

ACTIVE AND PASSIVE HEAT ACCLIMATION FOR EXERCISE PERFORMANCE IN FEMALE AND MALE ATHLETES

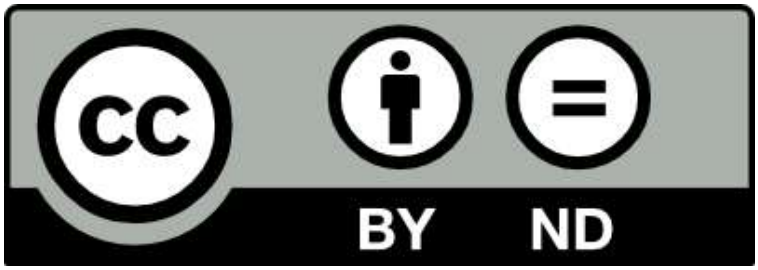
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ABSTRACT

Heat acclimation is an effective strategy to drive favourable physiological adaptations that attenuate exercise performance impairments in hot environments. However, there is limited data investigating heat acclimation in female participants. This thesis investigated novel aspects of active (heat stress during exercise) and passive (heat stress independent of exercise) heat acclimation; namely, short-term heat acclimation, post-exercise sauna bathing, heat acclimation decay and heat acclimation as an ergogenic aid for performance in cool conditions. This thesis focused particularly on the female athlete's response to these active and passive heat acclimation protocols.

Short-term heat acclimation (<7 days) is more cost- and time-efficient than traditional heat acclimation (~10 days) and has been repeatedly demonstrated to be effective in males. The first two experimental chapters of this thesis (**Chapters 4** & **5**) showed that short-term heat acclimation (4-5 days) did not improve exercise performance in hot or cool conditions in a female cohort. Conversely, 9-10 days heat acclimation did improve performance in both conditions in this female cohort, despite participants' reports of fatigue during time trials. Notably, performance improvements in cool conditions were not as meaningful for participants with better baseline performances. Overall, short-term heat acclimation appeared to be an ineffective strategy for females, whilst 9-10 days heat acclimation was fatiguing.

Though popularised as a more practical heat acclimation strategy for athletes, post-exercise sauna bathing had not previously been shown to improve exercise-heat tolerance. **Chapters 6-8** demonstrated that three weeks of intermittent post-exercise sauna bathing was an effective heat acclimation strategy, as it induced thermal, cardiovascular and sudomotor adaptations during exercise-heat stress without disrupting athletes' normal training. Body temperature and heart rate adaptations were similar, if not superior, in females as compared to males. Additionally, these investigations revealed a novel sex difference in the adaptation of physiological heat loss mechanisms, whereby females showed sudomotor adaptations whilst males showed improved forearm blood flow. Adaptations observed during exercise-heat stress were partially retained for two weeks post-intervention without exogenous heat stress. Specifically, body temperature and heart rate adaptations decayed by 25-33%, similar to rates of decay observed following consecutive-day, active heat acclimation. Extending the intervention from three to seven weeks induced only marginal additional physiological improvements during exercise-heat stress. Lastly, females and males similarly improved markers of exercise performance in temperate conditions following three weeks of intermittent post-exercise sauna bathing, more than

endurance training alone. These performance markers included: maximal aerobic capacity, running speed at 4 mmol·L^{-1} blood lactate concentration and time-to-exhaustion. Thus, post-exercise sauna bathing is an effective heat acclimation strategy, as well as an effective ergogenic aid, for both male and female athletes.

Overall, the findings detailed in this thesis provide useful information for female and male athletes undertaking heat acclimation to improve exercise performance in both hot and cooler conditions.

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LIST OF ABBREVIATIONS

3-Weeks	Tests following 3-weeks intervention
7-Weeks	Tests following 7-weeks intervention
ANOVA	Analysis of variance
ANCOVA	Analysis of co-variance
AUC	Area under the curve
BSA	Body surface area
CL	Confidence limits
CON	Control group
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric-oxide synthase
EPO	Erythropoietin
FBF	Forearm blood flow
Hct	Haematocrit
HIIT	High-intensity interval training
HR	Heart rate
HSP	Heat shock proteins
HTT	Running heat tolerance test
IUD	Intra-uterine device
NBM	Nude body mass
NO	Nitric oxide
η_p^2	Partial eta-squared
OCP	Oral contraceptive pills
Pre	Pre-intervention tests
RER	Respiratory exchange ratio
RH	Relative humidity

