage (Schull, Shaw & Kihl, 2013; Leberman & Shaw 2015; Lapchick, 2012; Hartmann-Tews & Pfister, 2003; Fink, 2015), as well as in the amount of scientific research focused on female performance (Costello et al., 2014).

Consequently, many exercise protocols and training recommendations are based on research that has been done almost exclusively on males, despite known physiological, biomechanical, and endocrinological differences between sexes (Costello, 2014). Given these known sex differences, using male-derived training recommendations to predict performance outcomes for female athletes is problematic. This study aimed to close the gap in the knowledge of performance outcomes of training interventions for female athletes, specifically when reacting and adapting to heat stimulus, better known as heat acclimation. Although heat acclimation in male athletes has been studied for decades, very few studies to date have examined female cohorts or sex differences in heat acclimation, and those that have were with an unmatched heat stimulus (Jay & Cramer, 2015) or have not structured their protocol with the aim to assess female endurance athlete performance outcomes. Additionally, as novel strategies exploring the ergogenic potential of heat acclimation are investigated, female cohorts have not been considered. As a result, there remains a lack of thorough understanding in the area of relevant heat acclimation performance outcomes for the female athlete. The

overall objective of this project is to determine female performance outcomes in hot and cool conditions following short- and long-term isothermic acclimation protocols (rectal temperature (T_{re}) aimed to be maintained at ~38.5°C). The specific aims were: 1) to determine if females can acquire meaningful thermoregulatory, cardiovascular and fluid regulatory adaptations following short-term (4-5 day) heat acclimation protocols or if long- term (9-10-day) heat acclimation protocols are required, and 2) examine how these adaptations translate to performance improvements in both hot and cool conditions.

2. Literature review: Heat Acclimation for the Female Athlete

The aim of this literature review is to provide the necessary background information to inform the reader of heat acclimation knowledge for females thus far, to highlight the areas in which the current research and recommendations are lacking, and to explore possible avenues to close these knowledge gaps and provide recommendations that are relevant and applicable for the female population. To do this, this review will first provide a basic summary of heat acclimation and its classic adaptations, followed by information on human thermoregulation that is pivotal to understanding how heat acclimation is achieved. Next, meaningful performance outcomes, the primary focus of the study, will be discussed by quantifying performance detriments experienced while under acute heat stress and how these can be minimised through heat acclimation. Novel strategies which are emerging to optimise adaptations will be examined, including short-term heat acclimation protocols and using heat acclimation as an ergogenic aid. Finally, the possible mechanisms facilitating these adaptations will be explained in further detail, predominantly addressing the variables measured in the study. Other possible mechanisms will be briefly recognised, however detailed description of these alternative mechanisms is beyond the scope of this review. The application of heat acclimation for females is central to the purpose of the study and will therefore be considered throughout.

2.1 What is Heat Acclimation?

Heat acclimation is a well-recognised means of improving temperature regulation and heat tolerance in occupational (i.e., military, firefighters) and athletic settings. Human adaptation to the heat is facilitated in two forms: acclimation and acclimatization. Acclimation is a laboratory-based method, often performed in an environmental chamber where conditions

such as temperature, humidity, and altitude can be manipulated and controlled. Acclimatization takes place in natural field conditions, in a hot environment where these factors are not controlled. During chronic heat stress sessions, temperature ranges between 35-49°C and relative humidity (RH) ranges between 20-79% (Tebeck et al., 2017). Active acclimation (exercise combined with hot conditions) has been observed to induce the greatest adaptations, as it drives body temperature increases (core and skin) and provokes sweat loss (Buono et al., 2009). This combination of stressors also stimulates a unique upregulation of genes which cannot be stimulated with exercise or passive heat individually (Kodesh et al., 2011).

Heat acclimation can be achieved using a variety of protocols. As with any intervention, optimizing frequency, duration, intensity, and type of protocol will yield the greatest adaptations (Sawka et al., 1993). Typically, a protocol consists of daily acclimation sessions (Gill et al., 2001) of approximately 90-minutes of submaximal exercise under heat stress (Chalmers et al., 2014, Guy et al., 2015) to prepare for endurance events in the heat. However, shorter session durations may be effective for team sport settings if intensity is high enough to produce a steep and immediate rise in core temperatures (i.e. HIIT training; Sunderland et al., 2008). Traditional "long-term" heat acclimation (LTHA) protocols last ~10-14 days (Garrett et al., 2011).

The intensity of the workload prescribed during the acclimation protocols may be of a fixed or varied intensity. When workload is fixed, it will be either relative to an athlete's VO_{2max} or at a rate of metabolic heat production relative to their body mass (Watts/kg protocol). The Watts/kg protocol developed by Cramer and Jay (2014), allows mechanisms of adaptation to be compared independent of size differences in participants by matching the stimulus in the form of metabolic heat production, which has often confounded past sex-

differences studies (Gagnon et al., 2008). Alternatively, workload can be manipulated to induce a rapid rise in core temperature to ~38.5°C, which is maintained for the duration of the session. This method, called an "isothermic" method, prioritizes maximal work done within the temperature zone commonly regarded to be "safe", while ensuring heat strain throughout the duration of the protocol (Taylor 2000). As participants begin to adapt to the heat, the intensity and impact of the heat stimulus begins to decrease. Therefore, protocols can be modified to progress with the participants by either increasing the fixed workload (%VO_{2max}, Watts/kg) or by raising the core temperature target for the session. This is the "progressive" method. Interestingly, Gibson et al., (2015a, b) observed no significant difference in markers of adaptation or in cellular thermotolerance response between fixed, isothermic or progressive isothermic 10-day protocols in male cohorts. All methods induce the classic markers of acclimation: decreased heart rate (HR) and temperature (at rest and during exercise), increased sweat rate, and improved aerobic performance in hot conditions (Sawka et al., 2011).

An alternative to active acclimation is sauna bathing following temperate endurance exercise, which may be effective in inducing heat acclimation and performance improvements, as it prolongs the elevated core temperatures that have been increased by exercise (Scoon et al., 2007; Zurawlew et al., 2016) and drives the fluid regulatory stimulus needed to significantly expand plasma volume (Stanley et al., 2015). In contrast, Creasy et al. (2003) observed that sauna bathing may be detrimental to performance during a 2,000 m rowing trial.

While active protocols, and possibly passive heating, seem to be sufficient to drive adaptations in males, modifications to duration and/or intensity may be needed to induce

similar effects for females (Sunderland et al., 2008; Mee et al., 2015, 2017), as described below.

2.2 Human Thermoregulation

2.2.1 Heat storage and exchange. The aim of both heat acclimation and acclimatization is to improve an athlete's ability to dissipate heat. The body stores and dissipates heat using four main pathways (Cheung, 2010):

Radiation: heat is exchanged between the body and surrounding objects via electromagnetic waves. Heat felt from the rays of the sun is a form of radiation.

Conduction: heat is exchanged when the body comes in contact with a stationary object and there is a temperature gradient between them.

Convection: heat exchange between the body to a moving substance, which allows heat to be moved away from the body immediately, i.e. air or water.

Evaporation: heat is dissipated from the body when water on the skin is vaporized. The body's ability to lose heat via evaporation is dependent on both the temperature and water carrying capacity or humidity of the surrounding air.

Therefore, Heat Exchange = Radiation + Conduction + Convection + Evaporation

Each pathway can be represented by positive or negative values, except for evaporation, which is always a form of heat loss, not heat gain. A positive value indicates heat loss from the body to the environment. When environmental conditions become similar to body temperatures, the body's ability to lose heat via conduction, convection, and radiation (dry heat exchange) are minimized (Hardy & DuBois, 1938). The body must then rely on its

ability to lose heat through evaporation. Sex is an important factor of thermoregulation in hot conditions, as females have reduced levels of sudomotor activity and sweat gland output in comparison to males at the highest levels of required heat loss (Gagnon & Kenny, 2012; Ichinose-Kuwahara et al., 2010), which limits females' capacity for evaporative heat loss in comparison to males (Gagnon & Kenny, 2012).

Heat exchange is not only influenced by the surrounding environment, but also by how much heat is stored in the body itself. Approximately 80% of the energy expended by skeletal muscles is released as heat, which accounts for between 70% (at rest) and up to 90% (during exercise) of total metabolic heat production (Cheung, 2010). Additionally, external work done affects heat storage. Active, concentric work is accounted for with a negative value, while passive (i.e. a contraction facilitated by an external body), eccentric work is accounted for with a positive value.

Therefore, Heat Storage = (Radiation + Conduction + Convection + Evaporation) + (Metabolic Heat Production – Work)

If heat is balanced, the equation will be equal to zero, and body temperatures will remain the same. This is because the amount of heat stress is compensable by the body. If the equation is positive, and the body cannot adequately dissipate metabolic heat, body temperatures will rise. This is known as uncompensable heat stress. The body's ability to dissipate metabolic heat can be improved with heat acclimation, especially by improving evaporative heat loss capacity, a factor in which sex-differences may have an effect.

2.2.2 Impact of female sex hormones, menstrual cycle phase, and hormonal contraceptives on thermoregulation. The menstrual cycle and its effects on heat stress and heat acclimation are not fully understood, so it is common to test subjects with a regular menstrual cycle during the "low hormone" follicular phase (Kolka et al., 1994). However, this makes it difficult to accurately apply these results when protocols stretch across different phases of the menstrual cycle, the participants have an irregular cycle (or commonly for endurance athletes, are amenorrheic; Bennell et al., 1997, Dale et al., 1979), or the participants are using a form of hormonal contraception (monophasic or triphasic oral contraceptive pill, contraceptive patch, injectable birth control, implantable rods, intrauterine devices [IUDs; copper or Levonorgestrel], or vaginal rings).

Internal core temperature may vary between 0.5-0.8°C over the course of the menstrual cycle (Baker & Driver, 2007; Stephenson & Kolka, 1999). As oestrogen rises during the follicular phase, there is lowering of body temperatures driven by vasodilation (Stephenson & Kolka, 1999; Kim et al., 2008). Following ovulation, progesterone steadily increases, peaking midway through the luteal phase and more rapidly declining in the few days preceding menstruation (Charkoudian and Johnson, 1999). Progesterone has direct effects on neurons of hypothalamus stimulating an increase of body temperatures during the luteal phase of the cycle (Charkoudian & Stachenfeld, 2016). Female sex hormones have been observed to elicit changes in thermoregulatory measures across the menstrual cycle during exercise in the heat (Pivarnik et al., 1992; Kolka et al., 2000; Stephenson & Kolka, 1999; Tenaglia et al., 1999; Janse et al., 2012) in untrained and recreationally trained eumenhorreic females ($\dot{V}O_{2max}$ ~42-45 ml/kg/min). Avellini et al., (1979; n = 4) and Lei et al. (2017) reported thermoregulatory differences in core temperatures and sudomotor activity across the menstrual cycle in highly trained females ($\dot{V}O_{2max}$ 49-57 ml/kg/min), however this is

somewhat conflicting, as other studies (Sunderland & Nevill, 2003; Kuwahara et al., 2005; Dervis et al., 2016) did not observe any effects of the menstrual cycle in highly trained females ($\dot{V}O_{2max}$ 49-52 ml/kg/min). This is likely because aerobic fitness minimises phase-related differences in internal temperatures and thermoregulatory response (Dale et al. 1979; Bullen et al. 1984; Kuwahara et al., 2005a, b).

Thermoregulatory effectors in oral contraceptive users appear to mimic eumenhorreic females, with slightly higher core temperatures during the second half of their pill cycle (Sunderland & Nevill., 2003; Grucza et al.; 1993); Although the effects may be less pronounced in combined (oestrogen and progestin), monophasic pill users than in triphasic or progestin-only pill users (Burrows & Peters, 2007; Joyce et al., 2013; Stachenfeld et al., 2000). A progestin-only pill has been observed to illicit higher internal temperatures and sweating thresholds than a combined pill, which contains both oestrogen and progestin (Stachenfeld et al., 2000), possibly because it skews the normal ratio of oestrogen and progesterone. Although minimal research has been conducted on thermoregulatory capacity of females using these different forms of hormonal contraception, between group differences (besides <0.5°C difference in rectal temperature at sweat onset) have not been observed in thermoregulation under exercise heat stress in eumenhorreic, oral contraceptive users, or users of depot medroxyprogesterone acetate contraceptive injection (Tenaglia et al., 1999; Armstrong et al., 2005). There has not been any research to date addressing the possible effects of the contraceptive patch, implant, coil or IUD on exercise in the heat. However, as the implant and IUD rely solely on progestin, there may be negative effects on thermoregulation, as progestin-only pills have been shown to increase body temperature (Stachenfeld et al., 2000). Alternatively, they may act in a similar way to the contraceptive injection of synthetic progesterone, and have no effect on thermoregulation and performance

(Armstrong et al., 2005). Although female sex hormones have been observed to have some effect on thermoregulation, their impact on performance is much less prominent, as described below.

2.2.3 Adjustable Set Point theory. Thermoregulation is a complex, integrative process that is still not completely understood. However, three distinct theories exist to attempt to explain the human ability to maintain thermostasis. The first is the Adjustable Set Point theory developed by Hammel et al., (1963), and is based on the analogy of the human body to a thermostat. When body temperature differs from the acceptable set/fixed temperature, a corrective response is initiated to restore homeostasis. However, this corrective response is graded, meaning that a more intense response is initiated the further body temperatures stray from the predetermined range. Afferent neurons from internal organs as well as peripheral limbs feed into the hypothalamus, which initiates a corrective response, with the intensity dependent on the variance from the predetermined "set point". The most common limitation of this theory is how it fails to account for the influence of multiple factors that can cause this "set-point" to vary. These include menstrual cycle, pyrogens, circadian rhythms, training status, and heat acclimation status (Gisolfi et al., 1984; Cabanac et al., 2006; Sawka et al., 2011), which suggest that the "set-point" is actually more of a dependent range than a single fixed temperature.

2.2.4 Reciprocal inhibition. Amendments to the Adjustable Set Point Theory lead to the development of the model of Reciprocal Inhibition by Bligh et al. (2006). While the set-point theory describes the signals from the cold and heat sensitive neurons combining to give a single temperature feedback signal, reciprocal inhibition proposed that these neurons

provide their own separate feedback. This implies that hot temperatures would not only send an excitatory stimulus to initiate a cooling response (sweating, vasodilation, etc.), but would also send an inhibitory signal to prevent the initiation of heating mechanisms (shivering, vasoconstriction, etc.). Thus, the effector response depends on a net thermal signal produced by the separate afferent signals. While the set-point theory does include a differing intensity of the response depending how severely temperature deviates from the set-point, reciprocal inhibition adds an additional layer. It suggests that a vasomotor response is initiated first to correct minor deviations, with a sudomotor response or shivering being initiated only once body temperature deviates beyond what can be corrected with a vasomotor response.

2.2.5 Heat regulation. The previous two models suggest that temperature is the main variable of which the body is defending and body heating or cooling is engaged in order to maintain this temperature and are the only pathways to thermal homeostasis. The proposal of the heat regulation model by Paul Webb (1995) takes a unique position that does not build on the theory of a set-point. Instead, the heat regulation model suggests that body temperature is a secondary priority as it is only a cause of underlying factors. The balance of total heat storage and dissipation are instead the main variables providing input for the initiation and intensity of thermoregulation. This model suggests that the body defends a heat production:dissipation balance as opposed to its deviance from a preset base temperature, which explains how the body tolerates a sustained higher temperature during prolonged exercise bouts. Although the body's temperature is higher than normal, as long as heat dissipation is matching heat production, thermal homeostasis is being sufficiently defended. This helps to explain the "steady state" that athletes often experience during endurance events. This steady-state (where thermoregulatory mechanisms are being sufficiently engaged

to match heat dissipation to heat production) becomes the new normal for the body to maintain. Finally, this theory has non-thermal inputs for thermoregulation, which has been demonstrated by the initiation of thermoregulation while clamping hypothalamus activity (Hammel et al., 1963). Muscle metaboreceptor stimulation and possibly muscle mechanoreceptor stimulation (non-thermoregulatory reflexes) have also been observed to affect skin blood flow, a primary mechanism of heat dissipation (Shibasaki et al., 2005; González-Alonso et al., 2008).

2.3 Performance in the Heat

2.3.1 Theories of fatigue in the heat. Various factors have been known to influence fatigue while under heat stress, with several mechanisms put forward as being the limiting factor depending on the type and intensity of exercise. These hypotheses have been grouped into theories to outline the complex and unique impact of heat stress on fatigue and performance.

2.3.1.1 Critical temperature and central fatigue. The commonly accepted theory of "critical temperature" describes performance detriments with core temperature increases, with fatigue occurring at the critical temperature of ~40°C. This is because, as core and brain temperatures are similar (Nelson & Nunneley, 1998), there is a central nervous system response inhibiting further activity as temperatures exceed a safe zone (Neilson & Nybo, 2003; Nybo, 2008). This can be further described as an anticipatory response (Tucker & Noakes, 2009) and reflects the "template" in which the brain has created anticipating the workload at which will lead to "maximal effort" being reached at the termination of exercise without exceeding "safe" temperatures and reaching premature fatigue. This central governor

model (Noakes 2007, 2012) proposes that skeletal muscle motor recruitment is adjusted by the brain after calculating a pacing strategy based on afferent feedback and anticipated duration of exercise (Nikolopolous et al., 2001; Pires & Hammond, 2011). This central fatigue is most apparent at submaximal exercise levels, as indicated by the inability of muscles to sustain activation for repeated maximal contractions (Nybo, 2004, 2014). Additionally, central fatigue may be influenced by the reduction in cerebral blood flow during exercise in the heat (Nybo, 2010; Nybo et al., 2014).

Higher core temperatures often observed in a hot environment coincide with higher thermal discomfort, which can severely impact a participant's willingness and motivation to continue, as well as the task being perceived as more difficult. It is suggested by Cheuvront and colleagues (2010) that the reason that the task is perceived to be more difficult is that the participant is working at a relatively higher VO_{2max} than is credited in order to attain the same power output in the heat as was attained in a trial in cooler conditions. As VO_{2max} is reduced in the heat, a fixed workload trial would mean that the participant is working at a higher $\%VO_{2max}$ than they would be in optimal temperatures. This also means that the participant would need to make behavioural adjustments to their pace during a time trial in order to complete the task without reaching fatigue prematurely (Cheuvront et al., 2010). An additional contributing factor to this central fatigue and thermal discomfort may be that pyruvate kinase, the rate limiting enzyme in glycolysis (Gupta et al., 2010), is highly heat sensitive and becomes impaired as muscle temperatures rise (Herman & Lee, 2009a, b, & c; Heller & Grahn, 2012). Additionally, at muscle temperatures of ~40°C, mitochondrial selective permeability becomes compromised (Brookes 1971), allowing protons to leak back into the intramembrane space, reducing the amount of ATP produced for a given number of protons moved through the electron transport chain (Parkin et al., 2011). Muscle strength has

been observed to be compromised as core temperature increases, regardless of local muscle and skin temperature, reinforcing that this higher core (and likely brain) temperature is critical (Thomas et al., 2006).

2.3.1.2 Temperature gradient. The theory of a critical temperature is well supported in the literature (Gonzalez-Alonso et al., 1999; Nielsen et al., 1993; Nielsen & Nybo, 2003; Nybo, 2007), however none of these studies manage to isolate core temperature as being the limiting factor, often with fatigue being accompanied by a high mean skin temperature and narrowed skin-core temperature gradient. This indicates that the rising core temperature may not actually be what limits exercise capacity, but instead the negative effect that the resulting narrowing of the skin-core temperature gradient has on the cardiovascular system. This alternative theory is supported by Ely et al., (2010) which observed similar "sub-critical" core temperatures during a time trial in both hot and temperate environments, but substantial (17%) performance detriments along with higher skin temperatures in the hot environment. Similarly, in time to exhaustion trials (MacDougall et al., 1974) many participants reached exhaustion at sub-critical core temperatures in hot environments (simulated by water perfusion suit) when skin temperatures were elevated to ~36-37°C (vs. ~29°C; Sawka 1992). Large cardiovascular drift was observed at fatigue, indicating that the blood flow redirected to the skin has a substantial enough effect on the cardiovascular system to accelerate fatigue, independent of core temperatures (MacDougall et al., 1974). This may explain how marathon runners and endurance athletes are able to sustain high core temperatures for extended periods during races (Byrne et al., 2006).

2.3.1.3 Cardiovascular strain. Exogenous heat stress creates a unique demand for skin blood flow that is not experienced in cooler environments (Gonzalez-Alonso et al., 2008). Blood is diverted to the skin by peripheral vasodilation (Gonzalez-Alonso et al., 2003), at the cost of central and splanchnic blood flow.

This elevated skin blood flow demand also competes with the demand for oxygen by the active muscles and causes severe strain to the cardiovascular system. The rapidly experienced cardiovascular drift and reduction of cardiac output leads to an arteriovenous difference (Rowell, 1974) which impairs the body's ability to deliver oxygen to working muscles (Gonzalez-Alonso et al., 2008). The resulting shift to anaerobic metabolism (Dimri et al., 1980) and eventual oxygen debt (marked by lactate accumulation) forces attenuation of pace during self-paced trials (Periard et al., 2011; Periard 2012) and earlier fatigue at a fixed workload (Arngrimmson et al., 2003 & 2004). The demand for skin blood flow in the heat leads to a level of cardiovascular strain greater than that experienced during exercise in temperate conditions, making it the limiting factor of performance, especially during maximal exercise in the heat (Rowell, 1974, Gonzalez-Alonso et al., 2008, Periard et al., 2011, Nybo et al., 2014).

2.3.1.4 Integrative fatigue. Besides the aforementioned factors leading to fatigue, the stressor of acute heat stimulus has a significant impact on the endocrine system and metabolism during aerobic exercise (Young et al., 1985; Sawka et al., 1985). Higher cortisol and adrenaline levels while exercising in the heat stimulates sympathetic activation (Neilson et al., 1993) and greater carbohydrate metabolism and muscle glycogen consumption during submaximal exercise (Dimri et al., 1980; Jentjens et al., 2002). These metabolic changes are detected by some of the many receptors offering afferent feedback for the brain to process in

order to inhibit or stimulate efferent physiological responses (Noakes, 2007, 2012). Whilst the greatest limiters of performance in the heat are still being investigated, collectively, these layers of physiological processes that have been described by each individual theory suggest that fatigue is integrative (Nybo et al., 2014). Fatigue is a complex development, meaning it is important to acknowledge that fatigue in the heat has multiple sources (temperature, cardiovascular, behaviour, CNS, neuromuscular, metabolism), and to not solely credit high core temperatures with being the cause of fatigue (Nybo & González-Alonso, 2015). Additionally, as fatigue under heat stress is further researched, physiological sex-differences explored below warrant the investigation of both female and male cohorts to determine the specific manifestation of fatigue in both sexes.

2.3.2 Quantifying performance detriments in the heat. The effects of heat stress and strategies for heat acclimation have been investigated in male cohorts since the 1930's (Dill 1931; Dresoti, 1935; Dill et al., 1938) and 1940's (Bean & Eichna, 1943; Robinson et al., 1943; Eichna et al., 1945). Heat stress has since been known to initiate an earlier onset of fatigue and cause exponential detriments to performance with every degree increase from "optimal" conditions (~10°C; Galloway & Maughan, 1997). VO_{2max} is degraded by ~7-18% from "optimal" conditions in male cohorts (Pirnay et al., 1970; Nybo & Nielson, 2001; Gonzalez-Alonzo et al., 2003; Lorenzo et al., 2010; James et al., 2016) and 4-17% from temperate conditions (21-25°C; Arngrimsson et al., 2003; Lafrenz et al., 2008, Sawka et al.; 1985; Klausen 1967). At maximal levels, it is suggested that heat stress resulting from hot ambient temperatures will degrade VO_{2max} performance for females in a similar capacity to males (Arngrimsson et al., 2004). It has also been reported that females do not differ in $\dot{V}O_{2max}$ under heat stress either across the menstrual cycle, or while using monophasic oral or

injectable contraceptives (Grucza et al., 1993; Armstrong et al., 2005; Janse de Jonge, 2003), although reductions in $\dot{V}O_{2max}$ have been observed in oral contraceptive users in temperate conditions (Joyce et al., 2013; Casazza et al. 2002, Lebrun et al. 2003).

Reductions in time trial (TT) performance in the heat in male cohorts range from 2% in short trials (4km TT; Altareki et al., 2009) up to 12% in longer trials (43km TT; Racinais et al., 2014), with common reductions falling within this range (~6-7%; Tyler et al., 2008; Tucker et al., 2004; Tatterson et al., 2000; Periard et al., 2011; Peiffer & Abbiss, 2011). Trials employing a Time to Exhaustion (TTE) type protocol on male cohorts usually record greater detriments of 19-47% in comparison to the control, (James et al., 2016; Dill et al., 1931; Galloway & Maughan, 1997; MacDougall et al., 1974; Morris et al., 1998; González-Alonso et al., 2003), however the reliability of a self-paced time trial is greater than a TTE trial (Hopkins et al., 2001). Repeated maximal muscle contraction is also impaired in the heat (Nybo & Nielsen, 2001; Brazaitis & Skurvydas, 2010), which may contribute to the reductions observed in TT and TTE performance in hot conditions. Although some information regarding physiological responses to heat in female cohorts have been documented, neither TT or TTE reductions in the heat in comparison to cooler conditions have been quantified for females. For a complete summary of relevant studies of time trials in hot conditions, refer to Appendix.

Behaviour is an important factor in performance in a self-paced time trial in the heat, which cannot be observed in a time to exhaustion protocol (Schlader et al., 2011). TTE trials involve a longer duration of submaximal exercise at a fixed intensity. This fixed intensity does not allow for behavioural adjustments, further impairing an athlete's ability to thermoregulate and making physiological challenges of heat stress more apparent. Females have been observed to be more tolerant to heat due to a lower rate of heat injury in a

retrospective military study by Druyan and colleagues (2012), as well as reduced detriments in self-paced marathon performance in the heat according to an analysis of IAAF International Association of Athletics Federation statistics (Guy 2015). These data indicate that females may exhibit different behavioural adjustments in comparison to males. Additionally, the pattern of performance drop-off or pacing adjustments of the time trial may differ between sexes, as drawn from evidence in cognitive investigations (Fine & Kobrick, 1985; Fine, 1987). Fine and colleagues reported that while males display a gradual reduction in performance of cognitive tasks with rising temperatures, females had a more abrupt pattern, showing minimal signs of performance detriments right up until the point at which heat can no longer be tolerated, and then declining rapidly to be similar to that of the males (Fine & Kobrick, 1985; Fine, 1987). How and if the cognitive performance differences observed between sexes in these studies could be observed in the pacing of time trials in the heat is unknown.

Female tolerance times and time trial performance in the heat have not been quantified in comparison to trials in cooler or temperate conditions, however, comparisons have been made in females across the menstrual cycle and whilst taking oral contraceptives, although results are somewhat conflicting. Exercise tolerance times reductions in the heat during the follicular phase were demonstrated by Tenaglia and colleagues (1999) who observed a 20% decrease during the mid-luteal (ML) phase in comparison to the early-follicular (EF) phase (107.4 [8.6] and 128.1 [13.4] min, respectively) in hot conditions (40°C, 30% RH), as the higher resting temperatures was theorised to cause women in the ML phase to reach their threshold for heat tolerance sooner. These findings were similar to Janse de Jonge et al. (2012), who observed a higher rate of core temperature change and earlier fatigue in the heat (32°C, 60% RH) during the luteal phase; with effects of a higher core temperature more

pronounced with the longer duration of these tolerance tests (≥60 minutes). However other studies, both in the heat (Lei et al., 2017; Sunderland et al., 2003; Kuwahara et al., 2005) and in temperate conditions (Bryner et al., 1996; Vaiksaar et al., 2011; Joyce et al., 2013) have observed that menstrual cycle does not affect performance. These mixed results may be attributed to the lower training status of participants in the former studies who observed performance differences (~40-44 $\dot{V}O_{2max}$ vs ~51-57ml/kg/min $\dot{V}O_{2max}$ in latter studies), as increased aerobic capacity has been shown to minimise hormonal effects (Kuwahara et al., 2005). For example, Sunderland et al. (2003) did not find any differences in physiological responses (i.e., core temperature, perceived exertion, estimated sweat rate, plasma lactate) or in performance (total distance run in high-intensity shuttle run test) in normally menstruating, unacclimated women ($\dot{V}O_{2max}$ 51.1 (0.7) ml/kg/min) between the follicular and luteal phases when exercising in the heat (31°C, 23% RH).

Despite possible detriments to VO_{2max} whilst using oral contraception, it does not appear to influence more sport specific endurance performance (Bennell et al., 1999, Joyce et al.; 2013, Lebrun et al., 2003). Oral contraceptive use has not been observed to influence exercise tolerance or TT performance in the heat (Tenaglia et al., 1999; Sunderland et al., 2003), and has been observed to have minimal to no impact in temperate conditions, especially when users were taking a low dosage, monophasic pill (Joyce et al., 2013; Giacomoni et al., 2000; Vaiksaar et al., 2011; Rechichi & Dawson 2012; Lebrun et al., 2003). For example, although Tenaglia et al. (1999) observed some differences in exercise tolerance times in normally menstruating females across their menstrual cycle, there was no such between group difference observed in exercise tolerance times in the heat (40°C, 30% RH) between normally menstruating women and oral contraceptive users.

Therefore, literature to date indicates that any differences between menstrual cycle phases or hormonal contraception users that is implied from mechanistic or VO_{2max} trials does not translate to practical performance outcomes. Physical size and aerobic capacity appear more important than menstrual cycle when comparing sex-differences in exercise under acute heat stress (Kenney et al., 1985; Gagnon & Kenny, 2012; Notley et al., 2017; Kuwahara et al., 2005). Female sex hormones are seen as a major barrier to research in female cohorts, and if research of female sport and female athletes is to ever catch up to rates of female participation and athletic success, menstrual cycle and hormonal contraceptives must be further investigated and addressed to construct well informed recommendations.

Performance during team sports has also been observed to be impaired in hot conditions. Mohr and colleagues (2012) showed that total distance run and the amount of running qualifying as "high intensity" in a football match was lower in hot conditions (~43°C vs ~21°C). However, team sport performance is tactical and skilful as well as dependent on strength, power, and endurance (Chalmers et al., 2014), so it should be noted that subjects completed significantly more successful passes and crosses during the match played in hot conditions compared to the temperate conditions (Mohr et al., 2012). Similarly, Bandelow et al. (2010) found accuracy was positively influenced by rises in core temperature when completing a variety of cognitive tests throughout football matches in the heat. In contrast, Malan et al. (2010) reported a significant impairment in reaction time in the heat after analysing recorded clips of male hockey goalkeepers.

In females, it is suggested that the addition of heat stress negatively impacts hockey skills, which Sunderland et al. (2005) observed to decline 6% more with exercise in hot (30°C, 38% RH) than in moderate (19°C, 51% RH) conditions.

Overall, heat stress has been observed to have a negative net effect on performance, however the severity of this effect may be expressed differently depending on the sport or activity, and the sex of the athlete may have a role.

2.3.3 Meaningful performance outcomes following traditional heat acclimation.

Early research into heat acclimation involved chronic bouts of exposure to heat stress in order to facilitate beneficial adaptations and thermal tolerance. Studies in the 1930's and 1940's focused on measuring physiological adaptations assumed to decrease risk of heat injury (Dresoti, 1935; Dill et al., 1938; Robinson et al., 1943; Eichna et al., 1945), and physiological adaptations gained from heat acclimation were later shown to translate to improved exercise tolerance in the heat (Pandolf & Young 1992). Recently, maximal and submaximal performance benefits have been quantified alongside physiological adaptations following traditional heat acclimation and acclimatisation protocols in male, or mostly male cohorts. Lorenzo et al., (2010) demonstrated performance improvements in 10 male and 2 female participants in hot conditions (38°C, 30% RH) following 10 days of acclimation, with an 8% improvement in $\dot{V}O_{2max}$, an 8% increase in mean power across a 1-h time trial, and a 5% increase in power at lactate threshold. In this study, the control group did not experience any significant performance improvements. Similarly, Keiser et al. (2015) demonstrated performance improvements in the heat both in mean and maximal power output during a 30-minute time trial and in $\dot{V}O_{2max}$ following 10 days of heat acclimation. Again, the control group did not experience any significant effects of training in temperate conditions (18°C, 30% RH) for 10 days.

As a result of 13 days training in hot conditions in Qatar, Racinais et al. (2014) observed that performance times during a 43-km cycling trial in the heat (~37°C) were

comparable to times in cooler conditions (~8°C), equating to an 18% improvement from the pre-acclimation trial in these male athletes. Although mean power output was still slightly lower, the drop off in power output throughout the trial originally observed in the heat was greatly reduced.

Studies that have investigated the physiological adaptations of heat acclimation have included female cohorts (Sawka et al., 1985; Mee et al., 2015; Armstrong et al., 2005; Avellini et al., 1980; Fein et al., 1975; Shapiro et al., 1980), however time trial performance improvements following traditional heat acclimation protocols (7-14 days) have yet to be quantified for females, exposing a large knowledge gap in real world application for females competing in hot conditions. Heat acclimation is normally undertaken in eumenhorreic females during the "low-hormone" follicular phase, and during the "quasi-follicular" or no pill/placebo stage for oral contraception users. This is to minimize confounding factors of sex, as men and women have been observed to experience similar thermoregulatory strain when women are in the low hormone phase of their menstrual cycle (Kolka et al., 1994). This is relevant for mechanistic based studies, however, as the literature suggests that possible physiological or VO_{2max} differences across the menstrual cycle or as a result of hormonal contraceptives do not influence the results of more applicable performance tests and time trials, this is an illusory barrier to investigating performance following heat acclimation. Heat acclimation is equally attainable for eumenhorreic females, oral contraceptive users, and users of hormonal injections, as demonstrated by Armstrong and colleagues (2005). They reported that there were no between group differences following heat acclimation in either physiological adaptations (thermal, metabolic, cardiorespiratory, and perceptual responses, besides minor differences in core temperature at sweat onset) or performance measures (sit-ups, push-ups, 4.6-km run time; Armstrong et al., 2005). Albeit, participants were relatively

untrained, and completed a 3-day/week acclimation protocol combined with other training over the span of 7-8wks – far from the traditional model of heat acclimation described above. Therefore, time trial type performance outcomes following heat acclimation have not yet been adequately quantified for females.

2.4 Novel Strategies for Heat Acclimation

2.4.1 Short-term heat acclimation. Despite known differences in males and females in response to acute heat stress, both sexes have historically been observed to adapt similarly to heat acclimation. Sawka et al. (1983) conducted an extensive study examining how females and males responded to both hot and dry heat at different hydration levels following a 10-day acclimation protocol. They concluded that there was no significant interaction of sex on acclimation, except that females may be more efficient sweaters under humid conditions. Other than that, both groups displayed classic signs of acclimation during a fixed intensity exercise heat stress test. Similarly, Avellini et al., (1980) observed similar results for male and female participants matched in aerobic fitness and size following a traditional 10-day acclimation protocol. A greater sweat rate in men (~7%) was the only observable difference, however it was statistically insignificant ($P > 0.05$). More recent acclimation research has explored shorter forms of acclimation (i.e., "short-term heat acclimation"), and, as detailed below, findings to date indicate that the duration of the acclimation stimulus may result in sex-dependent responses.

Short-term heat acclimation (STHA) is classified as being < 7 days (Garrett et al., 2011), while traditional, long-term heat acclimation (LTHA) protocols have typically lasted 10-14 days. Short-term heat acclimation offers the potential benefits of a reduction in time and cost, especially valuable during the recovery/tapering time in the days before an event.

Garrett and colleagues demonstrated the effectiveness of a STHA protocol first on physiological markers of acclimation in moderately trained males (2009), and then on meaningful performance improvements in highly trained male athletes (2012). Following 5-days of acclimation (39.5°C, 60% RH), athletes improved their time trial performance by an average of 4-seconds (1.5%), with a 10-second reduction at the highest level of improvement. The highly trained athletes were members of an international rowing team able to perform at 90% of the world record pace for the 2,000 m row. The 2,000-m test itself is a highly reliable test (0.6-0.7% coefficient of variance; Schabort 1999, Creasy 2002) and in the sport of rowing, disproportionally large increases in power output are required to achieve measurable increases in speed, indicating that these improvements were both meaningful and substantial. These initial observations by Garrett et al. have since been replicated and extended by others using STHA, all demonstrating significant performance improvements under heat stress (e.g., Costa et al., 2012; Chen et al., 2013; Best et al., 2013; Racinais et al., 2014; Gibson et al., 2015; James et al., 2016; Willmott et al., 2016 and Peterson et al., 2010).

The rationale for the STHA strategy was perhaps first supported by Armstrong and Maresh (1991), who suggested that ~75% of adaptations to the heat are attained within the first 4-6 days of heat acclimation, as the plasma volume expansion alleviates the cardiovascular strain experienced while exercising under heat stress. Indeed, Gibson et al. (2015) reported that 5 days may even be enough to reach full acclimation status when an isothermic protocol (rapidly increasing core temperatures to ~38.5°C and maintaining core temperature throughout) is implemented, as there were no additional physiological improvements gained from the latter 5 days of heat acclimation (LTHA) that followed the first 5 days (STHA). Periard et al. (2016) describes heat acclimation as being biphasic, meaning that some adaptations are achieved from the more transient STHA, with adaptations

stabilizing following LTHA. Although the adaptations attained during STHA may be transient, they do translate to meaningful performance outcomes in males. This biphasic response is supported by performance outcomes observed by Racinais et al., (2014), who reported short-term performance improvements for male cyclists in the heat, with additional improvements following the more stable second phase of LTHA (Periard et al., 2016).

While these positive results indicate possibilities for a contemporary protocol reducing valuable time and monetary cost to athletes, these studies were undertaken on all male cohorts. Physiological changes in female athletes following short-term and long-term heat acclimation were assessed in a sex comparison study by Mee et al., (2015). The results indicated that although females became partially acclimated (increased sweat rate), they were unable to attain the other classic markers of heat acclimation (reduced heart rate and core temperatures) after only 5 days of heat acclimation under controlled hypothermia, and may require a full traditional acclimation protocol of ≥10 days to alleviate this physiological strain. This highlights that female populations performing or working in the heat may not be as well prepared for substantial heat stress in the same capacity as their male counterparts after only undertaking a STHA protocol. In contrast, Sunderland et al., (2008) observed a 33% improvement in shuttle run endurance time in female soccer players in the heat following only four days of acclimation, albeit in the absence of physiological markers of acclimation. However, these were team sport athletes who may have exaggerated results compared to endurance athletes, who typically spend more time under heat stress and behave as if already acclimated (Taylor, 2000). Sunderland et al., (2008) attributes the success of the STHA protocol to its high-intensity nature – opting for intermittent interval training under heat stress in place of an isothermic protocol. Therefore, the traditionally prescribed 90-minute

isothermic protocol observed to induce markers of acclimation in males may not be a sufficient stimulus to induce the same results in females.

One potential explanation for the differential sex-related response to a 5-day acclimation protocol may be attributed to the typical size differences between males and females, leading to females experiencing a reduced metabolic heat production during an isothermic protocol in comparison to males, as described by Jay and Cramer (2014). The comparison of an isothermic heat stimulus for participants of different sizes is described using the analogy of altering the cooking time of a turkey according to its mass, as larger "turkeys" will need more heat to reach the same internal temperature, just as larger athletes required a greater heat stimulus to maintain a high core temperature (Jay & Cramer, 2014). Therefore, an isothermic protocol leads to a greater rate of metabolic heat production, and therefore greater heat stimulus, for larger participants.

A main requirement of a heat acclimation protocol is that it must induce sufficient sweating in order to drive adaptation (Buono et al., 2009). Jay and Cramer (2014) highlight that absolute evaporative requirements for heat balance are driven by metabolic heat production and are the main factor in determining sweat production. When internal heat production is matched under compensable conditions, so are sweat rates – a main requirement for driving heat acclimation (Neilson, 1998; Avellini et al., 1982; Henane et al., 1977).

As an isothermic protocol that maintains participants' core temperatures at ~38.5°C already provides the highest internal temperatures attainable within an ethically safe zone, Mee et al. (2017) investigated a possible solution in increasing the intensity of the heat stimulation during a STHA protocol for females by adding 20-minutes of passive heating while wearing a sauna suit before each session. This intervention yielded similar physiological adaptations for females during a running heat tolerance test to that which males

typically experience following STHA. These adaptations included reduced core temperatures and heart rate (at rest and during exercise), plasma volume expansion, increased rate of sweating, and improvements in ratings of perceived exertion and thermal comfort/sensation. This indicates that when acclimating females, the stimulus must either be more intense than that implemented for males, or they must spend more consecutive days under heat stress. However, there was no performance measure accompanying either study by Mee and colleagues. Therefore, the quantification of performance improvements in females following short-term vs long-term heat acclimation strategies is urgently needed to inform safety guidelines and preparation recommendations for real-world athletic events (Casadio et al., 2016).

2.4.2 Heat acclimation as an ergogenic aid. Research has been undertaken in male cohorts to determine the potential for heat acclimation to act in an ergogenic manner for performance in more temperate (20-23°C) or cooler (8-15°C) conditions. These temperatures better match conditions in which endurance athletes will ideally compete (of ~10-14°C; Ely et al., 2007), where heat stress is not imposed on athletes by the ambient temperatures. Performance improvements in temperate conditions (21-22°C) following HA for untrained athletes were first observed by Nadel et al. (1974), as participants experienced increases in $\dot{V}O_{2max}$ of ~190mL following a 10-day HA protocol. A similar improvement of 4% in the $\dot{V}O_{2max}$ of male soldiers was observed with a nearly identical protocol by Sawka et al. (1985). More recently, ergogenic effects were observed by Lorenzo and colleagues (2010), who reported that power at lactate threshold, $\dot{V}O_{2max}$, and maximal power output during a 1-h TT increased 5-6%, in cool conditions (13°C) following heat acclimation, whilst the control group did not experience any improvements. Improvements for power at lactate threshold and

peak power output in temperate conditions (22°C, 60% RH) were reported by Neal and colleagues (2016a, b) following short and longer-term protocols, although $\dot{V}O_{2max}$ was unaffected. Additionally, Buccheit and colleagues (2011) observed a 7% improvement in intermittent shuttle test results in temperate conditions (22°C) following in-season heat acclimatisation of trained male football players. One such investigation in females was undertaken in international-level female soccer players, who recorded a 1.5% increase in speed during the 30–15 Intermittent Fitness Test (IFT) in temperate conditions (20°C), setting a personal best for the team in this highly familiar test. The small, but meaningful effect observed in elite female soccer players warrants further investigation into the ergogenic potential for HA in female athletes. The effect of HA on endurance performance in a time trial style test has not yet been investigated in females.