RPE	Rating of perceived exertion
SAUNA	Sauna intervention group
STHA	Short-term heat acclimation
SR_{BSA}	Estimated sweat rate relative to body surface area
TT_{COOL}	15-min cycling time trial in cool conditions
TT_{HOT}	15-min cycling time trial in hot conditions
T_{rec}	Rectal temperature
T_{rec75}	Mean T_{rec} for the final 75 min of active heat acclimation sessions
$T_{recRISE}$	Rise in rectal temperature
T_{sk}	Mean weighted skin temperature
TTE	Time-to-exhaustion
VEGF	Vascular endothelial growth factor
$\dot{V}O_{2max}$	Maximal aerobic capacity
$\dot{V}O_{2peak}$	Peak oxygen consumption
W	Watts
$\%HR_{max}$	Percentage of age-estimated maximum heart rate
[Hb]	Haemoglobin concentration
$[La^-]$	Blood lactate concentration

RESEARCH DISSEMINATION

Journal publications related to this thesis

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Conference proceedings from additional work

Kirby, N.V., Smith, B., Skinner, B., Wightman, L., Lucas, R., & Foster, C. (2018). A rapid review of the evidence regarding physical activity for disabled adults: recommendations for public health. Presented as a research poster at the 23rd Annual Congress of the European College of Sport Science (ECSS), Dublin, Ireland

Kirby, N.V., Smith, B., Skinner, B., Wightman, L., Lucas, R., & Foster, C. (2018). Physical activity for disabled adults: recommendations for public health. Presented as an oral communication at the 2018 Meeting of Clinical Champions and Physical Activity Leads, Public Health England, London, UK

1. INTRODUCTION

1.1 Historical perspective: Environmental heat stress and adaptation

Humans survive within extremely narrow physiological limits, with their underlying physiological processes constantly fighting for homeostasis when exposed to varied environmental stressors that they may purposefully, or accidentally, encounter (Cotter & Tipton, 2014). When applied correctly, the chronic strain from environmental stressors results in physiological adaptations that allow humans to better cope with a given environment - though this is not necessarily limited to the specific environment that caused the strain (Hale, 1969).

Environmental heat stress is one such environmental stressor. During heat stress, the body must defend a narrow core body temperature homeostatic range. For example, a deviation in core body temperature of just 1°C is likely to provoke noticeable discomfort and regulatory physiological responses (e.g. sweating; Benzinger, 1959). Like other stressors, repeated heat strain has been observed to initiate integrative adaptive responses that yield a systemic adaptation at the level of the whole organism, known as heat acclimation (Fox et al., 1963b; Nielsen et al., 1993; Sawka et al., 2011). Early reports of this particular adaptive response can be found in the 1771 essays of James Lind, who wrote that Europeans relocating to East and West Indian climates frequently experienced what we now know to be heat illness. He described this heat illness as a “malignant fever”, being “an indisposition of the body, attended commonly with an increase of heat; a thirst; often with a head-ach; more frequently with a remarkable quickness of the pulse, or at least a great change from its natural state” (p.10). However, with time, these European migrants eventually became habituated, even comfortable, in their new hotter environment (Lind, 1771). In 1925, over a century after Lind’s observational findings, Dr. Aldo Dreosti became the first to *purposefully* employ heat acclimation to improve physical work capacity in the heat. Dreosti repeatedly tested and modified an underground heat acclimation protocol, which aimed to optimise the work capacity (and survival) of the gold-mining workforce in the Witwatersrand Basin (near present-day Johannesburg, South Africa), who were required to perform intense physical work whilst surrounded by rocks with temperatures of up to 60°C (Dreosti, 1935). Dreosti’s descriptions of the 7-14-day heat acclimation protocols laid the foundation for the traditional combined exercise- and ambient-heat stress acclimation protocols that are still widely studied today. Moreover, Dreosti’s research was part of the seminal work in the area, which prompted the questions: How do humans respond and adapt to extreme environments such

as extreme heat? How can exercise performance in the heat be improved and optimised through adaptation?