The main mechanisms that could facilitate ergogenic outcomes following heat acclimation are: a) plasma volume expansion; b) an increased thermoregulatory capacity, and c) substrate metabolism shifts and metabolic adaptations. It would seem that a combination of these main mechanisms, along with smaller, underlying adaptations and the individuals' responses to them, yields the small percentage of performance improvement sought out by high-level athletes (Minson & Cotter, 2016).

The adaptation that has been most credited with providing ergogenic potential, as well as being highly debated, is the plasma volume expansion experienced as a result of heat acclimation. Individual performance improvements in temperate conditions have been observed to be highly correlated with plasma volume (and likely blood volume) expansion (Buccheit et al., 2011, Scoon et al., 2007). Minson and Cotter (2016) proposed that this is the central mechanism driving ergogenic effects, although it is specified that evidence is mainly applicable to sub-elite and team sport athletes. Nybo and Lundby suggested that in elite

athletes, plasma volume expansion does not improve performance (Nybo and Lundby, 2016). Elite endurance athletes may already be at an optimal, hypervolemic blood volume in which any further plasma volume expansion may reach the point that the increased cardiac output is no longer beneficial enough to counteract the reduced O_2 carrying capacity resulting from haemodilution (Keiser et al., 2015; Neal et al., 2016). These athletes may also be approaching the limit of their diastolic reserve capacity (Warburton et al., 1999). The optimal balance of cardiovascular benefits and haemodilution varies on an individual basis (Racinais et al., 2012), suggesting that some individuals could benefit from plasma volume expansion, while others may not. However, there appeared to be no clear performance trend when elite athletes' plasma volume was expanded by heat acclimation in a controlled, counterbalanced study (Keiser et al., 2015). Additionally, plasma volume expansion along with an increased sensitivity to thirst resulting from heat acclimation could help to combat dehydration in any condition, a factor that can be critical athletic and mental performance (Baker et al., 2007, Devlin et al., 2001).

Plasma volume responses to HA in females is not well understood. Sex differences in the endocrine response responsible for plasma volume expansion (detailed in Section 2.5.5; Stachenfeld et al., 2001), presents the possibility that even elite female athletes may respond differently to HA than males. Additionally, as plasma volume has been observed to fluctuate during the menstrual cycle (Fortney et al., 1988; Stachenfeld & Taylor, 2005), heat acclimation could be used to prevent plasma volume reductions and fluctuations across the cycle.

As stated, "optimal temperatures" for endurance events is ~10-14°C (Ely et al., 2007), and performance decreases exponentially as conditions deviate further (Galloway & Maughan, 1997). Despite this, real-world endurance events may take place in temperatures

outside this “optimal” zone. Small changes in ambient temperature can be detrimental to performance, even as the temperature remains relatively cool, as rising internal temperatures may be a contributing factor in fatigue for endurance activities in any temperature (MacDougall 1974). Increased skin blood flow and sweating during exercise in cooler conditions further indicates that thermal strain is still a factor contributing to fatigue.

Additionally, this knowledge can be applied practically for sports other than just cycling. Many sports with a substantial endurance requirement (i.e. soccer/football, field & ice hockey, lacrosse, etc.), require athletes to wear jerseys or uniforms that do not allow for the same optimal heat dissipation that is experienced by athletes in the lab, who are often able to wear minimal clothing (i.e. cycling shorts, sports bra). These jerseys or uniforms are extra clothing that create a microenvironment next to the skin with little airflow, and the sweat-wicking properties of popular materials may inhibit heat loss by evaporation, impairing an athlete’s ability to dissipate heat (Cheung, 2010). In this scenario, there may be more of a thermal effect than anticipated, limiting performance even in temperatures that are considered “optimal” or cool. The thermoregulatory adaptations of heat acclimation, including improved sudomotor function and attenuated internal temperatures, allow the body to not only thermoregulate more effectively, but to also better tolerate these higher core temperatures (Maron et al., 1997; Byrne et al., 2006). Optimising an athlete’s thermoregulatory capabilities through heat acclimation training prepares them for the variable conditions that they may encounter in real-world competition in any ambient temperature.

Metabolic adaptations have also been observed in both hot and cool conditions following heat acclimation (Sawka et al., 1985). A shift towards aerobic metabolism (Young et al., 1985) indirectly increases glycogen sparing, which may be particularly beneficial in cooler conditions, where glycogen stores are more severely depleted at the onset of fatigue, as

compared to heat stress (Parkin et al., 2011). A decreased lactate accumulation in both the muscle and plasma during exercise in cool environments (Young et al., 1985) may be an indication of this shift to aerobic metabolism. Additionally, an increase in VO_{2max} following acclimation would increase oxygen delivery and decrease lactate production, and contribute to the reduction in lactate accumulation in the muscle and plasma during exercise.

Substrate utilization in females has been studied during bouts of acute heat stress (Vaiksaar et al., 2011), but not following heat acclimation. However, it would be interesting to observe their responses and how it could affect their performance in cooler conditions, considering females' elevated lipolytic sensitivity to epinephrine and the known effects of sex hormones on fatty acid mobilisation (Jensen et al., 1994; Pedersen et al., 2004; Horton et al., 2006; Williams, 2007; Tarnopolsky, 2008; Oosthuyse & Bosch, 2012).

Some endurance performance tests that have been undertaken following heat acclimation in temperate and cool conditions have yielded minimal (0.4%) or insignificant improvements (Neal et al., 2016, Karlsen et al., 2015, Morrison et al., 2002, Keiser et al., 2015). These studies argue that plasma volume expansion is not ergogenic for more elite endurance athletes whom are already close to peak muscle O_2 delivery (Keiser et al., 2015, Coyle et al., 1990). However, some limitations should be considered. Karlsen et al., (2015) undertook time trials in cool, outdoor conditions of 8°C, where participants were dressed in clothing which covered the majority of their skin, including gloves, which may have impaired their cooling mechanisms and increased heat storage, counteracting the benefits gained through heat acclimatization. Participants in Morrison et al. (2002) only undertook 7 days of heat acclimation, which can still be considered a short-term protocol (Garrett et al., 2011), and the heat acclimation phenotype may not have been fully developed and/or stable (Periard et al., 2016). In the study by Keiser and colleagues (2015), pre-heating before the performance

tests may have also interfered with the thermoregulatory benefits of heat acclimation, such as a lowered body core temperature. Additionally, the statistical analysis of data may have been underpowered by the sample size of seven participants, as the numerical mean increase in peak power of 10W (3%; P = 0.19) and in absolute VO_{2max} of 200 mL (4%; P = 0.08) are not statistically significant, however are very similar to those found by Lorenzo and colleagues (12 W or 3%; P < 0.01 and 180 mL or 4%; P < 0.01, respectively; Lorenzo et al., 2010).

Research to date has been limited, but presents interesting hypotheses for future avenues of ergogenic research. One idea is that these 10-14 day HA protocols are only "medium-term", and truly stable adaptations may require an even longer protocol (Horowitz, 1998). Rodent studies by Horowitz and Kodesh (2010), Horowitz et al., (2011), and Kodesh et al., (2011) demonstrated that prolonged heat acclimation (30-days) produced a stable phenotype with genomic adaptations. Maloyan et al. (2005) also observed that rodents heat acclimated for one month showed elevated hypoxia-inducible factor-1 (HIF-1) activation, which upregulates genes including vascular endothelial growth factor (responsible for angiogenesis) and erythropoietin (responsible for red blood cell production). This suggests that it is possible that prolonged heat acclimation could induce some of the desirable and ergogenic adaptations that have been observed following altitude training (Levine & Stray-Gundersen, 1997; Ashenden et al., 1999; Richalet & C. J. Gore, 2008).

If the ergogenic potential of a long-term acclimation protocol is to be explored, female endurance athletes especially, who are prone to anemia and low iron levels, and also lose more iron through sweating (Lamanca et al., 1988), may need to consider using iron supplementation while undertaking a long-term heat acclimation protocol to maximize benefits. Iron is necessary for red blood cell production, and is commonly suggested to

maximise results of altitude training (Stray-Gundersen et al., 2001; Wehrlin et al., 2006; Clark et al., 2009).

Alternatively, as elite athletes have a smaller scope for adaptation (Periard et al., 2015), it is possible that only high responders will experience measurable improvements in temperate conditions (Neal et al., 2016). Individual differences in response to heat acclimation have been reported by Racinais et al., 2012, just as individual responses have been observed in athletes undergoing altitude training (Levine & Stray-Gundersen, 1997). It should be noted that females are able to obtain these performance benefits from altitude training in a similar way to males (Stray-Gunderson et al., 2001).

Varying the intensity and duration of the HA protocol has been observed to elicit different results in performance in the heat (Wingfield et al., 2016), and therefore may elicit different results in cooler conditions. The findings of Wingfield and colleagues (2016) indicated that 30-minutes of high intensity interval training in the heat will significantly improve peak power output by ~6% more than a low intensity protocol, possibly linked to the heat activated m-TOR pathway (Kakigi et al., 2011; Yoshihara et al., 2013). However, the 90-minute lower intensity protocol was more efficient in improving 20-km time trial performance. Combining these intensities into a 90-minute protocol that also includes high-intensity intervals is a possible strategy to improve both maximal and submaximal performance in cooler conditions. It is also reiterated that females require a more intense protocol to obtain the heat acclimated phenotype (Mee et al., 2017), making these interval-long duration combination protocols a possible, and perhaps optimal, option.

Alternatively, when designing these HA protocols for ergogenic use, it may be most effective to keep HA sessions as low-intensity session complimentary to normal training (Minson & Cotter, 2016). Since heat strain impairs exercise, athletes attempting to complete

all training sessions in the heat could see their performance suffer from a reduced exercise intensity during training sessions (Minson & Cotter, 2016). Completing all training sessions in the heat would also add the fatigue of an unfamiliar stressor, possibly impairing performance test results following an intense heat acclimation protocol. Therefore, keeping HA sessions to a lower intensity could be necessary to elicit endurance performance improvements. This practice has been successfully implemented as it relates to altitude training ("live-high train-low"), and evidence indicates that a "live-hot train-cool" model may be the new prescription for heat acclimation for ergogenic performance gains (Corbett et al., 2014). Ergogenic effects may not be as easily attained as the acclimated phenotype, so finding the right way to combine training strategies is key to uncovering the ergogenic potential of heat acclimation.

In summary, there is strong evidence that heat acclimation can be ergogenic for sub-elite and team sport athletes, and the possibility exists that carefully designing an optimal protocol could positively benefit even the most elite athletes, to whom even the smallest percentage of performance improvement is pursued. Considering possible female differences in adapting to heat stress, how females respond to an acclimation protocol designed with the intention of facilitating performance improvements in cool conditions is unknown.

2.5 Mechanisms of Adaptation

2.5.1 Core and skin temperatures. During exercise, blood perfused through the active muscles are heated and upon returning to the central areas of the body, cause internal temperatures to rise. Hot ambient temperatures impair the body's heat loss mechanisms and internal and skin temperatures rise at a greater rate. Thermal response is estimated to be 90% influenced by core temperatures (mean skin temperature influences the remaining 10% of

response; Nadel et al., 1971), however, in a hot environment as skin temperatures deviate heavily, the influence of skin temperature on thermal response may increase (Sawka et al., 2011).

Rising core and skin temperatures are a central factor in heat injury and declining performance in the heat, making temperature adaptations a main target in heat acclimation (Neilson et al., 1993). Heat acclimation reduces core and skin temperatures during exercise, as well as resting core temperatures (Sawka et al., 2011). It is well documented that males can achieve these desired reductions in temperature after only completing a STHA protocol (< 7 days; Garrett et al., 2011, 2012; Chen et al., 2013; Best et al. 2013, Racinais et al. 2014, Gibson et al. 2015), however females may require additional stimulus through a longer, ~10-day protocol in order to similarly alleviate thermoregulatory strain (Mee et al., 2015).

2.5.2 Skin blood flow. Warmed blood is delivered from the core to the surface to dissipate heat through the evaporation of sweat. Acclimation improves the body's ability to dissipate heat via skin blood flow by initiating this cooling mechanism at a lower temperature threshold (Roberts et al., 1977).

A greater core to skin temperature gradient becomes increasingly important during exercise, as it lessens the demand for skin blood flow by allowing ambient and skin temperatures to cool the warm blood from the core (Cheuvront et al., 2003; Sawka et al., 2011). If skin temperatures can remain low, less blood flow is required. Interestingly, a higher core temperature (often thought to have negative effects on all areas of thermoregulation) also increases this gradient, and therefore decreases skin blood flow (Sawka et al., 2011).

Unacclimated females, who are typically smaller than males, rely more on skin blood flow and vasodilation to cool down. However, at compensable heat stress levels this is mainly

a product of physical size differences, not sex (Notley et al., 2017; Gagnon & Kenny, 2012). Additionally, females' autonomic control of vasodilation may be influenced by their varying levels of sex hormones (Charkoudian et al., 2016).

2.5.3 Cardiovascular. Although necessary for heat dissipation, the cardiovascular strain to meet the increased blood flow demands in the heat can compromise the system's ability to provide O_2 to the brain and active muscles (Neilson & Nybo, 2003; Nybo & Secher, 2004; González-Alonso et al., 1998; Rowell, 1974). These competing demands, as well as decreased blood pressure as plasma volume is lost through sweating, drives the higher heart rate observed during exercise in hot conditions (Cheuvront et al., 2003). This results in less time spent in the diastolic phase. Both of these lead to a decrease in ventricular filling, and therefore a decrease in stroke volume (Rowell, 1966; Trinity et al., 2010). For cardiac output to be maintained, heart rate must increase further in parallel with stroke volume reductions, known as cardiovascular drift (Montain & Coyle., 1992; Lafrenz et al., 2008). Heat stress is characterized by an increased heart rate in comparison to the same workload in cooler conditions and an accelerated onset of cardiovascular drift (Sawka et al., 1992).

Cardiovascular stability is attained through heat acclimation, primarily as a result of plasma volume expansion (Nielsen et al., 1993; Garrett et al., 2009), which simply provides an increased supply of body fluid to better meet demands. Therefore, heat acclimation lowers heart rate at rest and during exercise, increases ventricular filling, and increases stroke volume and cardiac output.

Additionally, genetic adaptations of the myocardium have been observed following the heat acclimation of rats (Horowitz et al., 2011, Schwimmer et al., 2006). Myocardium contractions are facilitated by calcium-induced calcium release. calcium binds to receptors on

the cardiac muscle, which stimulates a further release of calcium from the sarcoplasmic reticulum into the cell, and contraction is initiated (Roderick et al., 2003). An upregulation of calcium signalling genes caused by heat acclimation improves cardiac muscle excitation-contraction coupling, which increases force generation of the cardiac muscle (Cohen et al., 2007). This increase in contraction force allows for greater expulsion of blood from the heart and a decrease in end diastolic volume (Horowitz et al., 1986). Therefore, cardiac efficiency can also be improved with heat acclimation, however it may be achieved sooner in males than in females (Mee et al., 2015).

2.5.4 Sudomotor activity. Depending on the sport or activity, sex, size, and training status of the athlete, sweat rate can range from 0.5-2 liters/hour (Sawka et al., 2007). Trained athletes sweat more as a result of multiple factors, including a high density of active sweat glands and sweat onset being initiated at lower core temperatures.

Warm skin increases sweat rate, as it stimulates local thermal neurons, and also because of the demand it creates for blood flow, making more plasma readily available for sweating (Charkoudian, 2003). Sweat rate is an important adaptation of chronic heat stress, as it allows heat dissipation via evaporation, the only avenue for heat loss as ambient temperatures surpass skin and core temperatures (Cheung, 2010). Historically, males have been credited with sweating more, however at a submaximal level, this may be primarily a function of size and body surface area to mass ratio (Notley et al., 2017, Gagnon & Kenny 2012). At the highest requirements for heat loss, women exhibit a reduced capacity for evaporative heat loss, due to a reduction in sweat gland output (Gagnon & Kenny, 2012). However, in humid environments, females may be more “efficient sweaters”, as they are more

sensitive to wetted skin, preventing unnecessary fluid loss when already secreted sweat has not yet been evaporated (Sawka et al., 1985).

Sweat is primarily secreted through the eccrine sweat glands, which may increase in size following heat acclimation (Bouno et al., 2009). Additionally, sodium that is excreted in the sweat can be resorbed by cells lining the duct portion, conserving electrolytes (mainly sodium, however chloride, potassium, calcium, and magnesium are also secreted; Maughan, 1991), an ability which is improved with heat acclimation (Allan & Wilson, 1971). This is beneficial, as reductions in sweat sodium concentration will allow easier evaporation of sweat, and therefore increase evaporative cooling capacities. Sweat gland sensitivity also is enhanced following heat acclimation (Buono et al., 2009), and as females have a reduced cholinergic sensitivity in comparison to males (Madeira et al., 2010), there may be greater potential for immediate peripheral improvements, explaining sweat rate improvements in females following STHA. Although this increases evaporative capacity, the increased sweat rate may add further strain to the female cardiovascular system that has not yet established stability in the heat (Mee et al., 2015; Taylor, 2014).

2.5.5 Plasma volume expansion. Heat acclimation provides a large stimulus for fluid regulatory adaptations through sweat loss, which leads in the expansion of plasma volume (typically ~7-14%; Guy et al., 2015, Patterson et al., 2004, 2014) through a number of processes. In order for adaptation to take place, dehydration levels of the intracellular fluid must pass the "osmotic threshold" of an approximate 2% increase in plasma osmotic pressure (Andreoli et al., 2000; Cheuvront & Kenefick, 2014), roughly measurable by a 2% loss in body weight. Once this threshold has been exceeded, adaptive mechanisms of anti-diuretic hormone (ADH) secretion by the hypothalamus and thirst sensitivity improvements by neural

signalling are triggered (Andreoli et al., 2000, Bourque et al., 2008). The most rapid response is the secretion of aldosterone and ADH, which activates the renin-angiotensin-aldosterone system, and increases sodium chloride retention and decreases urine output (Nagashima et al., 2001, Akerman et al., 2016). This, combined with an increased water consumption through an improved sensitivity to thirst, results in an increased extracellular fluid volume and net fluid retention (Nose et al., 1998, Convertino et al., 1980, Cheuvront et al., 2013). Albumin synthesis resulting from fluid regulatory stimulus increases circulating intravascular proteins, and subsequently, the colloid pressure of the vessels. This pulls water into the circulatory system, and further increases plasma volume (Convertino et al., 1980).

The advantages of an increased plasma volume counteract any possible performance detriments that may accompany an increase in body mass. An increased plasma volume better matches the demand for skin blood flow, allowing for greater thermoregulation (Periard et al., 2016). Cardiovascular stability is also improved as a result of increased vascular filling pressure (Senay et al., 1976). Additionally, plasma volume expansion has been the only physiological mechanism statistically linked to predicting individual performance improvements following heat acclimation (Racinais et al., 2012)

Plasma volume expansion in males may be transient if the acclimation protocol includes a fixed workload and does not provoke a sufficient stimulus (Shapiro et al., 1981, Periard et al., 2016), which also does not drive the same percentage of plasma volume expansion in females (possibly due to females resisting renin activity; Dustin et al., 1970; Tarazi, 1976), however with the implementation of an isothermic protocol, this response becomes more stable in men. In females, a traditional isothermic protocol still does not induce the cardiovascular and thermoregulatory stability typically accompanying plasma volume expansion, until ~10 days (Mee et al., 2015), which indicates that stable plasma volume

expansion may not be as established, and a longer or more intense protocol may be required (Mee et al., 2017). This could be a result of females retaining less salt as osmolality of the blood increases, as oestrogen may mediate aldosterone and ADH secretion (Spruce et al., 1985; Stachenfeld et al., 1998), as well as females having a higher renal sensitivity to ADH (Stachenfeld et al, 2001). Additionally, males have been observed to have increased levels of plasma ADH at a similar plasma osmolality, indicating a higher sensitivity to changes in plasma osmolality (Stachenfeld et al., 2001). There has been some research on plasma volume expansion following heat exposure in females, however it has been in sedentary or untrained participants, and produced confounding results because of the known plasma volume expansion that accompanies endurance exercise (Fellmann et al., 1992).

2.5.6 Lactate and substrate utilization. The added stressor of a hot environment results in a shift to a greater percentage of anaerobic substrate utilization (Dimri et al., 1980), resulting in a greater accumulation of blood lactate during submaximal exercise in the heat (Young et al., 1985), as it is an output of anaerobic metabolism. Blood lactate measurements are indicative of both the production of lactate and the body's ability to remove it, making it a consequence and a measurable marker of fatigue (although it is not necessarily a cause; Heller & Grahn, 2012; Brooks, 2001). It is therefore a useful measure to compare to direct findings and hypotheses of muscle metabolism, and allows us to speculate about the processes and adaptations happening at the cellular level.

It has been demonstrated that HA reduces aerobic metabolic rate and improves muscle glycogen sparing in males (Young et al., 1985; Febbraio et al., 1994). This is partially as a result of increased capacity for lactate removal by changes in regional blood flow and increased lipid oxidation (Kirwan et al., 1987), but reductions in epinephrine are likely to be

of greater influence (Febbraio et al., 1994). These factors influence the reductions in muscle and blood lactate following HA (Young et al., 1985). Acclimation may also increase power at lactate threshold, as observed during a graded exercise test in hot conditions in a study by Lorenzo et al. (2010).

There are known sex differences in the factors above, which may influence the reductions in muscle and blood lactate following HA. At low exercise intensity levels, females rely more on fat oxidation for fuel (Romijn et al., 2000, Horton et al., 1998) because of sex differences in the capacity for fatty acid metabolism (Soler-Argilaga & Heimberg 1976; Tarnopolsky et al., 1990; Jensen et al., 1994; Tarnopolsky et al., 2008), and glycogen and epinephrine levels are reduced in females (Horton et al., 2006). Females also exhibit an increased lipolytic response to this epinephrine (Crampes et al., 1989), however as females experience elevated levels of epinephrine when exposed to heat stress (Jezova et al., 1994), the gap in substrate utilization between males and females may be narrowed when exercise is undertaken in hotter conditions. Blood lactate response to endurance exercise has been observed as similar between sexes (Ohkuwa et al., 1988, Korhonen et al., 2005), however, these observations have been in temperate conditions, and possible sex differences in substrate utilisation and blood lactate responses following acute and chronic heat stress have not been investigated.

One caveat when using blood lactate as a measure of fatigue in hot conditions is that higher muscle glycogen stores have been observed at the point of fatigue in the heat in comparison to temperate (18°C) conditions. This indicates that other factors limit maximal exercise in the heat before glycogen can be depleted enough to make metabolism a limiting factor (Parkin et al., 2011). Therefore, blood lactate measures may be a marker that is more closely related to the causes of fatigue in cool than in hot conditions.

2.5.7 Heat shock response. Behind the systemic appearance of many physiological adaptations to chronic heat stress, there are cellular mechanisms at work protecting the organism. Heat shock proteins (HSPs) are the molecular response to heat stress, which drives thermotolerance at the cellular level and may contribute to other heat stress adaptations (Horowitz, 2014; Amorim et al., 2015; Akerman et al., 2016). HSPs can be divided into intracellular HSPs (iHSPs) and extracellular (eHSPs). Heat stress appears to have a greater impact on intracellular HSPs than extracellular HSPs (Tyler et al., 2016). Intracellular HSPs are activated by heat stress (among other stressors) and are responsible for refolding denatured polypeptides, keeping thermally damaged proteins from clustering (chaperoning), accelerating repair of these denatured proteins and maintaining structural proteins (Mosely et al., 1997; Asea, 2005). This prevents cell death, allowing the cells to withstand stressors that would have otherwise been fatal, and therefore acquire thermotolerance (Landry et al., 1982; Kregel, 2002). Thermotolerance may explain why some marathon runners, among other endurance athletes, are able to tolerate internal temperatures of 41.5°C, far past clinical heat stroke thresholds (Maron et al., 1997). This thermotolerance extends to other organs, such as the gut, where HSPs may be linked to a reduction in the heat-induced permeability of the epithelial monolayer and could decrease endotoxin leakage (Moseley et al., 1994).

HSP72, specifically, is found in the nucleus and cytosol of the cell, and is responsible for protein folding and cytoprotection, with other members of the HSP70 family found in the mitochondria having the additional role of molecular chaperone (Mayer & Bukau, 2005). Some of the HSPs are released from the cells in vacuoles formed from the cell membrane, called exosomes, where they then become extracellular HSPs (Lancaster & Febbraio, 2005). These eHSPs trigger an immune response and cytokine release (Asea et al., 2005), which, combined with their known chaperoning function, has earned HSPs the name of

"chaperokines" (Asea et al., 2003). Acute heat stress causes an upregulation of HSPs, although as acclimation develops, the heat shock response to acute heat exposure is reduced (Maloyan et al., 1999).

Heat shock proteins may have a threshold for activation, requiring a protocol considerably more intense than the athlete's normal training level, and even greater than the minimal requirements for physiological adaptations of heat acclimation (Gibson et al., 2014). In a study by Hom et al. (2012) participants cycled at 50% of their VO_{2max} for 90-minute sessions in 33°C, which induced classic physiological adaptations of heat acclimation, albeit in the absence of increases iHSPs. Cycling at only 50% VO_{2max} may not have been intense enough to meet the threshold for a heat shock response to be initiated. Gibson and colleagues (2014) suggest that this threshold can be reached by driving core temperatures to ~38.5°C during HA sessions. Once protocol intensities have reached this threshold and a heat shock response is initiated, it appears that an individual's heat shock response is linked with their ability to heat acclimate effectively (McClung et al., 2008). McClung and colleagues (2008) observed that an increased blunting of HSPs was correlated with the individual's ability to thermoregulate whilst exercising in hot conditions (49ºC, 20% RH).

While the heat shock protein response has been relatively well established and investigated in males for decades, sex-specific differences in humans were not observed until 2009 by Morton and colleagues. They observed that whilst males experienced a meaningful increase in HSPs (38 ± 41%) following 6 weeks of interval training, females did not experience any significant increase. This difference was hypothesized to be linked to findings in rodent studies, where female rodents have been observed to already exhibit higher basal levels of HSPs, therefore diminishing the necessity for further production, with the proposition that oestrogen is regulating this process (Paroo et al., 2002; Voss et al., 2003;

Shinohara et al., 2004; Hamilton et al., 2004; Rayner et al., 2008; Bombardier et al., 2009; GIllum et al., 2012). Oestrogen stimulates the release of HSPs in the unstressed state to increase basal HSP levels, providing cytoprotection when the cell comes under stress (Voss et al., 2003; Hamilton et al., 2004). This is supported by rodent studies in which female rodents exhibit reduced vascular (Otsuka et al., 1996; Hinojosa-Laborde et al., 2000; Rayner et al., 2008) and skeletal muscle damage (Paroo et al., 2002; Bombardier et al., 2009) following various stresses. Furthermore, evidence is drawn from various studies of rat hearts, one of which showing reduced basal HSP levels of rats which have had their ovaries removed, with HSP levels restored with oestrogen therapy (Paroo et al., 2002).

Although it has been suggested that females require greater levels of stress to upregulate HSP activity on account of their elevated basal HSP levels (Voss et al., 2003), there are no sex differences in transcription of HSP mRNA following chronic heat stress (Mee et al., 2016). This means that the both sexes are receiving similar signals for genetic upregulation of HSPs, and oestrogen's role in mediating the heat shock response is further downstream from transcription. The evolving linkage between oestrogen and HSPs may provide a source of explanation for some of the differences in responsiveness to heat stress between males and females.

2.5.8 Psychophysical and behavioural. Athletes commonly report higher ratings of perceived exertion (RPE; Borg 1962) with exercise under heat stress as a result of greater discomfort of the warmer ambient and skin temperatures, as well as greater perceived effort in the heat caused from the elevation in cardiovascular strain (Cheuvront et al., 2010). Ratings of thermal comfort and thermal sensation (Toner et al., 1986) reported by the athlete are also important indicators of how the environment may be impacting their performance. Overall,

RPE ratings and thermal sensation have an inverse relationship with muscular and aerobic performance (Nybo, 2014; Cabanac, 2006). These may be partially overridden, however, by external motivation factors, such as head-to-head competition (Corbett et al., 2017). Heat acclimation has been observed to improve RPE and thermal comfort and sensation in both males and females, however the temporal patterns of their improvements may differ.

Sunderland et al. (2008) reported an improvement in thermal sensation for females in hot conditions after only four days of heat acclimation, whereas James et al., (2016), Neal et al. (2016), and Gibson et al. (2015) failed to find any significant improvements in males following a STHA protocol, with perceptual changes only manifesting after completing the full 10-day protocol (Neal et al., 2016, Gibson et al., 2015). These improvements may stem from the reduction in cardiovascular strain following heat acclimation, allowing athletes to work at a lower $\%VO_{2max}$ than in pre-acclimation heat stress tests (Cheuvront et al., 2010), although comfort may also develop with the experience of performing in the heat during the acclimation protocol. While acclimation improves these psycho-physical ratings, it is still recommended that athletes wear clothing that allows sweat wicking when competing, as an accumulation of sweat adds a level of discomfort that cannot be combated with heat acclimation (Aoyagi et al., 1998).

Behavioural modifications also develop with heat acclimation, as athletes may improve their pacing strategies with heat acclimation (Racinais et al., 2014). In summary, although there has been little information investigating the role of psycho-physical adaptations on performance in the heat, the importance of its influence cannot be ignored.

2.6 Summary

There is still much to be done for sport science research to catch up to the success of female athletes, especially as it pertains to heat acclimation. As heat stress can cause severe physiological and cognitive detriments that affect performance, the investigation of optimal protocols for females to efficiently adapt is urgently needed, especially as it has been observed that females may require longer durations or elevated intensity to acquire the same cardiovascular and thermoregulatory stability as their male counterparts. Various factors, including size and sudomotor differences, sex hormones' effects on thermoregulation, endocrinological differences affecting fluid regulatory responses, and oestrogen's cytoprotective properties may be partially responsible for these discrepancies. Although evidence indicates that menstrual cycle and hormonal contraception have minimal, if any significant effect on performance, additional information is needed to confirm this. Finally, as new research emerges relating to heat acclimations ergogenic potential, female cohorts should be tested alongside males in a way that reflects their now similar participation and success in high level sport.

3. Methods

3.1 General Overview and Design

An overview of the study design is displayed in Figure 1. This study was approved by the University of Birmingham Ethics Committee, and conformed to the standards set by the Declaration of Helsinki. All participants were informed of the experimental procedures and possible risks involved in the study before their written consent was obtained. Each participant also completed a general exercise questionnaire and a menstrual cycle questionnaire. All testing procedures were completed in the environmental chamber laboratory in the School of Sport, Exercise and Rehabilitation Sciences building at the University of Birmingham. Participants performed all heat acclimation (HA) and testing sessions at the same time of day (± 2 hours), and at similar times to their normal training sessions so as not to disrupt their normal circadian rhythms (Winget et al., 1985; Reilly & Brooks 1986). Following preliminary procedures and familiarisations, 15-minute time trials were conducted in hot (40°C, 30% RH) and cool (15°C, 30% RH) conditions pre-acclimation, following 4-5 days HA, and following 9-10 days HA. One recovery day was taken before all baseline time trials, before all cool time trials (15°C, 30% RH), which coincided with a rest day between 5-day HA blocks, to minimise the carryover effect of fatigue. This experiment was conducted in the UK from April-June, when mean ambient temperatures were below 20°C (exclusive of n = 3 days; mean daily temperatures of 23°C, 24°C, and 27°C). The protocol was performed in a complementary fashion to normal training sessions (i.e., weight training and normal conditioning such as swimming and running). Participants were asked to refrain from alcohol and overly strenuous sessions outside of the laboratory 48 hours before time trials.

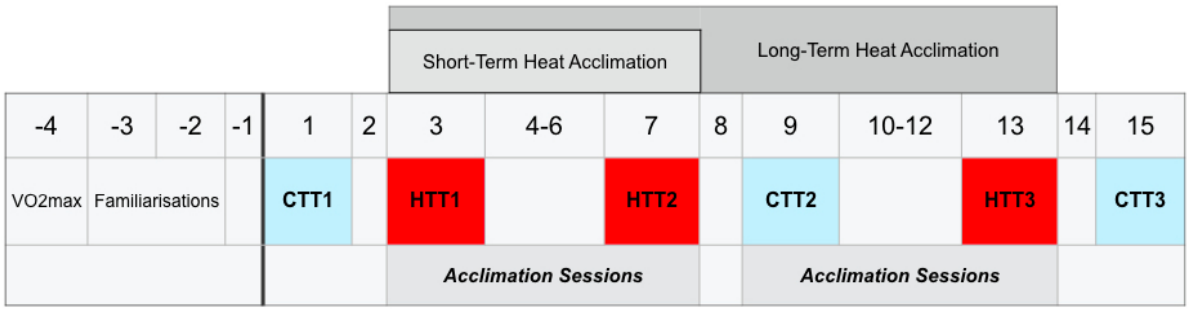

Figure 1. Schematic diagram of the experimental protocol. VO_{2max} tests and a minimum of two familiarisation trials were conducted pre-acclimation, with one day's rest before beginning the protocol. Time trials were conducted in hot (HTT; 35°C, 30% relative humidity (RH)) and cool conditions (CTT; 15°C, 30%RH), before (1) and after short-term (4-5 days; (2)) plus long-term (9-10 days; (3)) heat acclimation. Heat acclimation sessions (40°C, 30%RH) followed an isothermic protocol (where exercise intensity was manipulated for 90-minutes to attain rectal temperatures of ~38.5°C) with permissive dehydration. There was at least one day's rest between time trials.

3.2 Participants

Seven recreational endurance athletes aged 23-35 years volunteered for and completed this study. An additional participant (Participant 6) volunteered, but dropped out after preliminary testing, and was not included in the results. All participants were familiar with competitive, race-style endurance events, and corresponded to performance level of 3 according to classifications set for participants in sport-science research (De Pauw et al., 2013), when VO_{2max} is compared for similar female scores (Shvartz & Reibold, 1990). Participants were eumenhorreic or using various forms of hormonal contraceptives, including OCPs (oral contraceptive pill), contraceptive implant, and IUDs (intrauterine device), and did not report any negative premenstrual symptoms that could have affected performance during time trials (Giacomoni et al., 2000). All participants were previously unacclimated and had not been in hot conditions for the past three months. Personal characteristics are summarised in Table 1.

Table 1. Personal characteristics of participants

Participant	Age (years)	Height (cm)	$\dot{V}O_{2max}$ (ml/kg/ min)	Body mass (kg)	Contraceptive
1	32	168	40.0	54.1	OCP (Cilest)
2	28	168	44.9	58.8	Implant
3	25	176	42.5	64.8	Implant
4	23	165	43.4	55.3	EU
5	35	173	41.8	68.9	EU
7	32	172	52.7	63.0	IUD Coil
8	23	165	52.7	61.1	OCP (Yasmin)

OCP, Oral contraceptive pill user (pill brand); IUD Coil, Copper coil intrauterine device; EU, eumenhorreic natural cycle

3.3 Preliminary Testing

3.3.1 VO_{2max} test. During the first visit to the laboratory, participant height was recorded to 1 cm using a fixed stadiometer (Seca 217, Seca, Hamburg, Germany), and nude body mass recorded to 0.1 kg using digital scales (Seca 877, Seca, Hamburg, Germany). To determine VO_{2max}, a graded exercise test was performed at room temperature (~18°C) using a cycle ergometer (Sport Excalibur, Lode, Groningen, The Netherlands). During a 3-5 minutes warm-up, power output was adjusted according to ratings of perceived exertion (RPE; Borg 1962), until reaching an intensity level reported as 11 (i.e. "light"). The test began at this self-selected intensity, and resistance was applied to the flywheel to gradually increase intensity by 20W/min. The test was terminated when participants reached volitional exhaustion and/or the cadence could no longer be maintained at 80 ± 5 rpm despite strong verbal encouragement. Expired air was measured using a metabolic cart (Vyntus CPX, Jaeger, Wuerzberg, Germany) and VO_{2max} was determined by absolute peak VO_2 relative to body mass. Heart rate (HR) was recorded in the final minute of each stage using a HR monitor (Polar Electro, Kempele, Finland).

3.3.2 Familiarisations. Each participant completed a minimum of two familiarisation sessions, where they were re-briefed on the time trial protocol. Participants completed an

identical time trial protocol to that described below on a Velotron cycle ergometer (Velotron, RacerMate Inc., Seattle, WA). The familiarisations took place in cool conditions (15%°C, 30%RH), however HR was the only measurement collected. Participants were encouraged to become familiar with the gearing and the pacing strategies that they would use for the time trials.

3.4 Heat Acclimation Sessions

Participants were instructed undertake permissive dehydration by restricting water intake during the acclimation sessions as well as ~30-minutes after the sessions. Upon arrival to the laboratory, participants voided their bladder to provide a urine sample, which was analysed for urine osmolality (Osm_u; Osmocheck, Vitech Scientific Ltd., West Sussex, UK). Towel-dried, nude body mass was recorded before and immediately after each session as a measure of estimated sweat loss. Conditions during HA sessions were set to 40°C, 30%RH with a fan-generated airflow of ~3 m/s facing participants. All heat acclimation sessions and time trials were completed using a Velotron cycle ergometer, which was calibrated according to manufacturer instructions for each temperature and confirmed to exhibit < 1% deviation from calibration settings before each use. Following a 5-minute, self-selected warm-up, participants completed 15-minutes of high-intensity intervals, where maximum effort was given for 15-seconds, with 45-seconds of active recovery. The aim of the high-intensity intervals was to rapidly increase rectal temperature (T_{rec}). This was followed by an additional 5-minutes of self-paced active recovery, and 70-minutes of cycling at an intensity manipulated with the aim of further increasing T_{rec}, and maintaining it at ~38.5°C (Patterson et al., 2004; Garrett et al., 2012). On days that time trials (TT) preceded HA sessions, the TTs were used in place of the high-intensity intervals. Time exercising under heat stress totalled

90 minutes, plus a 5-minute warm up, aiming to increase T_{rec} to 38.5°C. RPE (Borg, 1962), Thermal Sensation, and Thermal Comfort (Toner et al., 1986) were recorded at 15-minute intervals during the HA sessions. Participants were instructed to refrain from fluid consumption as much as could be tolerated during HA sessions to induce the added stressor of dehydration (Garrett et al., 2014). Water bottles were weighed to 0.001 kg before and after HA sessions and the difference was added to estimated sweat loss. Exercise was terminated (zero incidences) if $T_{rec} \geq 39.5$ °C, or the participant withdrew due to volitional exhaustion, or the participants could no longer maintain exercise intensity despite strong verbal encouragement. Heat acclimation involved two, five consecutive day blocks of HA sessions (STHA, plus LTHA), with 1-day's rest in between.

Participants inserted a rectal thermistor (Mon-a-Therm, Covidien, Mansfield, MA) 10 cm past the anal sphincter before each session to measure T_{rec}. Skin temperature (T_{sk}) was recorded using skin thermistors (Squirrel Thermal Couples, Grant Instruments, Cambridge, UK) attached to four sites: the mid-point of the right pectoralis major (T_{chest}), midpoint of the triceps brachii lateral head (T_{arm}), right rectus femoris (T_{thigh}), and right gastrocnemius lateral head ($T_{lower\ leg}$). Skin and rectal thermistors were connected to a Squirrel temperature logger (Squirrel 2020 series, Eltek, Ltd., UK) and were recorded at 30-second intervals throughout HA sessions and TTs. Heart rate (Polar Electro, Kempele, Finland) was also recorded throughout each session. Power output and distance cycled were recorded by the Velotron Coaching Software (Velotron CS 2008, RacerMate Inc., Seattle, WA).

3.5 Time Trials

Participants were instructed to maintain normal hydration before each time trial, which was verified with an Osm_u value of ≤ 700 mOsm/kg (Sawka et al., 2007). Towel-dried, nude body mass was recorded before and immediately after each trial as a measure of estimated

sweat loss. Participants lay supine for 10 minutes of stabilisation prior to each trial in order collect resting measures of HR, T_{rec}, blood lactate, Haemoglobin (Hb) and Haematocrit (Hct). Blood lactate measures were recorded by extracting a finger-tip blood sample using an automated lancet (Unistik 3 Comfort, Owen Mumford, Oxfordshire, UK) and then immediately analysing the sample with a Lactate Plus analyser (Lactate Plus, Nova Biomedical, Waltham, MA). Hb and Hct were recorded using a syringe (15-mL BD Plastipak, BD Medical, Madrid, Spain) for venous blood draw from the antecubital vein. The blood sample was immediately pushed from the syringe directly onto a sample cartridge (i-STAT 6+ Cartridge, Abbott, Maidenhead, UK) and analysed using an i-STAT blood analyser (i-STAT 1, Abbott, Maidenhead, UK). Hb and Hct measures were recorded for n = 3 participants. Supplies for measures of Hct and Hb were not yet available whilst the other participants (n = 4) were undergoing the HA protocol. Participants entered the environmental chamber and commenced a 5-minute warm up at a self-selected pace, before completing a 15-minute, self-paced time trial. Power output and distance cycled were recorded continuously by the Velotron Coaching Software (Velotron CS 2008, RacerMate Inc., Seattle, WA). Participants were aware of the time elapsed, as displayed by a stop-clock mounted to the handles of the cycle ergometer, however they did not have access to any other physiological or performance feedback (i.e., HR, power output, distance cycled, etc.) so as not to influence motivational factors. Participants were given equal verbal encouragement by the same researchers at similar time points during the trial. Free drinking was permitted during TTs, and pre- and post-trial water bottle mass was considered when accounting for estimated sweat loss. RPE and blood lactate were recorded immediately following the TTs. Participants then completed 5 minutes of self-paced active recovery before exiting the environmental chamber.