In the present day, these questions remain relevant for athletes, military and occupational personnel, who perform physical work and competition in extreme environments. Firefighters knowingly enter burning buildings and athletes test the limits of human performance in hot environments (e.g., the Marathon Des Sables, the Summer Olympic Games and the Hawaii Ironman). Meanwhile, amid the ongoing climate crisis, average daily summer temperatures are steadily rising, and the frequency of extreme heat events is expected to increase exponentially in coming years (Lehner et al., 2018; Power & Delage, 2019). Furthermore, technology and infrastructure advancements have rapidly increased urbanisation in the hottest parts of the planet (e.g., Dubai's population has approximately tripled since the year 2000; Dubai Statistics Centre, 2019). Consequently, major international sporting events are held in hot locations at an increasing frequency (e.g., Doha 2019 World Athletics Championships, Tokyo 2020 Olympic Games, 2022 FIFA World Cup in Qatar). Therefore, whilst some athletes may have purposefully sought out these extreme environments in the past and present, the sportspeople of the near future are likely to have to perform under extreme environmental heat stress in their home countries, as well as at international competitions.

Alongside these marked environmental changes, a growing number of women are entering more physically demanding occupations (Ministry of Defence Report, 2014) and female sport has experienced unprecedented growth in the last half-century (Lopiano, 2000; Joyner, 2017). Naturally, this means *more* women are now performing physical work or exercise under extreme environmental conditions *more* often. However, female participants are particularly underrepresented in research on the human response and adaptation to heat stress. The discrepancy in this field and the social/cultural implications were noted by Hardy and DuBois in their 1940 paper, *Differences between men and women in their response to heat and cold*:

> "There have been a large number of publications dealing with the reactions of men to changes of environmental temperature, but very few studies with women. Just why there has been such a neglect of half our population is not clear. Air conditioning is theoretically based on physiological studies, and politeness alone would demand more consideration of the ladies." (p. 389)

Unfortunately, despite efforts of inclusion and awareness from a number of individual researchers, there remains a dearth of heat stress and heat acclimation research that includes female participants. Tyler and colleagues conducted a meta-analysis in 2016 to

investigate the physiological, perception and exercise performance responses to heat acclimation. Of the 96 manuscripts with >1000 study participants included in this meta-analysis, only 7% were female. Similarly, in an umbrella review (i.e. pooling the results of 36 systematic reviews) Morris et al. (2020) investigated methods to mitigate occupational heat strain, with the aim of providing scientific-based guidance for practitioners. Only 9% of the 12,684 participants from the original research articles sampled were female. Thus, responses from female participants are underrepresented in the data currently informing policy and recommendations. Finally, Benjamin et al. (2019) reviewed exercise *performance* changes following heat acclimation and reported that only 3% of participants were female. This underrepresentation of females in heat acclimation and thermoregulatory literature appears greater than that observed in the broader field of exercise sciences, as Costello et al. (2014) reported that females made up 39% of participants in research studies from three major sports and exercise medicine journals from 2011-2013. Shifts in basal body temperature and thermoregulatory thresholds across the menstrual cycle (see details described in **Chapter 2**) are factors that likely contribute to the severity of underrepresentation of female participants in this particular area. Nevertheless, using mostly male-derived training recommendations to predict sport and exercise performance outcomes in the heat for female athletes is problematic and concerning, considering known physiological, biomechanical and endocrinological differences between sexes (Costello et al., 2014). This theme underlies the rationale for the investigations detailed within this thesis, which contributes to the growing knowledge base of female exercise physiology, and specifically, females' responses to acute and chronic heat stress.

1.2 Historical perspective: Women in sport

Before further addressing the sex differences related to exercise and competition in the heat, a brief historical perspective is necessary to frame the problem and better understand the factors contributing to the long-standing marginalisation of female sport and the research that surrounds it.

Although female sport has progressed considerably, differences related to gender as it pertains to sociological aspects and attitudes of sport and exercise today have roots in historical inequalities (Fink, 2015). 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, whilst female "sport" was limited to dances and exercises that would have an aesthetic benefit, as well as better prepare women for healthy childbearing (Hartmann-Tews & Pfister, 2003). In the 20th century, the passing of the 1972 United States legislation, *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, which included sport and athletics (Ware, 2013). This Act was a springboard for female sport, with the U.S.A. experiencing a 600% increase in girls' sport participation in the six years following the implementation of the *Title IX* legislature (Kaestner & Xin, 2010). This era of anti-sexism revolution also included the official allowance of women to enter the famed Boston Marathon in 1972 and eventually, the creation of a women-only Olympic marathon event in 1984. Fast-forward approximately three decades to the most recent Summer Olympics in Rio de Janeiro in 2016 and 45% of all competitors were women; a substantial increase from the previous record of 29% reached in 1992 in Barcelona (International Olympic Committee, 2016). Most recently, the International Olympic Committee estimated that 49% of competitors at the Tokyo 2020 Olympic Games will be female (International Olympic Committee, 2020).