Time trials were performed in cool conditions (15°C, 30%RH) pre-acclimation (CTT1), following STHA (CTT2), and following LTHA (CTT3). A schematic describing the CTTs is displayed in Figure 2. Time trials were also performed in hot conditions (40°C, 30%RH with a fan-generated airflow of 0.30 m/s facing participants) on the first day of heat acclimation (HTT1), on the final day of STHA (HTT2), and on the final day of LTHA (HTT3; Figure 2). Ratings of Thermal Comfort and Thermal Sensation (Toner et al., 1986) were reported inside the environmental chamber, preceding the warm-up for HTTs, as well as immediately after. Sweat gland activity was also recorded immediately following HTTs. Active sweat glands were quantified using a modified-iodine paper technique with computer aided analysis (Gagnon et al., 2012). This technique involves impregnanting 100% cotton paper with iodine for 48 hours before use, and cutting papers of exactly the same dimensions (28mm diameter circle) using a craft punch. Three pre-cut papers were applied evenly to the dorsal forearm using a flat plastic surface for 6-seconds immediately following the HTTs. When the paper is applied to the skin, purple dots appear where the fluid secreted by the sweat glands contacts the saturated paper. Each paper was scanned at the highest resolution (600x600dpi) and the clearest paper was subjectively selected for analysis. The dots were then quantified with computer aid (ImageJ, available from the NIH at http://rsbweb.nih.gov/ij/; Gagnon et al., 2012).

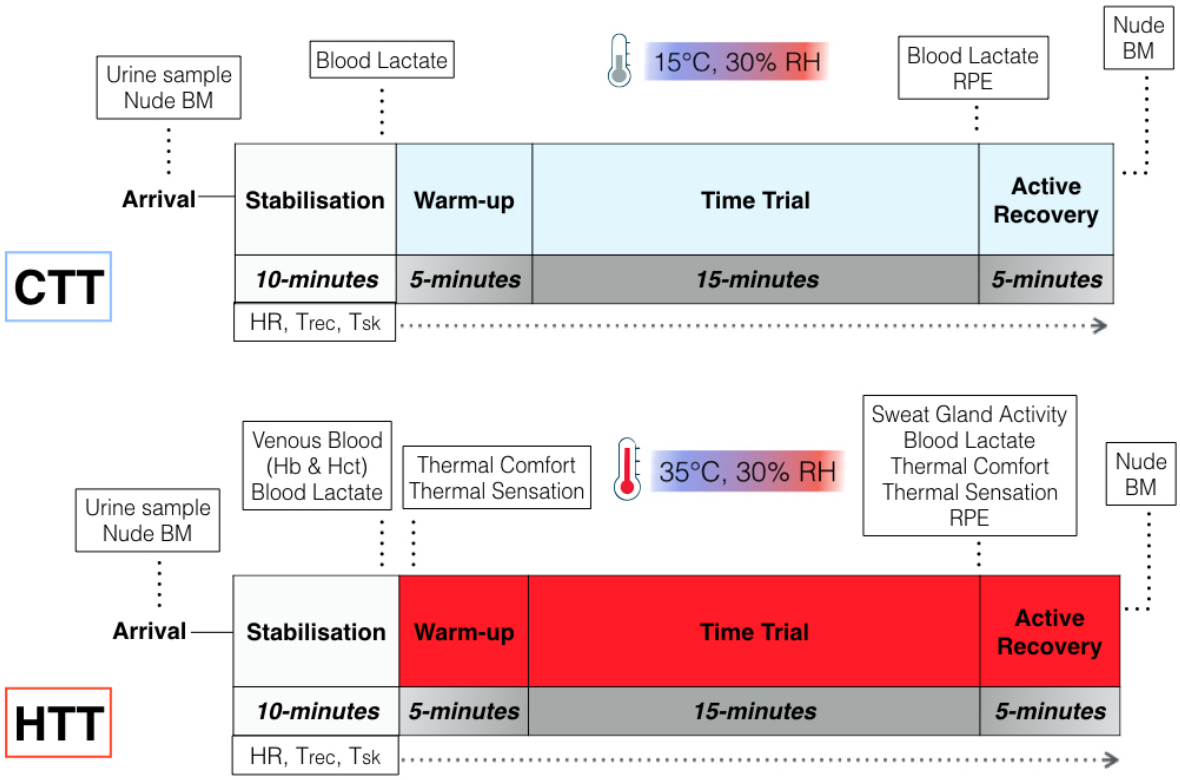

Figure 2. Schematics of physiological measurements taken during time trials in cool (CTT) and hot (HTT) conditions.
HR, heart rate; T_{rec}, rectal temperature; T_{sk}, skin temperature; BM, body mass; RPE, rating of perceived exertion (Borg, 1962); Hb, haemoglobin; Hct, haematocrit; RH, relative humidity

3.6 Data Analysis

Mean rectal temperatures for the final 75 minutes of the session, which followed the 15-minute high-intensity intervals, is represented by T_{rec75}. Maximum rectal temperature recorded during the session (Max T_{rec}) was used to calculate rectal temperature increase from rest (ΔT_{rec}). T_{sk} was calculated as a weighted average according to Ramanathan (1964):

$$T_{sk} = 0.3 \times (T_{chest} + T_{arm}) + 0.2 \times (T_{thigh} + T_{lower\ leg})$$

Estimated sweat rate relative to body surface area (SR_{BSA}) was calculated from changes in nude body mass (NBM) from pre- to post-session with considerations for water consumed, relative to body surface area (BSA; calculated using the formula derived by Du Bois & Du Bois, 1916), as normalised for exercise time:

Estimated sweat loss (g) = (pre-trial NBM – post-trial NBM) + (water bottle pre-trial – water bottle post-trial)

$$BSA\ (m^2) = 0.007184 \times (height^{0.725} \times body\ mass^{0.425})$$

$$SR_{BSA}\ (g/h/m^2) = (estimated\ sweat\ loss) / (1\text{-}hour / exercise\ time) / (BSA)$$

Two values were obtained for measurements of resting blood lactate and an additional two values were obtained for blood lactate immediately following TTs. The results were averaged to yield a single value for each time point (pre- and post-trial). Extreme outliers falling outside the physiological range were excluded, and only the rational value was used (Goodwin et al., 2007; n = 3).

Relative changes in plasma volume (n = 3) were determined using changes in the concentration of Hb and Hct, as described by Dill and Costill (1974). The subscripts 1 and 2 refer to time points in chronological order. Blood volume 1 (BV_1) is assumed as 100%, and therefore 100.

Blood volume

$$BV_1 = 100\%$$

$$BV_2 = BV_1 \times (Hb_1/Hb_2)$$

Red blood cell volume

$$CV_1 = BV_1 \times (Hct_1)$$

$$CV_2 = BV_2 \times (Hct_2)$$

Plasma volume

$$PV_1 = BV_1 - CV_1$$

$$PV_2 = BV_2 - CV_2$$

Percent changes

$$\Delta BV, \% = 100 \times (BV_2 - BV_1) / BV_1$$

$$\Delta CV, \% = 100 \times (CV_2 - CV_1) / CV_1$$

$$\Delta PV, \% = 100 \times (PV_2 - PV_1) / PV_1$$

Power output (watts) was recorded per second during TTs, and an average of each minute's power output was used to calculate area under the curve (AUC; Pruessner et al.,

2003). AUC was also calculated for T_{rec} (recorded at 30-second intervals) during TTs in both environmental conditions.

All data were analysed using SPSS statistical software (SPSS version 24.0.0, SPSS, Chicago, IL). To assess performance and physiological differences during STHA and LTHA, between HTT1, HTT2, and HTT3, and between CTT1, CTT2, and CTT3, a repeated-measures one-way analysis of variance (ANOVA) was performed. AUC comparison of power output and T_{rec} were also analysed using a one-way ANOVA. If significance was found, Bonferroni-corrected post-hoc comparisons were undertaken to isolate the effect. All data are expressed as means ± SD, and the threshold for significance was set at the $p < 0.05$ level for each analysis.

4. Results

4.1 Heat Acclimation Sessions

4.1.1 STHA vs. LTHA. Mean T_{rec75} was lower during long-term heat acclimation (LTHA) sessions (Days 6-10) as compared to short-term heat acclimation (STHA) sessions (Days 1-5; $p = 0.020$). No other significant physiological or psychophysical differences were observed between STHA and LTHA ($p > 0.05$). Heat acclimation (HA) results are summarized in Table 2. Athletes' average RPE ratings for sessions were between 14-15 ("Hard"), and mean power output during the sessions were not different between STHA and LTHA.

Table 2. Performance, physiological, and psycho-physical responses across short-term heat acclimation (Days 1-5) as compared to long-term heat acclimation (Days 6-10). Data are presented as mean ± SD

	STHA	LTHA
Mean Power Output (W)	108.1 ± 18.6	112.9 ± 21.6
Mean T_{rec75} (°C)	38.3 ± 0.2	38.1 ± 0.2*
Resting T_{rec} (°C)	37.3 ± 0.4	37.2 ± 0.3
Max T_{rec} (°C)	38.6 ± 0.2	38.5 ± 0.2
ΔT_{rec} (°C)	1.3 ± 0.4	1.2 ± 0.4
Mean HR (bpm)	149 ± 6	145 ± 7
SR_{BSA} (g/h/m^2)	680 ± 138	713 ± 127
Urine Osmolality (mmol/kg)	383 ± 91	476 ± 120
RPE	15 ± 2	14 ± 2
Thermal Sensation	10 ± 1	10 ± 1
Thermal Comfort	5 ± 1	5 ± 2

STHA, short-term heat acclimation (Days 1-5); LTHA, long-term heat acclimation (Days 6-10); T_{rec}, rectal temperature; $T_{rec75,}$ rectal temperatures recorded during the final 75-minutes of the session; ΔT_{rec}, change in rectal temperature from rest; HR, heart rate; SR_{BSA}, estimated sweat rate relative to body surface area; RPE, ratings of perceived exertion. *Significantly different from STHA ($p < 0.05$)

4.1.2 Resting measures. Resting rectal temperatures decreased across the 10 days of HA (main effect: $p = 0.038$, Table 3). Post-hoc comparisons revealed that resting T_{rec} was lower following 10 (-0.26 ± 0.17 °C; $p = 0.028$) but not 5 days of HA ($p > 0.05$).

There were not differences in HR at rest (Table 3), although there was a trend for improvement ($p = 0.08$), as 6 of 7 participants experienced a decrease in resting heart rate averaging 8 ± 5 bpm following 10 days of HA.

Table 3. Resting physiological measures recorded following stabilisation at room temperature. Data are presented as mean ± SD

Resting measure	Pre-Acclimation	Post-STHA	Post-LTHA
HR (bpm)	64 ± 10	63 ± 12	58 ± 11
T_{rec} (°C)	37.44 ± 0.18	37.28 ± 0.29	37.18 ± 0.26*

STHA, following 5 days of heat acclimation; LTHA, following 10 days of heat acclimation; HR, heart rate; T_{rec}, rectal temperature.
*Significant increase from pre-acclimation ($p < 0.05$).

Plasma volume data obtained at rest prior to HTTs (n=3), as calculated via haematocrit (Hct) and haemoglobin (Hb) content, showed no change ($p > 0.05$). Individual data are shown in Table 4.

Table 4. (A) Haematocrit and haemoglobin levels measured at rest before trials pre-acclimation (HTT1), following 4 days of heat acclimation (HTT2), and following 9 days of heat acclimation (HTT3), and (B) relative percent change in plasma volume across the protocol.

A.

	HTT1		HTT2		HTT3	
Participant	Hct (%PCV)	Hb (g/dL)	Hct (%PCV)	Hb (g/dL)	Hct (%PCV)	Hb (g/dL)
5	37	12.6	39	13.3	34	11.6
7	43	14.6	38	12.9	41	13.9
8	39	13.3	35	11.9	37	12.6
mean	40	13.5	37	12.7	37	12.7

B.

	Relative Plasma Volume Change (%)		
Participant	HTT1 ⇒ HTT2	HTT2 ⇒ HTT3	HTT1 ⇒ HTT3
5	-8.3	24.1	13.8
7	23.1	-11.7	8.7
8	19.1	-8.5	9.0
mean	11.3	1.3	10.5

Hct, haematocrit; Hb, haemoglobin; (n = 3).

4.2 Hot Time Trials

4.2.1 Performance. Distance cycled during time trials in hot conditions increased 260 m ± 150 m (3.3% ± 2.0%) from HTT1 to HTT3 (main effect: $p = 0.008$; Figure 3). Post-hoc analysis indicated that distance was greater in HTT3 ($p = 0.017$) as compared to HTT1, but not HTT2 as compared to HTT1 ($p > 0.05$). Additionally, distance cycled increased from HTT2 to HTT3 ($p = 0.022$; Table 5).

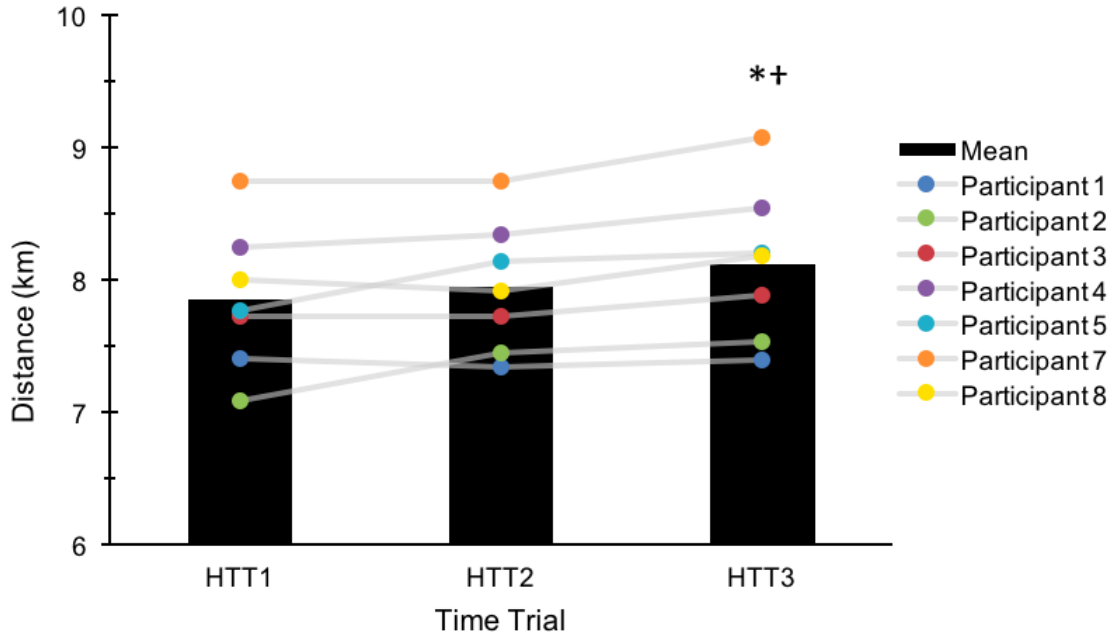

Figure 3. Distance cycled during time trials in the heat pre-acclimation (HTT1), following 4-days heat acclimation (HTT2), and following 9-days heat acclimation (HTT3). *Significant increase from HTT1 ($p < 0.05$); †Significant increase from HTT2 ($p < 0.05$).

Table 5. Performance measures during time trials in the heat. Data are presented as mean ± SD

	HTT1	HTT2	HTT3
Distance (km)	7.85 ± 0.50	7.95 ± 0.46	8.11 ± 0.54*†
Average power (W)	174.1 ± 28.9	179.0 ± 26.8	189.4 ± 32.8*†
Peak power (W)	225.6 ± 37.9	237.7 ± 52.1	268.0 ± 58.8*†

HTT1, pre-acclimation trial; HTT2, trial following 4-days heat acclimation; HTT3, trial following 9-days heat acclimation. *Significant increase from HTT1; †Significant increase from HTT2 ($p < 0.05$).

These results were paralleled by mean power output across the HTTs (main effect: $p = 0.004$). Post hoc analysis revealed that improvements were attained in HTT3 as compared to HTT1 (+15.3 W ± 8.7 W (+8.8% ± 5.4%); $p = 0.015$), but not in HTT2 as compared to HTT1 ($p > 0.05$; Table 5). Additionally, power output during HTT3 was greater than in HTT2 (+10.4 W ± 7.4 W (+5.8% ± 4.1%); $p = 0.040$). AUC for power output during HTTs were calculated from minute averages (Figure 4) and a main effect was observed between HTTs ($p = 0.005$). Post hoc comparisons revealed that power output AUC during HTT3 was greater than in HTT1 ($p = 0.016$) and showed a trend towards increases from HTT2 ($p = 0.057$).

Power output AUC was not different between HTT1 and HTT2 ($p > 0.05$). Peak power output also increased as a result of LTHA (main effect; $p = 0.028$), with post hoc comparisons revealing that increases were observed in HTT3 in comparison to HTT2 ($p > 0.05$; Table 5). All significant performance improvements from HTT1 were observed during HTT3 only.

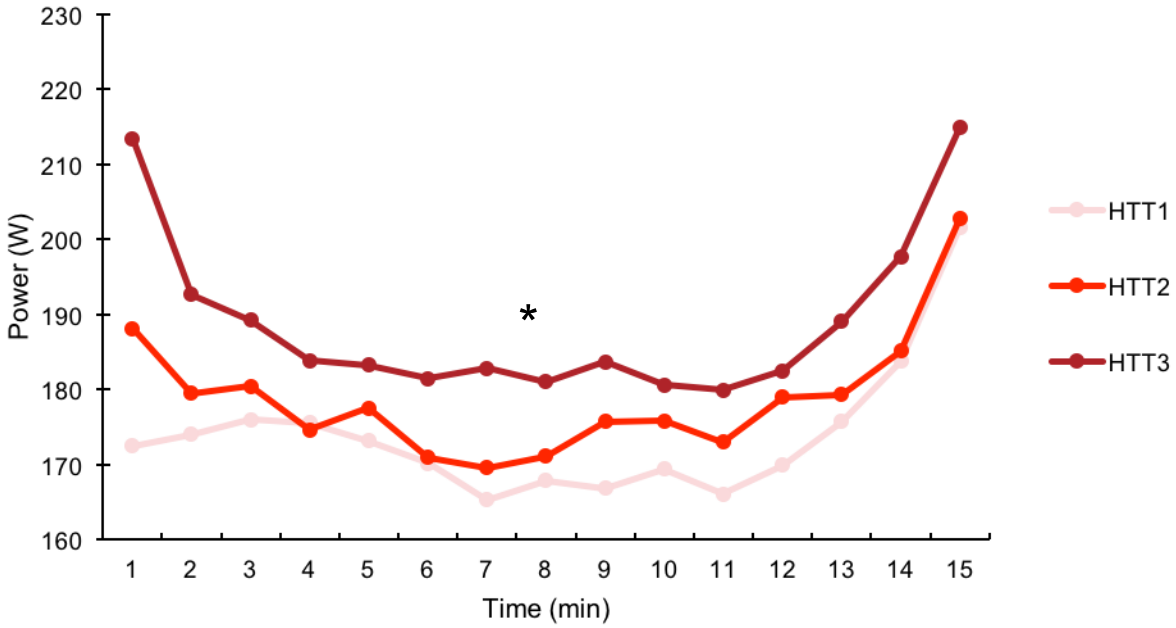

Figure 4. Power output during 15-minute time trial in hot conditions (40°C, 30% RH) performed pre-acclimation (HTT1), following 4-days heat acclimation (HTT2), and following 9-days heat acclimation (HTT3). Each data point is an average of the preceding minute. *Area under curve (AUC) significantly different from HTT1 ($p < 0.05$).

Power output during the time trial was also separated into the start, middle, and "end-spurt", which consisted of minute 0-2, minute 2-13, and minute 13-15, respectively (Figure 5). There was a main effect observed between the starting segments of the HTTs ($p = 0.039$), with an increase in power output of 29.8 W ± 28.8 W (20% ± 17%) at the start of HTT1 as compared to the start of HTT3, however post-hoc analysis could not locate the location of the differences.

There was a main effect between mean power outputs during the middle portion of the HTTs ($p = 0.011$; Figure 5). Post-hoc comparisons revealed that power output during the middle of HTT3 was greater than in the middle of HTT1 (+12.8 W ± 9.2 W (+8% ± 6%); $p =$

0.03), but not HTT2 as compared to HTT1 ($p > 0.05$). There was a main effect between mean power outputs during the end-spurt of the HTTs ($p = 0.006$; Figure 5). Post-hoc analysis indicated that power output during the end spurt was greater in HTT3 as compared to HTT1 (+13.6 W ± 4.4 W (+7% ± 2%); $p = 0.001$), but not HTT2 as compared to HTT1 ($p > 0.05$).

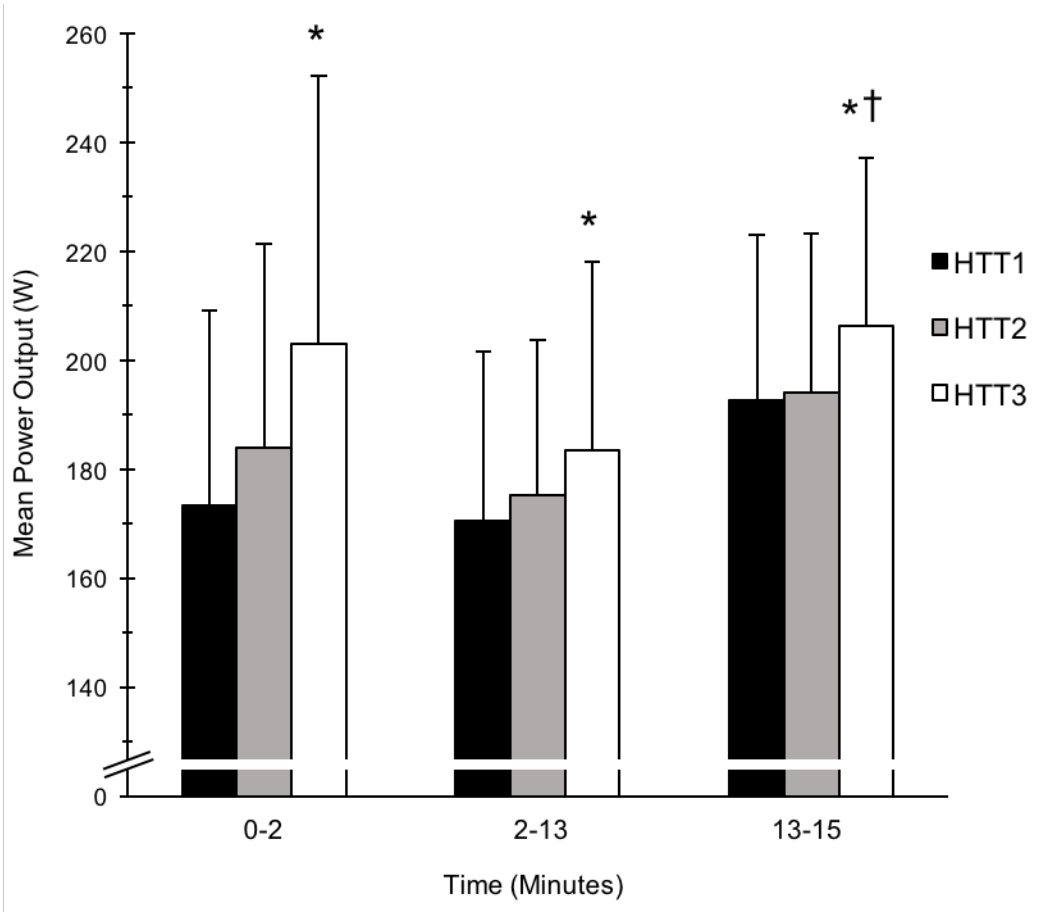

Figure 5. Mean power output during of start, middle, and "end-spurt" of time trials in hot conditions performed pre-acclimation (HTT1), following 4-days heat acclimation (HTT2), and following 9-days heat acclimation (HTT3). Data are an average of power output during minutes 0-2, minutes 2-13, and minutes 13-15. *Significant increase from HTT1 ($p < 0.05$); †Significant increase from HTT2 ($p < 0.05$).

4.2.2 Thermoregulatory. There were no statistically significant differences in rectal temperatures during the HTTs (Figure 6). Mean and maximum skin temperatures (T_{sk}) during HTTs were not affected by HA ($p > 0.05$; Table 6).

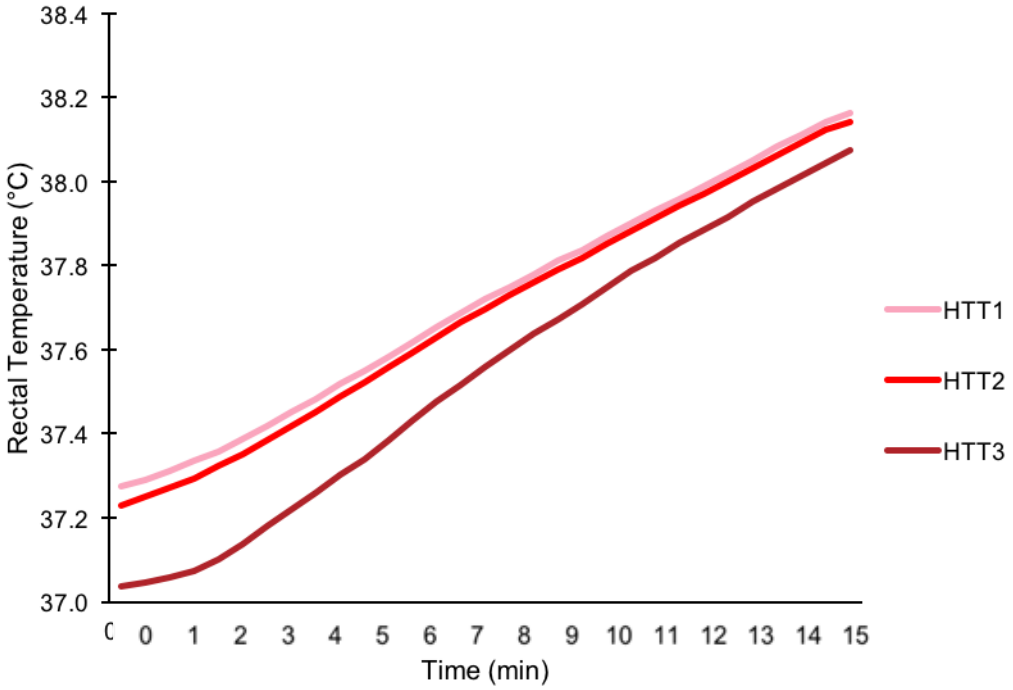

Figure 6. Rectal temperature recorded at 30-second intervals during time trials in hot conditions pre-acclimation (HTT1), following 4-days heat acclimation (HTT2), and following 9-days heat acclimation (HTT3).

4.2.3 Cardiovascular. There were no significant differences in HR during TTs in hot conditions (Table 6; $p > 0.05$).

Table 6. Physiological measures recorded during time trials in hot conditions. Data are presented as mean ± SD

	HTT1	HTT2	HTT3
Thermoregulatory			
Mean T_{rec} (°C)	37.64 ± 0.23	37.69 ± 0.32	37.54 ± 0.28
Max T_{rec} (°C)	38.03 ± 0.29	38.07 ± 0.32	38.09 ± 0.46
ΔT_{rec} (°C)	0.88 ± 0.50	0.91 ± 0.33	1.04 ± 0.65
Mean T_{sk} (°C)	33.86 ± 2.09	34.02 ± 1.18	34.06 ± 1.00
Max T_{sk} (°C)	34.97 ± 0.59	34.75 ± 0.65	34.81 ± 0.47
Cardiovascular			
Mean HR (bpm)	167 ± 14	164 ± 9	167 ± 12
Sudomotor response			
SR_{BSA} (g/h/m^2)	613 ± 105	676 ± 252	772 ± 114*
Sweat loss (%BW)	2.6 ± 0.5	2.8 ±1.0	3.2 ± 0.5*
Active sweat glands/sq. inch	395 ± 135	422 ± 153	494 ± 157*†
Blood lactate			
Pre-Test (mmol/L)	1.2 ± 0.7	1.0 ± 0.4	1.0 ± 0.5
Post-Test (mmol/L)	11.8 ± 3.6	11.9 ± 2.0	13.4 ± 2.1

HTT1, pre-acclimation trial; HTT2, trial following 4-days heat acclimation; HTT3, trial following 9-days heat acclimation.
T_{rec}, rectal temperature; ΔT_{rec}, change in rectal temperature during trial; T_{sk}, weighted mean skin temperature; HR, heart rate; SR_{BSA}, estimated sweat rate relative to body surface area; Sweat Loss $_{BSA}$, estimated sweat loss during trial relative to body surface area.
*Significant increase from pre-acclimation trial ($p < 0.05$); †Significant increase from HTT2 ($p < 0.05$).

4.2.4 Sudomotor. There were no significant differences in measures of sudomotor activity following STHA. Sudomotor activity was elevated following LTHA, as demonstrated by an increased estimated sweat rate relative to body surface area (SR_{BSA}) from HA Day 1 (613 ± 105 g/h/m^2) to HA Day 10 (772 ± 114 g/h/m^2; $p = 0.018$), as well as estimated sweat loss relative to percent body mass (2.6 ± 0.5% to 3.2 ± 0.5*%; p* = 0.034). Active sweat gland density during HTTs also increased following LTHA, from 395 ± 135 active sweat glands/sq. inch immediately following HTT1 to 494 ± 157 active sweat glands/sq. inch immediately following HTT3 ($p = 0.016$); with *post-hoc* analysis locating the difference to be between HTT2 and HTT3 ($p < 0.01$; Figure 7). Results are summarized in Table 6.

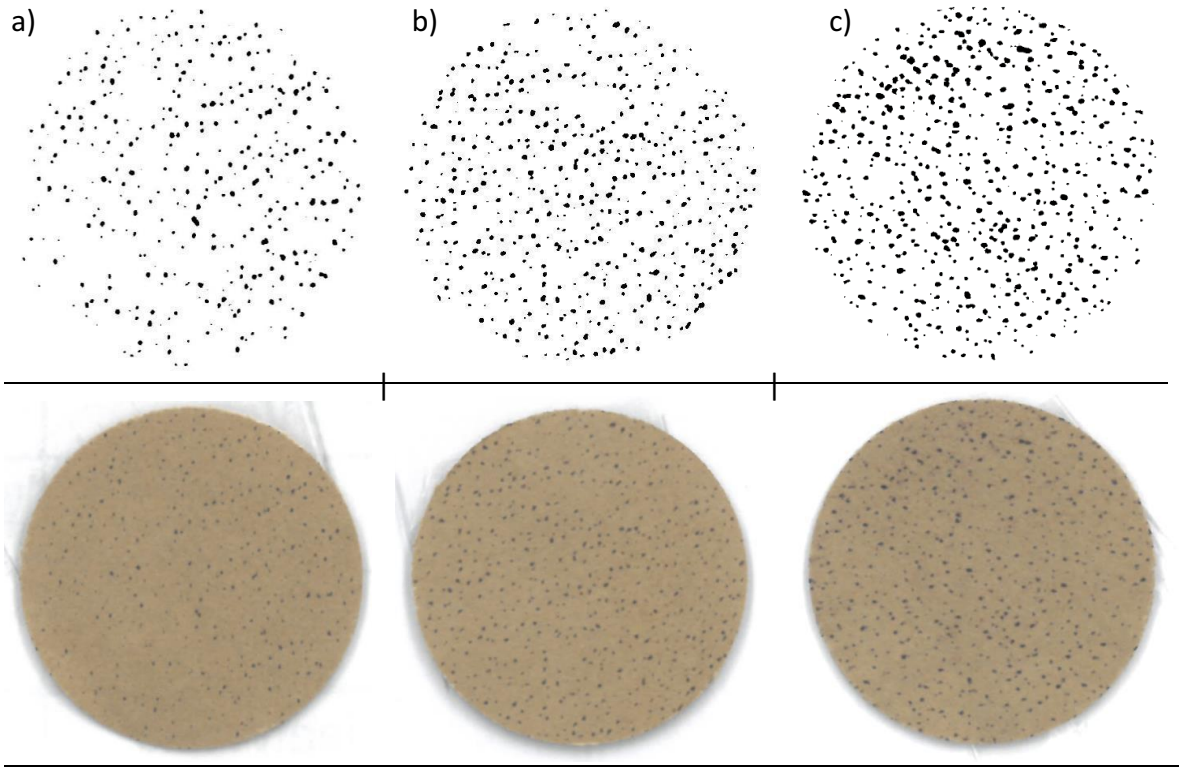

Figure 7. Example of sweat gland activity measured immediately following trials a) pre-acclimation (HTT1), b) following 4 days of heat acclimation (HTT2), and c) following 9 days of heat acclimation (HTT3). Bottom row images are scanned copies of iodine-cotton paper applied to participant's skin. Top row of images are the same images following computer processing (ImageJ, available from the NIH at http://rsbweb.nih.gov/ij/; Gagnon et al., 2012).

4.2.5 Blood Lactate. There were no differences in blood lactate across HTTs at rest or immediately following any of the HTTs ($p > 0.05$), although blood lactate did increase from pre- to post-HTT ($p < 0.01$; Table 6).

4.2.6 Psychophysical. Mean RPE ratings at the end of HTTs were 18 ± 1 (HTT1), 18 ± 1 (HTT2), and 19 ± 1 (HTT3). This confirms all participants gave a maximal effort during performance test and that the effort given was not different between tests ($p > 0.05$).

Psycho-physical ratings of thermal comfort and thermal sensation (Toner et al., 1986) did not change across the heat acclimation protocol (Table 7; $p > 0.05$).

Table 7. Thermal comfort and thermal sensation ratings reported immediately before and after each time trial in hot conditions. Data are presented as mean ± SD

	HTT1	HTT2	HTT3
Thermal comfort			
Pre-Test	2 ± 2	2 ± 1	2 ± 1
Post-Test	5 ± 1	5 ± 2	4 ± 2
Thermal sensation			
Pre-Test	9 ± 1	9 ± 1	8 ± 1
Post-Test	10 ± 1	10 ± 1	10 ± 1

HTT1, pre-acclimation time trial; HTT2, time trial following 4-days of heat acclimation; HTT3, time trial following 9-days of HA.

4.3 Cool Time Trials

4.3.1 Performance. Participants cycled 210 ± 150 m (2.4% ± 2.3%) further in CTT3 than in CTT1 (8.21 ± 0.52 m vs. 7.99 ± 0.64m, respectively; main effect: $p = 0.039$; Figure 8). Post-hoc comparisons revealed that improvements were only observed following LTHA ($p = 0.038$), with no improvement in distance cycled from CTT1 to CTT2 ($p > 0.05$; Table 8).

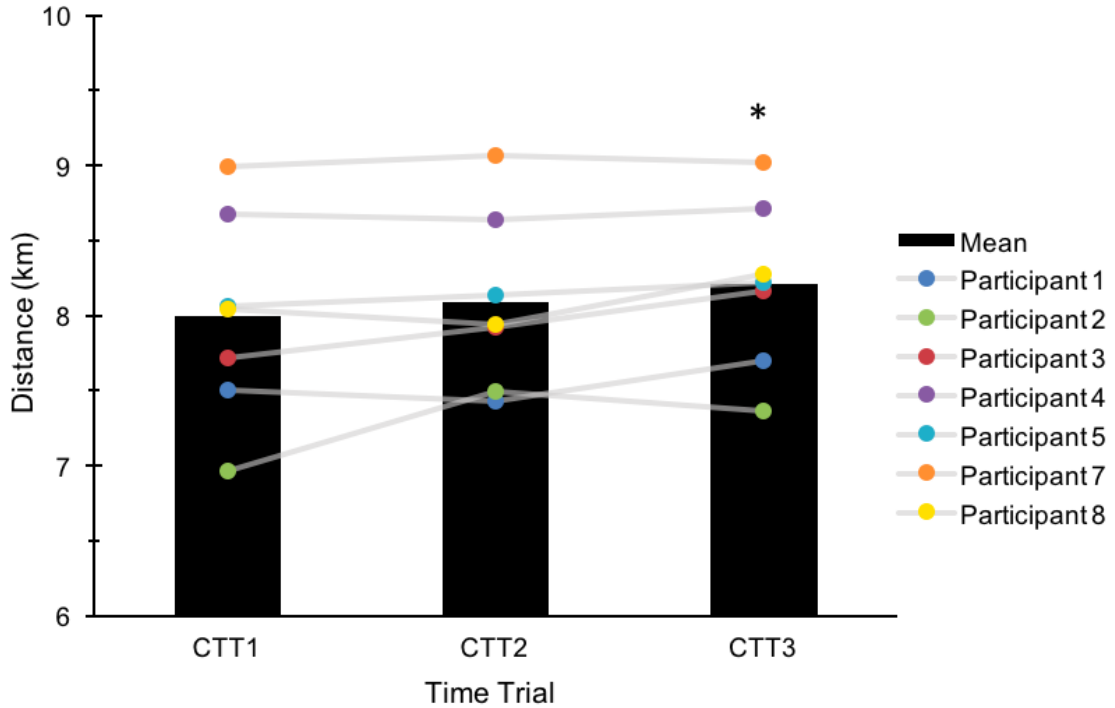

Figure 8. Distance cycled during cool time trials (15°C, 30% RH) pre-acclimation (CTT1), following 5-days heat acclimation (CTT2), and following 10-days heat acclimation (CTT3). *Significant increase from CTT1 ($p < 0.05$)

Table 8. Performance measures during trials in the cool conditions (CTT; 15°C, 30% RH). Data are presented as mean ± SD

	CTT1	CTT2	CTT3
Distance (km)	7.99 ± 0.64	8.09 ± 0.55	8.21 ± 0.52m*
Average power (W)	183.6 ± 37.0	187.7 ± 33.7	194.4W ± 31.5*
Peak power (W)	247.0 ± 50.8	247.7 ± 56.9	278.0 ± 68.4

CTT1, pre-acclimation trial; CTT2, trial following 5-days heat acclimation; CTT3, trial following 10-days heat acclimation. *Significant increase from HTT1 ($p < 0.05$).

Mean power output increased 10.7 W ± 7.7 W (6.8% ± 5.1%) from CTT1 (183.6 W ± 37.0 W) to CTT3 (194.4 W ± 31.5 W; main effect: $p = 0.034$). Post-hoc tests revealed differences between CTT1 and CTT3 ($p = 0.040$), but mean power output was not increased in CTT2 in comparison to CTT1 ($p > 0.05$). There was no effect of heat acclimation on peak power output, although a trend towards an increase was observed ($p = 0.058$; Table 8). AUC for power output during CTTs was calculated from minute averages (Figure 9) and a main effect was observed between CTTs ($p = 0.033$). Post-hoc analysis indicated an increase in AUC in CTT3 in comparison to CTT1 ($p = 0.034$), but not for CTT2 as compared to CTT1 ($p > 0.05$).

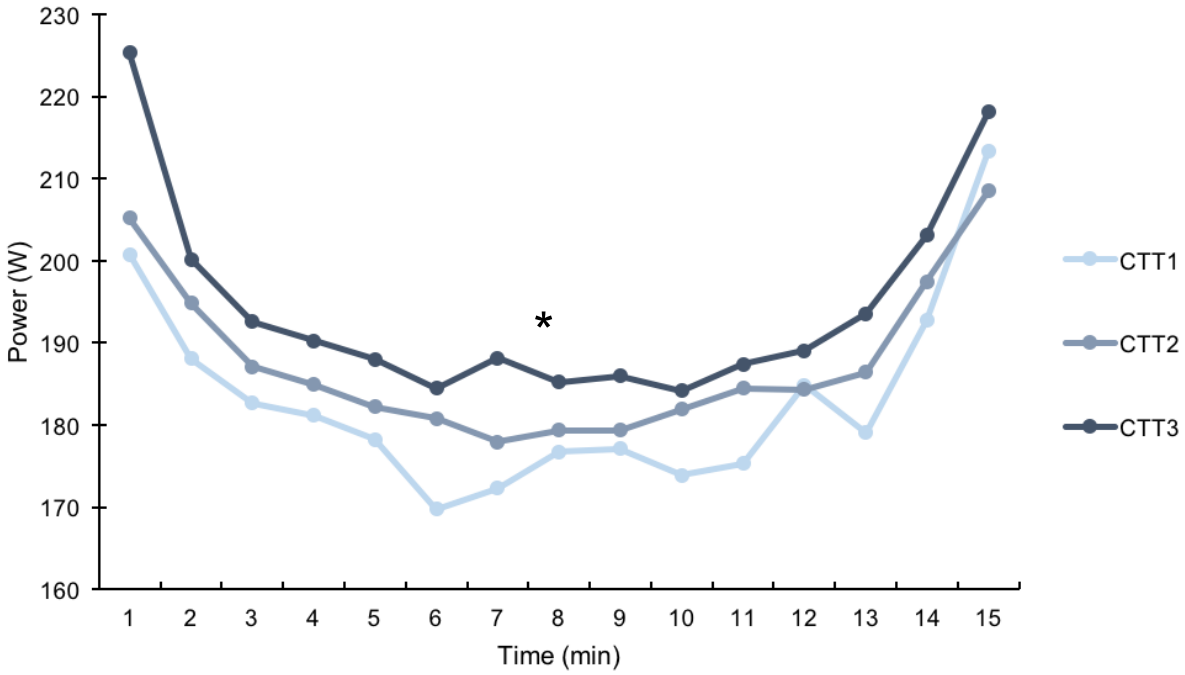

Figure 9. Power output during 15-minute time trials in cool conditions (15°C, 30% RH) performed pre-acclimation (CTT1), following 5-days heat acclimation (CTT2), and following 10-days heat acclimation (CTT3). Each data point is an average of the preceding minute. *Area under the curve (AUC) different from CTT1 ($p < 0.05$).

There was no significant difference between the start, middle, and "end-spurt" segments of any CTT (main effect: $p > 0.05$; Figure 10). However, there was a trend towards a main effect of mean power output between the start portion of CTTs ($p = 0.087$), as well as a trend for a main effect of mean power output between the middle portion of CTTs ($p = 0.051$).

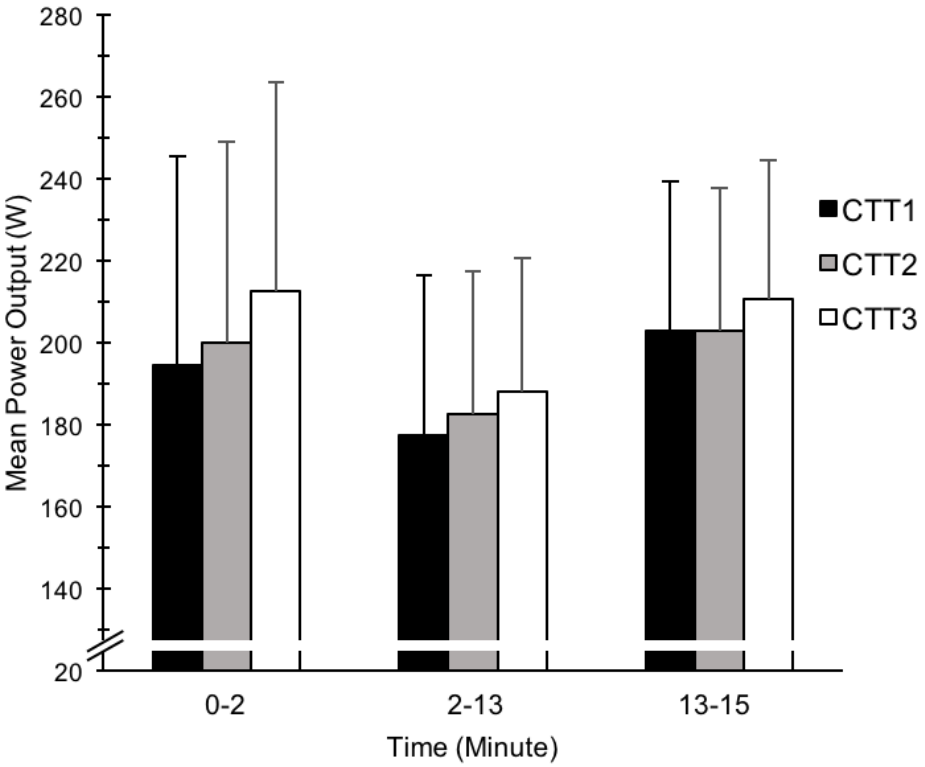

Figure 10. Mean power output during of start, middle, and "end-spurt" of time trials in cool conditions (15°C, 30% RH) performed pre-acclimation (CTT1), following 5-days heat acclimation (CTT2), and following 10-days heat acclimation (CTT3). Data is an average of power output during minute 0-2, minute 2-13, and minute 13-15.

4.3.2 Thermoregulatory. AUC for rectal temperature during CTTs was calculated from 30-second measurements (Figure 9) and a main effect was observed between CTTs (p = 0.049). Post-hoc analysis indicated an increase in AUC in CTT3 in comparison to CTT1 (p = 0.034), but not for CTT2 as compared to CTT1 ($p > 0.05$; Figure 11). Mean and maximum skin temperatures during CTTs were not affected by HA ($p > 0.05$; Table 9).

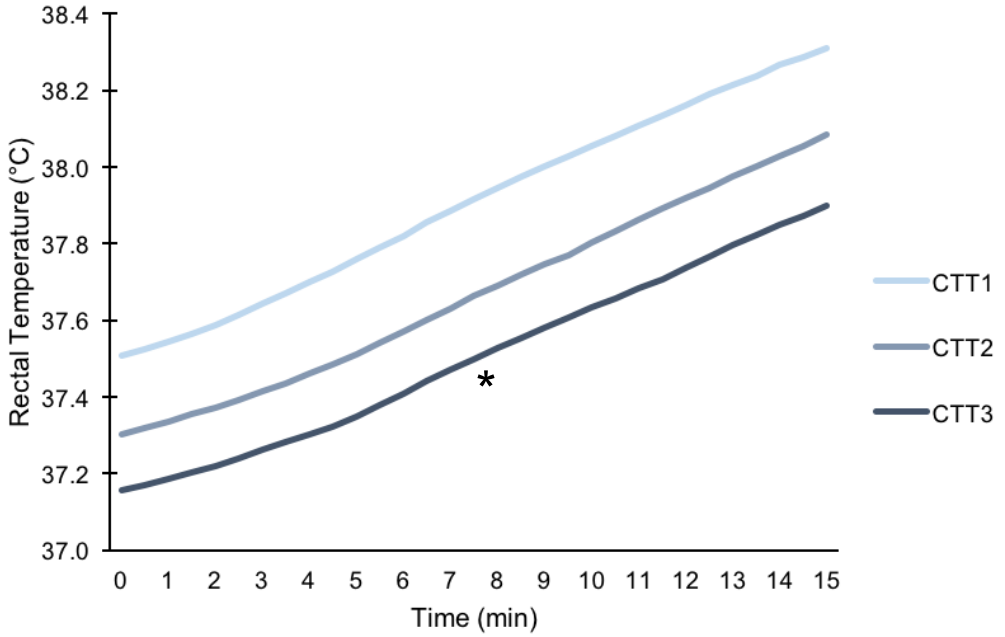

Figure 11. Rectal temperature recorded at 30-second intervals during cool time trials (15°C, 30% RH) pre-acclimation (CTT1), following 5-days heat acclimation (CTT2), and following 10-days heat acclimation (CTT3). *Area under the curve (AUC) different from CTT1 ($p < 0.05$).

Table 9. Physiological measures recorded during time trials in cool conditions (CTT; 15°C, 30% RH). Data are presented as mean ± SD

	CTT1	CTT2	CTT3
Thermoregulatory			
Mean T_{rec} (°C)	37.9 ± 0.3	37.6 ± 0.3	37.6 ± 0.2
Max T_{rec} (°C)	38.3 ± 0.4	38.1 ± 0.3	38.0 ± 0.3
ΔT_{rec} (°C)	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3
Mean T_{sk} (°C)	28.5 ± 1.2	27.8 ± 1.2	28.1 ± 0.8
Max T_{sk} (°C)	29.1 ± 1.0	28.6 ± 1.1	28.8 ± 0.7
Cardiovascular			
Mean HR (bpm)	167 ± 14	167 ± 11	167 ± 11
Sudomotor response			
Sweat loss $_{BSA}$ (g/m^2)	272 ± 46	--	259 ± 50
Blood lactate			
Pre-Test (mmol/L)	1.4 ± 0.5	1.4 ± 0.5	1.9 ± 0.4
Post-Test (mmol/L)	13.2 ± 1.9	13.5 ± 2.3	12.7 ± 3.0

CTT1, pre-acclimation trial; CTT2, trial following 5-days heat acclimation; CTT3, trial following 10-days heat acclimation.
T_{rec}, rectal temperature; ΔT_{rec}, change in rectal temperature during trial; T_{sk}, weighted mean skin temperature; HR, heart rate; SR_{BSA}, estimated sweat rate relative to body surface area; Sweat Loss $_{BSA}$, estimated sweat loss during trial relative to body surface area.
*Significant increase from pre-acclimation trial ($p < 0.05$); †Significant increase from HTT2 ($p < 0.05$).

4.3.3 Cardiovascular. Heart rate was similar during CTTs (Table 9; $p > 0.05$).

4.3.4 Sudomotor. There were no differences in measures of sudomotor activity during CTTs. Results are summarized in Table 9.

4.3.5 Blood lactate. There were no differences in blood lactate across cool time trials at rest or immediately following any of the CTTs ($p > 0.05$), although as expected blood lactate did increase from pre- to post-CTT ($p < 0.01$; Table 9).

4.3.6 Psychophysical. RPE scores were reported immediately following CTT1 (18 ± 1), CTT2 (19 ± 1), and CTT3 (19 ± 1). This confirms all participants gave a maximal effort during performance test, but effort given was not different between tests ($p > 0.05$).

5. Discussion

This study was designed to determine whether females could achieve the performance improvements in hot conditions following STHA that have been observed in males, or whether LTHA is required. This study also aimed to investigate whether the ergogenic endurance performance effects of HA that has been observed in males could also be observed in females. Heat acclimation (HA) was undertaken using two, 5-consecutive day bouts of 90-minute isothermic HA sessions. In females, STHA did not significantly improve time-trial performance; however, LTHA resulted in performance improvements TTs in hot and cool conditions, with a lower rectal temperature observed in CTT3. Significant sudomotor adaptations were observed in hot conditions following LTHA, including increased sweat rate and sweat gland activity. These adaptations, in union with a decrease in resting core temperature and a trend for a decrease in resting heart rate, contributed to an augmented performance in both conditions. These results were consistent with the study hypothesis, which predicted that females would require a greater heat stress stimulus than is typically administered using a STHA protocol in order to make the physiological adaptations needed to transfer to performance enhancements in the heat, and that these performance enhancements would also be observed in cool conditions.

5.1 Heat Acclimation for Performance

5.1.1 Short-term heat acclimation. There were no performance improvements in distance cycled or mean or peak power output observed in either hot or cool conditions following STHA. There was also no change in rectal or skin temperature, heart rate, sweat rate, blood lactate, thermal comfort or thermal sensation at rest or during time trials following STHA. Collectively, these data indicate that the lack of physiological adaptations to heat

stress in females following STHA documented in our study and in the literature (Mee et al., 2015) are reflected in endurance performance. The significant improvement from STHA to LTHA in this study is in stark contrast to findings in male cohorts observed by Gibson and colleagues (2015). They found that males had no significant additional physiological adaptations following LTHA, and that a STHA protocol was sufficient to attain stable acclimation when an isothermic protocol was used (as was used in the current study). This has been reflected in a number of studies on male cohorts, where males have been observed to improve a range of endurance performance measures in the heat following STHA (Garrett et al. 2012; Costa et al., 2012; Chen et al., 2013; Best et al. 2013, Racinais et al. 2015, Gibson et al. 2015, James et al. 2016, Willmott et al. 2016 and Peterson et al. 2010).

Two participants may have been exposed to additional heat stimulus during STHA, as mean daily ambient temperatures for the Birmingham, UK area reached 23°C, 24°C, and 27°C on days that they were undergoing STHA. Regardless, as there were no significant improvements in any measure following STHA, this additional stimulus did not appear to be enough to drive additional adaptations or influence our results.

Although the performance results mirrored the timeline of mechanistic adaptations observed by Mee and colleagues (2015), it is possible that the environmental conditions selected for the heat acclimation sessions may not have been stressful enough to elicit adaptations and performance improvements following STHA.

Maximum T_{rec} for HA sessions was ~38.5°C, though this was not the rectal temperature that was maintained for the majority of the sessions (Mean T_{rec75} was ~38.1-38.3°C). This is slightly lower than what was desired and what is normally maintained during isothermic protocols (typically maintained at ≤ 38.5°C). This may not have been sufficient to

induce adaptations, indicating a possible explanation for the lack of significant performance improvements following STHA.

5.1.2 Long-term heat acclimation. The increase in overall mean power output as a result of LTHA was significant in hot conditions ($p < 0.01$), indicating a meaningful improvement in this performance measure. This improvement was confirmed using area under the curve, which allowed us to compare pacing during time trials and showed that power output was significantly elevated throughout the time trial ($p < 0.05$; see Figure 4). Additionally, when comparing the mean power output of minutes 3-13 of the HTTs (which excludes starting pace and end spurt), participants had a significantly higher power output in HTT3 than HTT1, indicating that heat acclimation improved participants' ability to maintain pace when working aerobically in the heat, which is well documented in male cohorts (Costa et al., 2012; Chen et al., 2013; Best et al., 2013; Racinais et al., 2014; Gibson et al., 2015; James et al., 2016; Willmott et al., 2016).

Significant improvements in power output during the "end spurt" of the final minute of HTT3 alongside increases in peak power output (relative to HTT1) may be attributed to the high-intensity intervals that were incorporated into the HA sessions, as heat acclimation adaptations may be specific to the type of exercise done during HA (Wingfield et al., 2016). The stimulation of the m-TOR pathway by maximal efforts under heat stress may also underlie improvements in peak power output in HTT3 (Kakigi et al., 2011; Yoshihara et al., 2013). This type of mixed-intensity HA may be an efficient way to heat acclimate athletes for self-paced events in the heat that require both aerobic stability and spurts of maximal effort, which is typical of many race-type scenarios (i.e. triathlon, cycling, etc.).

The 8.8% improvement in power output observed in HTT3 following LTHA is comparable to that observed in male cohorts in hot conditions. Keiser et al. (2015) showed that male participants experienced a 10% improvement in power output during a 30-min TT following HA, and Lorenzo and colleagues (2010) found that male participants had an 8% improvement during a 1-h TT following HA.

However, changes in mean power output do not always proportionally translate to distance covered during TTs, as pacing and cadence also contribute. Distance cycled increased on average ~260 m (3%) in HTT3 from HTT1. Although the range of performance results were relatively large (7.09 - 8.75 km in HTT1, 7.39 - 9.08 km in HTT3; 6.96 - 8.99 km in CTT1, 7.36 - 9.02 km in CTT3), the ability of participants to successfully improve performance following heat acclimation was not related to initial time trial results. For example, the participant that scored the lowest (7.09 km) in HTT1 improved ~6% (440 m) in HTT3, and the participant that scored the highest in HTT1 (8.75 km) improved ~4% (330 m). Both of these scores were above the mean improvement of 3%. Additionally, performance improvements following heat acclimation were unlikely to be related to participants' VO_{2max}. Therefore, performance improvements in hot conditions following acclimation are achievable for females falling within a $\dot{V}O_{2max}$ range of 40.0 mL/min.kg-1 to 52.7 mL/min.kg-1, which is typically classified as being recreationally or moderately fit.

Every participant was able to successfully increase their distance cycled in cool conditions following LTHA (CTT3). This is in agreement with the hypothesis that 10-days heat acclimation can be used as an ergogenic aid in females. Female athletes aiming to improve their performance in an upcoming event should therefore consider heat acclimation as a complementary segment to their preparation. Even the lowest recorded performance improvement in this cohort (0.3% or 30 m) is meaningful in a short 15-minute performance

test, especially for athletes competing in endurance sport where fractions of a second can separate placing and qualifying times. Although this protocol was deemed "long-term", 10-days is not synonymous with what is normally thought of as being a long-term training intervention. The performance improvements observed in cool conditions following a protocol that can be completed in under two weeks should not be overlooked.

The 6.8% improvement in mean power output in CTT3 is similar to the 6% observed by Lorenzo et al. (2010) in a mostly male cohort completing a 1-h TT in 13°C conditions. This indicates that females undertaking a LTHA protocol can expect to experience performance enhancements in cool conditions similar to that of their male counterparts. Analysis of the start, middle, and end spurt did not reveal any segment of the CTT to be a statistically significant source for improvement ($p > 0.05$). However, the trend for an increase in power output during the middle of CTT3 ($p = 0.051$), as well as the trend for an increase in peak power output across CTT3 ($p = 0.058$), indicates that although the augmented power output during the "steady state" or aerobic portion of the trial may be responsible for the majority of the overall improvement, other mechanisms which increase muscle strength or anaerobic performance, such as m-TOR pathway stimulation by heat stress (Kakigi et al., 2011; Yoshihara et al., 2013), may play a role in improving endurance performance in cool conditions.

It is possible that there was a training and/or learning effect in TT performance induced independently of HA. Participants were completing all sessions on the Velotron cycle ergometer and increased familiarity with the equipment and the test is possible (Nybo & Lundy, 2016). However, it seems unlikely that there was a learning effect, as a minimum of two familiarisation time trials were undertaken before commencing the protocol, and trials following STHA would have been the forth (HTT2) and fifth (CTT2) time that the

participants completed the 15-minute time trial. In spite of this experience, there was no performance improvement following STHA. However, the lack of a comparative control cohort makes it difficult to decisively exclude the possibility of a training effect, even for a protocol involving only ten training sessions.

The range in improvements in distance cycled in CTT3 in comparison to CTT1 (0.3-5.7%), provides evidence for the theory that there may be high and low responders to heat acclimation, and that responses could be dependent on the individual's balance of haemoconcentration and plasma volume (Racinais et al., 2012). Two participants experienced minor improvements (0.3%) in CTT3, whilst two experienced relatively large performance improvements (~6%). The remainder scored very close to the mean of 2.7% improvement. When applying HA to an athlete's training protocol, their individual responses should be considered when predicting their expected performance outcomes.

Although there is a mixed consensus in the literature concerning the utilisation of HA as an ergogenic aid, all previous investigations have been undertaken on male cohorts. It has been generally agreed that HA can be an effective training component for performance in cool conditions for trained, moderately-trained, and team sport athletes; although its effectiveness for elite level endurance athletes is highly contested (Nybo & Lundy, 2016). Sub-elite athletes can benefit from the plasma volume expansion known to follow heat acclimation, as their blood volume is not maximally expanded within the optimal limits to avoid excessive haemodilution (Schmidt, 1988; Hopper et al. 1988; Luetkemeier & Thomas, 1994). This determines that these athletes have a greater potential for performance augmentation resulting from the plasma volume expansion and cardiovascular stability that typically follows HA (Sawka et al., 2011; Minson & Cotter, 2016). The results of the present study provide further

evidence for this theory and for heat acclimation's effectiveness as an ergogenic aid for sub-elite athletes by demonstrating performance improvements in female endurance athletes.

A consideration when evaluating these results is that high intensity exercise bouts were often implemented to drive a rise in T_{rec}, especially during LTHA sessions, and participants were consistently rating sessions as between 14-15 on the RPE scale (Borg, 1962), or "Hard". Anecdotal feedback from the participants indicated that they felt fatigued from the intensity of the HA protocol and that the single day's rest between sessions and time trials was insufficient to alleviate factors of soreness and general exhaustion. This may have caused the positive effects of HA on performance during the final time trials to be underestimated. Furthermore although, the 15-minute time trial was a reliable test time length (Hickey et al., 1992), the extent of the pre-acclimation detriments in HTT1 caused by heat stress and post-acclimation benefits (heart rate and temperature adaptations) in HTT3 may not be as prominent as that observed in more prolonged efforts (i.e., 30-60 minutes). Therefore, the performance benefits reported in the heat may be underestimated for endurance events longer than 15 min.

Additionally, this study did not control for menstrual cycle. Studies on lesser trained cohorts have led to the general recommendation that investigations involving females should control for menstrual cycle and hormonal contraception (Avellini et al. 1979; Stephenson & Kolka, 1993; Kolka & Stephenson, 1997; Tenaglia et al. 1999; Janse de Jonge et al. 2012). However, in light of recent investigations concluding that performance under heat stress is not affected by menstrual cycle in trained female athletes (Lei et al., 2017), we did not control for menstrual cycle. Furthermore, it has been suggested that the combined monophasic pills that were used by participants in this study are unlikely to have a significant effect on temperature regulation and performance (Stachenfeld et al., 2000; Burrows & Peters, 2007; Joyce et al.,

2013). Participants were randomised in their phases, with both eumenhorreic participants being in opposite phases and both oral contraceptive pill users being in opposite phases. None of the other three participants (contraceptive implant or copper IUD) were menstruating. Despite this, it is possible that there could have been some effect of menstrual cycle and hormonal contraceptives on performance.

5.2 Physiological Adaptations to Heat Acclimation

5.2.1 Core & Skin Temperatures. As hypothesised, LTHA was required to observe a significant drop in resting rectal temperatures in females. This drop in resting rectal temperature contributed to a lower rectal temperature sustained throughout the time trial in cool conditions, however only following LTHA (CTT3). STHA, using a standard isothermic protocol, was an insufficient stimulus to drive this classic thermoregulatory adaptation (Sawka et al., 2011). It is well documented that males can achieve these desired reductions in body temperature after only completing a STHA protocol (< 7 days; Garrett et al., 2011, 2012; Chen et al., 2013; Best et al. 2013, Racinais et al. 2014, Gibson et al. 2015). However, consistent with Mee and colleagues (2015), findings from the current study indicated that females may require additional stimulus and a longer ~10-day protocol in order to similarly alleviate thermoregulatory strain.

It was important for performance to be evaluated alongside mechanistic adaptations, as the lack of thermoregulatory adaptations observed in STHA by Mee and colleagues (2015) can only imply, not confirm, performance outcomes. For example, Sunderland and colleagues (2008) observed a 33% improvement in intermittent running performance times following 4-days HA in the absence of differences in T_{rec} and HR_{peak}. This highlights that a reduction in core temperature is an indirect outcome of the underlying physiological adaptations of the

thermoeffectors, and does not necessarily solidify this marker as an explicit requirement for performance improvement (Nybo & González-Alonso, 2015). Likewise, our results indicated that core temperatures reaching similarly high levels during all time trials in hot conditions did not appear to be limiting performance improvements following LTHA, although the full effects of core temperature adaptations may have been underestimated and may be more prominent in a longer time trial.

Assuming that females are typically smaller than males, size differences often contribute to sex differences in metabolic heat production during an isothermic protocol, which may be a source of the temporal differences observed in attaining adaptations to chronic heat stress (Gagnon & Kenny, 2012; Jay & Cramer, 2014). Men, who are typically larger and carry more muscle mass, require greater internal heat production to reach the typical 38.5°C core temperature threshold of an isothermic protocol, leading to a greater stimulus (Jay & Cramer, 2014). However, while isothermic protocols do not account for body mass, they still provide the highest relative intensity and stimulus for the individual within the ethical safety limits set by core temperatures. The typically smaller females cannot attain a greater stimulus by further increasing exercise intensity, as they are limited by their rising core temperature. To match their stimulus, (larger) males would need to reduce the intensity of their HA sessions, which is not ideal when investigating a practical and applicable protocol that aims to maximise heat as a stimulus. This is problematic for female athletes, who are also aiming to maximise the benefits of HA, and requires them to instead lengthen their sessions to be under heat stress for longer to attain the same thermoregulatory adaptations as men following STHA (Mee et al., 2017).

Reductions in core temperature are facilitated by adaptations of the thermoeffector responses, including vasomotor and sudomotor activity. As the body fights for equilibrium of

the heat balance equation, the thermoeffector response adapts in three ways: 1) earlier initiation of the thermal effector responses (i.e. vasodilation and sweating); 2) an increase in responsiveness to thermal changes in the body (i.e. a greater response initiated for a lesser temperature disturbance), and 3) the thermal effectors become more efficient by dissipating the same amount of heat at a lower grade of effector response (Gisolfi & Wenger, 1984; Gagnon & Kenny, 2012). Therefore, sex hormones and their effect on vasodilation (Charkoudian et al., 2016), as well as differences in female sudomotor activity described above in Section 2.5.4, (e.g. reduced evaporative capacity and peripheral modulation of sweating (Gagnon & Kenny, 2012)) may also influence thermoeffector adaptations. This may subsequently contribute to the observed differences in core temperature adaptations following STHA.

Although it was suggested above that it is unlikely that menstrual cycle or hormonal contraceptives had an effect on performance results, female sex hormones are known to affect measures of thermoregulation, and the validity of the more mechanistic results, such as body temperatures, cardiovascular adaptations, and sudomotor activity, could be questioned (Lei et al., 2017).

Mean skin temperatures were not significantly different across HTTs (all ~34°C, see Table 6), indicating that participants were experiencing similar demands for skin blood flow in each HTT (Smith & Johnson, 2016). Despite these high demands, participants were able to perform better in the final trial in hot conditions (HTT3). This may be as a result of plasma volume expansion enabling the cardiovascular system to direct blood flow to meet these demands, whilst also providing more oxygen to the brain and active muscles, which would have been compromised in the unacclimated state (Rowell, 1974; Neilson & Nybo, 2003;

Nybo & Secher, 2004). However, as measurements for the calculation of plasma volume (n = 3) were insignificant, this is only speculative.

5.2.2 Cardiovascular. Six of seven participants experienced a drop in resting heart rate following LTHA, and an overall trend was observed ($p = 0.08$). This was not observed following STHA ($p > 0.05$), providing further evidence of the longer or more intense protocol required to acclimate females.

Again, we speculate that the trend for a reduction in heart rate at rest was facilitated by plasma volume expansion and an increased stroke volume (Senay, 1986). However, it should be considered that resting heart rate was measured following stabilisation in the laboratory instead of immediately after waking up in the morning, and an anticipatory response of the time trial that was to follow may have impacted results. This methodological design likely impacted the accuracy in reporting a true "resting heart rate", and could explain why only 6 of 7 participants experienced a drop in resting heart rate following a full LTHA protocol. This also may have interfered with detection of a possible drop in resting HR following STHA. Additionally, a major limitation of this study is that there were only seven participants. A lack of power may explain why some of the expected physiological adaptations were observed only as trends, if at all.

Although peak and mean heart rate during exercise were unchanged in all CTTs and HTTs ($p > 0.05$), the workload was not standardised. It is likely that cardiovascular stability following LTHA contributed to the improvement in time trial performances in both conditions, especially during the middle segment HTT3, when participants were cycling at a submaximal pace. Submaximal performance in the heat pre-acclimation is known to be limited by the poor ability to meet flow demands of both the skin and the active muscles

(Rowell, 1974; González-Alonso et al., 1998; Nybo et al., 2014), which is improved with acclimation (Wyndham et al., 1976).

5.2.3 Sudomotor activity. In the current study, sweat rates in hot conditions were not significantly increased following STHA, but were significantly increased following LTHA. This timeline of sudomotor adaptation was in accordance with the hypothesis, although it was not in accordance with data recorded by Mee et al., (2015). Mee et al., (2015) observed increases in sweat rate following STHA, and also observed increases of a greater magnitude following LTHA. Participants in this study exhibited sweat rates of 613 ± 105 g/h/m^2 pre-acclimation (HTT1), and 772 ± 114 g/h/m^2 following LTHA (HTT3), whilst participants investigated by Mee and colleagues exhibited sweat rates of only 326 ± 156 g/h/m^2 before improving to reach 798 ± 229 g/h/m^2 following LTHA. The higher pre-acclimation sweat rates observed in our study possibly indicate a higher training status in our participants prior to acclimation. However, as we measured sweat rates whilst employing a maximal effort time trial, it is difficult to compare to the fixed-workload submaximal test used by Mee et al. (2015). Some of these differences could also be attributed to differences in methodological design between our study and that of Mee and colleagues (2015). Our study was designed with the foremost aim of investigating endurance performance augmentations during self-paced time trials, so workloads were not standardised. Sweat rates during the sessions were assumed to be driven by internal temperature changes (Nadel et al. 1971; Gisolfi & Wenger, 1984; Wenger et al. 1985), which were similar during our isothermic protocol, however without a standardized workload, mechanistic changes across HA cannot be confirmed. Although insignificant, there was an increase in mean power output from HA day 1 to HA

day 10, which could have been partially responsible for the increase in sweat rate observed here.

High sweat output during heat acclimation sessions is required for meaningful sudomotor adaptations (Buono et al, 2009). We are confident that the sweat rates recorded during HA sessions were enough of a stimulus to elicit a sudomotor adaptation. The ~600-800 g/h/m^2 recorded during HA sessions in our cohort far surpasses the ~200-400 g/h/m^2 recorded by Mee and colleagues (2015) during HA sessions in their female cohort. Despite this, participants studied by Mee and colleagues (2015) more than doubled their sweat rate during the submaximal running heat tolerance tests following 10-days HA, whilst our cohort did not achieve an adaptation of quite the same magnitude following LTHA (~25% increase).

An auxiliary detail of our protocol was the undertaking of voluntary dehydration during HA sessions, which was not included in the study by Mee and colleagues (2015). The usefulness of voluntary dehydration as an added stimulus for HA has been questioned in recent studies by Neal and colleagues (2016a, b). The core temperature threshold for sweating onset (Montain et al., 1995) and the thermoeffector's sensitivity to changes in core temperature (Gonzalez-Alonso et al., 2000, Montain et al., 1995) are both impaired by dehydration. Given that maximising sweating during HA sessions is critical for initiating adaptation (Buono et al., 2009), dehydration during HA may have subsequently reduced the stimulus for sudomotor adaptation during our study (Armstrong & Maresh, 1991).

There was no change in sweat rate during CTTs, which is likely due to the 15-minute duration of the trial in 15°C, 30% RH being too short to drive a strong enough thermal stimulus for a significantly different thermoeffector response (Gagnon & Kenny, 2012), especially as the effector response may be graded and may employ vasodilation prior to sweating to correct for lower levels of thermal disturbance (Bligh et al., 2006).

We observed an increased density of active sweat glands on the forearm following LTHA, which may have been related to an increased cholinergic sensitivity, as observed by Buono and colleagues (2009) following HA in males. Additionally, the dots left by the sweat output of the sweat glands on the iodine paper appeared larger in diameter, as well as having a deeper colouring (See Figure 7). These observations were purely subjective, as the reliability of the iodine paper technique has been verified to quantify number, not size or sweat output of the sweat glands. These seemingly larger dots indicate that more moisture was absorbed per dot (i.e., per sweat gland) in the same amount paper-to-skin contact time, following HTT3 vs HTT1. This may indicate an increase in sweat output by the individual glands, which is facilitated by an improved size of the sweat gland and its efficiency in secreting sweat for a given length of secretory coil (Sato and Sato, 1983; Sato et al., 1990; Bouno et al., 2009).

It should be noted that these measures of sweat gland activity were taken from sites on the forearm and it is not suggested that they are a precise indication of whole body sweat gland adaptations. While the increased activity shows a better use of body surface area to dissipate heat, heat acclimation has been shown to increase local sweat rates of the forearms more than areas that already exhibit a high sweat output in the unacclimated state, such as the back and chest (Havenith et al., 2008; Poirier et al., 2016). Females in the unacclimated state exhibit a more evenly distributed sweat rate than males (Havenith et al., 2008), meaning it is possible that the increase in sweat gland activity of the forearm is a better representation of whole body sweat glands than would be in males.

Other factors possibly contributing to the increase in sweat rate observed in our investigation following LTHA include sweat onset being initiated at lower core temperatures (Wyndham, 1967; Nadel et aI., 1974; Tipton et al., 2008) as well as an increased sensitivity of

the sudomotor response to deviations in core temperature (Kondo et al., 2009). However, these mechanisms were not measured in the current study.

5.2.4 Plasma volume measures. Although the measures of Hb and Hct in three participants yielded insignificant results, plasma volume expansion following HA is heavily documented in the literature, and typically accompanies the other main markers of the heat acclimation phenotype (Wyndham et al., 1968; Senay, 1979; Shapiro et al., 1981; Nielson et al., 1993; Patterson et al., 2004, 2014). Nevertheless, even with just three participants' data and the general trend observed it seems likely that plasma volume expansion did occur for our participants, and was likely one of the contributing mechanisms responsible for the decreased resting core temperature and the trend for decreased resting heart rate observed in this study. It is also the main mechanism credited with enhancing VO_{2max} and aerobic performance in sub-elite endurance athletes (Schmidt, 1988; Racinais et al., 2014; Minson & Cotter, 2016) which was also observed in the current study.

The heavy sweating during both STHA and LTHA sessions (2-4% BM of water loss), with the addition of voluntary dehydration, should surpass the "osmotic threshold" of plasma osmotic pressure required for the implementation of adaptive mechanisms to increase plasma volume (Andreoli et al., 2000; Cheuvront and Kenefick, 2014). However, as males have been observed to have increased levels of plasma ADH at a similar plasma osmolality (indicating a higher sensitivity to changes in plasma osmolality; Stachenfeld et al., 2001), this osmotic threshold could be higher for females. This offers some explanation for the difficulty experienced in attaining the heat acclimated phenotype following STHA that was observed here as well as in the literature (Sunderland et al, 2008; Mee et al., 2015).

5.2.5 Lactate and muscle metabolism. Blood lactate was not significantly affected by STHA or LTHA in both hot and cool conditions when recorded at rest and immediately post-trial. Although heat acclimation has been shown to reduce muscle and blood lactate accumulation in both hot and cool conditions (Young et al., 1985) in a time trial-style performance test, it is more difficult to interpret these mechanistic results than during a fixed-workload type test. The high blood lactate recorded post-trial is likely as a result of the maximal effort "end spurt", and therefore was elevated to a similar level pre- and post-acclimation. However, as blood lactate is a measure of both lactate production and lactate clearance (without distinction), it is not certain that the weight of this balance stayed similar both pre- and post-acclimation. For example, during the middle, steady-state portion of the trials, it is possible that blood lactate may have been reduced, or may have not have changed, alongside the higher power output that was recorded. The improvement in aerobic capacity and assumed increase in lipid oxidation during HTT3 (Kirwan et al., 1987) may have allowed for the participants to set their pace at a greater mean power output, and subsequently produce more lactate, but an increased blood flow allowance to the liver and the inactive muscles (facilitated by plasma volume expansion) may have slightly improved the clearance of this blood lactate (Rowell, 1968; Lorenzo et al., 2010). Additionally, reductions in epinephrine levels observed in male cohorts following HA are thought to largely influence the reduction in blood lactate accumulation (Febbraio et al., 1994). However, an already reduced epinephrine level in females during exercise in comparison to males (Horton 2006) may influence metabolic responses to chronic heat stress.

5.2.6 Psycho-physical and behavioural. Similar T_{rec} and T_{sk} during HTTs and a variable workload may explain the lack of significant improvements in either thermal comfort

or thermal sensation reported immediately following the trials in the heat. However, evidence of psycho-physical changes lies in the improved time trial performance results. As it was a self-paced test, increased power output may be largely a behavioural result of assessment of physiological factors by a central governor to increase intensity (Noakes 2007, 2012; Tucker & Noakes, 2009; Nikolopolous et al., 2001; Pires & Hammond, 2011). Behavioural modifications are known to develop with heat acclimation, as athletes may improve their pacing strategies with chronic heat exposure (Racinais et al., 2014).

Performance tests with a standardised workload have provided evidence that heat acclimation has been observed to improve perceived exertion and thermal comfort and sensation in both males and females, however the temporal patterns of their improvements may differ (Sunderland et al. 2008; Gibson et al., 2015: Neal et al., 2016; James et al., 2016). These improvements may stem from the reduction in cardiovascular strain following heat acclimation, allowing athletes to work at a lower $\%VO_{2max}$ than in pre-acclimation heat stress tests (Cheuvront et al., 2010), although comfort may also develop with the experience of performing in the heat during the acclimation protocol.

5.2.7 Other possible mechanisms of adaptation. As the performance results of this study mirrored the temporal patterning of female physiological adaptation in the sex differences study by Mee and colleagues (2015), we are left to speculate the possible reasons that females appear unable to adapt to chronic heat stress using a 5-day isothermic protocol. One of these possibilities is the sex-differences that have been observed in heat shock protein synthesis following heat acclimation (Hamilton et al., 2004; Bombardier et al., 2009; Morton et al., 2009; Gillum et al., 2012, 2013). The upregulation and functioning of HSPs appears to be necessary for the appearance of the markers of the heat acclimated phenotype, although it

is unclear exactly how they are involved (Kuennen et al., 2011). HSP72 mRNA has been observed to be similar in males and females during isothermic heat acclimation (Mee et al., 2016), indicating that the isothermic heat stimulus is sufficient to surpass the activation threshold of the heat shock response (Gibson et al., 2015) in both males and females; however the obstructer of their eventual synthesis and appearance in females is unknown. Oestrogen may (Paroo et al., 2002; Voss et al., 2003; Shinohara et al., 2004; Hamilton et al., 2004; Bombardier et al., 2009; Chu et al., 2017) or may not (Gillum et al., 2012) play a role. The connection and direct interaction between these sex-differences observed at a cellular level and the female performance augmentations observed in our study is unknown.

5.3 Perspectives

These results contribute to a currently sparse collection of research that informs the expected performance outcomes of HA for female athletes in a quantifiable manner and confirms the translation of physiological adaptations to a positive effect on performance in hot conditions. Previously, any performance outcomes that were anticipated for females were either derived from male cohorts or predicted based on mechanistic approaches. Therefore, these results provide valuable information to female athletes who are considering heat acclimation to prepare for an event in a hot climate, as they offer applicable information on the performance improvements that they can expect to gain following a typical heat acclimation protocol. The results of this study indicate that while HA can be an effective training component for competition in both hot and cool temperatures, female athletes utilising heat acclimation as an ergogenic aid should expect to undergo at least a LTHA protocol (if not longer) before experiencing any changes to their performance.

While participants in this study were moderately-trained endurance athletes, their VO_{2max} may be comparable to a higher level of team sport athletes, who's primary focus is not endurance and aerobic conditioning, although it is an important component of their sport (i.e. football, hockey, netball, etc.). Additionally, the improvements observed in a time-trial lasting only 15-minutes highlights that the use of heat acclimation as an ergogenic aid should not be limited to long-distance endurance athletes, and can be explored by any athlete requiring aerobic proficiency in their sport or event.

The sex-differences in adaptations to STHA observed by Mee and colleagues (2015), as well as in our study in comparison to aforementioned studies on males, is concerning for female athletes. Previous to the publication of this information, females assuming the effectiveness of STHA protocols may have been exposed to a safety risk when competing in hot conditions without proper acclimation, in addition to the poorer performance than anticipated.

Besides females being unable to attain thermoregulatory adaptations following STHA, one particular concern involves discrepancies in the observations of temporal patterning of sudomotor adaptations in females. Data from the current study and a study by Sunderland and colleagues (2008) did not indicate any sudomotor changes following STHA, however data from Mee and colleagues (2015) did show a difference. Females undergoing HA should be aware that sudomotor adaptations may precede other adaptations that accompany the heat acclimated phenotype (Sawka et al., 2011; Mee et al., 2015), and that this increased fluid loss could add further strain to a cardiovascular system which may not have yet established stability in the heat via accelerated dehydration (Taylor et al., 2014).

5.4 Future Directions

Sex hormones' effects while under acute and chronic heat stress remain highly debated (but considerably less investigated). Thus, the ability of trained female athletes to acclimate, regardless of their menstrual cycle and/or contraception method, should be investigated for more definite and inclusive HA prescriptions that can be confidently implemented for the safety and benefit of all athletes. As records of HA being investigated date back to the 1930's, and it has been well-known for decades that sex hormones may have an effect on females' ability to acclimate, the delay in this investigation by avoidance is unacceptable. The common practice of testing females only during the low-hormone phase masks this avoidance as "control" and renders results unreliable for athletes that must compete during all phases of their menstrual cycle. Considering the success that female athletes have experienced on the current side of the 21st century, this information is urgently needed.

Elite female athletes are a population that also warrant unique investigation for use of HA as an ergogenic aid. Sex-differences in fluid regulation (Stachenfeld et al, 1998, 2001), thermoeffector response (Gagnon et al., 2012), and heat adaptation (Mee et al., 2015) may result in a more positive outcome than previously observed in elite males (Keiser et al., 2015; Nybo & Lundy, 2016).

Whilst the blood lactate measurements in this study allow for speculations described above, amongst other possible speculations about the metabolic adaptations of female athletes following HA, future research should investigate these theories with more precise measures (i.e., muscle biopsies, gas exchange analysis, indirect calorimetry, etc.). Longer trials should also be investigated in female cohorts to determine if this increased thermoregulatory capacity can have a greater impact as heat accumulation becomes a more influential element of

performance in temperate conditions. Additionally, in the future, a more taper-like protocol should be investigated to: 1) replicate the preparation an athlete would take before an event or competition, and 2) document performance results devoid of the fatigued state caused by an accumulated training load.

Overall, future research should incorporate a practical, real-world focus on performance for female athletes. To do this, factors in plasma volume expansion, substrate utilisation, and the heat shock response interaction with sex hormones, along with longer time trials and time trials in cool conditions, should be further investigated.

6. Conclusion

Moderately-trained females did not improve performance in either hot or cool conditions for a 15-minute time trial following STHA. Performance improvements were observed following LTHA in both hot and cool conditions, providing the first quantifiable evidence of time trial performance improvements for females following HA. This also provides more evidence for the use of heat acclimation as an ergogenic aid, a possibility that has not previously been investigated in females.

The mixed-intensity HA protocol that involved both high-intensity intervals and lower intensity aerobic training could be beneficial to athletes that are required to vary their pace when competing (i.e. a faster pace during the start and end spurt than during the middle). This HA protocol has proved ergogenic for this cohort of moderately-trained female endurance athletes after 10-days of exposure, likely by achieving a more beneficial balance between plasma volume expansion and haemoconcentration, amongst an amalgamation of other underlying factors. Performance results experienced in both conditions in a self-paced time trial lasting only 15-minutes indicates that heat acclimation can improve performance in short and longer distance time trials This was the first study to investigate the effects of a full long-term heat acclimation protocol on female endurance performance using a self-paced, time trial style performance test. Previously, heat acclimation research for females has involved heat tolerance tests at a fixed workload (Mee et al., 2015; Avellini et al., 1980; Fein et al., 1975; Shapiro et al., 1980; Sawka et al., 1985), heat tolerance tests in the form of intermittent sprint test duration (Sunderland et al., 2008) or sedentary participants (Armstrong et al., 2005).

A decrease in resting T_{rec} may have helped to improve performance in both time trial conditions following LTHA, although lack of significant differences in mean, peak, and delta T_{rec} during HTTs indicates core temperature adaptations may not be the most important

adaptation for performance enhancement. A trend for cardiovascular stability following LTHA may have been facilitated by plasma volume expansion and/or cardiovascular efficiency.

There were no physiological or psychophysical adaptations following STHA. Thus, data from the current study indicated that classic physiological markers of heat acclimation (i.e., reduced heart rate and core temperatures, increased sweating; Sawka et al., 2011) must be present in order to improve time trial performance in hot conditions for females. Females in the current study not exhibiting cardiovascular and thermoregulatory stability following STHA that has been demonstrated in male cohorts may be due to sex-differences in the following factors: fluid regulatory factors leading to plasma volume expansion; cytoprotective thermotolerance; thermoregulatory control; body size and resultant differences in metabolic heat production during an isothermic protocol; evaporative capacity at the highest requirements for heat loss. The current study's results may also have been influenced by fatigue during time trials resulting from the length and intensity of the protocol, the absence of a standardised workload, not controlling for menstrual cycle and hormonal contraception, and a slightly lower T_{rec} maintained during HA sessions.

The additional support and performance element that this study provides for the temporal patterning of adaptation to chronic heat stress in female athletes should prompt a re-examination of exercise prescriptions in the heat. These recommendations have traditionally been constructed based on research involving primarily male cohorts, and this study provides meaningful information that has been missing for female athletes implementing heat acclimation into their training.

Appendix

	Time/Length	Control	Heat	Performance Reduction
Time Trial				
Altareki et al., 2009	4 km	13°C	35°C	2%
Tyler et al., 2008	90-minute	14°C	30°C	7%
Tucker et al., 2004	20 km	15°C	35°C	6%
Racinais et al., 2014	43 km	8°C	37°C	12%
Tatterson et al., 2000	30-minute	23°C	32°C	7%
Periard et al., 2011	40 km	20°C	35°C	7%
Peiffer & Abbiss, 2011	40 km	17°C	32°C	6%
Castle et al., 2011	Intermittent Sprints	22°C	32°C	Power in 4/20 sprints significantly reduced
Drust et al., 2005	Power during intermittent cycling sprints	20°C	40°C	10%
TTE				
Dill 1931		12°C	34°C	25%
James et al., 2016		13°C	32°C	19%
González-Alonso et al., 2003		+1°C skin temperatures	+10°C skin temperatures	28%
Galloway & Maughan, 1997		21°C	31°C	36%
Morris et al., 1998		20°C	30°C	22%
MacDougall 1974		29°C *	35°C *	47%

*skin temperature manipulated by water perfusion suit

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