Despite increased participation and athletic success, equality in media coverage of female sport lagged (and continues to lag) behind (Fink, 2015). The magnitude of the historical media disparity can be exemplified by a strikingly recent observation: media coverage of women's sports did not supersede that of dogs and horses until 1992 (Lopiano, 2000). This is acutely relevant because the presentation of issues and content by the media at large impacts how the public perceives reality (Pan & Kosicki, 1993). Combined with historical sport participation barriers, it is not surprising that lingering discrepancies remain between female and male sport as it relates to funding, salary, leadership positions, and facilities (Hartmann-Tews & Pfister, 2003; Schull et al., 2013; Burton, 2015; Fink, 2015; Leberman & Shaw, 2015). These discrepancies appear to have influenced the amount of scientific research focused on female exercise and sports medicine (Costello et al., 2014). As a result, current sex differences in exercise and sports science often represent an intersect between gender (based on societal attitudes and ideals) and sex (the biological state of being male, female or inter-sex). Specifically, social factors surrounding gender impact on athlete development and sports culture, and thus differences rooted in gender become integrative to sex differences (**Figure 1.1**).

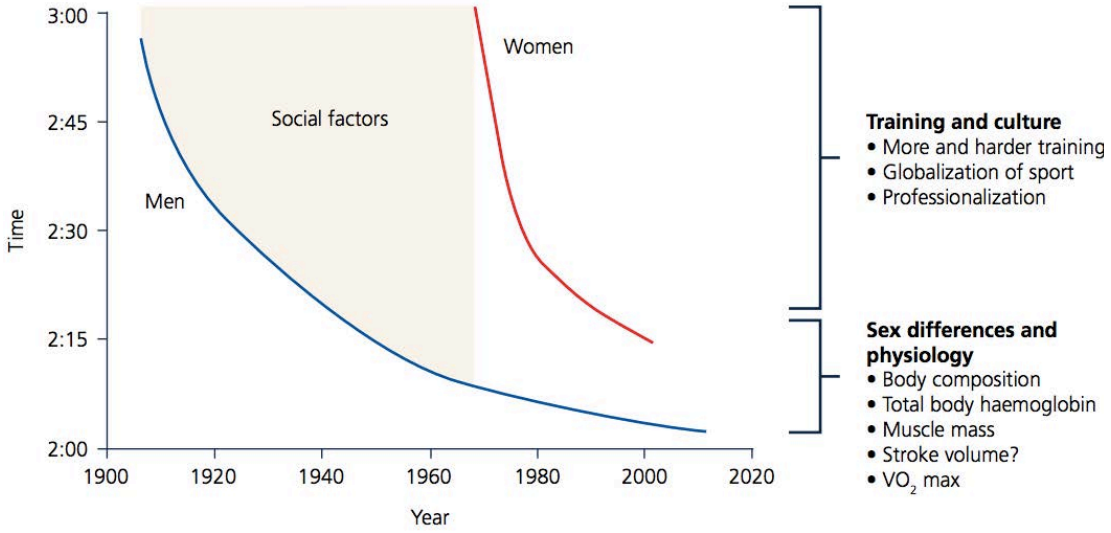


Figure 1.1 Historical world record marathon times in men and women, demonstrating the intersection of sex and gender on sports performance. The difference in record marathon times between men and women in the late 1960's and early 1970's can largely be credited to social factors. The rapid improvements in women's world record marathon times thereafter represent a combination of increased training volume and intensity and more competitive opportunities, more aligned with what was previously only available to male athletes. Taken from Joyner et al. (2017). $\dot{V}O_{2max}$, maximal aerobic capacity

1.3 The case for research investigating females exercising in the heat

As previously alluded to, 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 (Tyler et al., 2016; Morris et al., 2020), and even fewer have structured their protocol with the aim to assess female athlete endurance performance outcomes (Benjamin et al., 2019). Furthermore, novel strategies investigating alternative methods of heat acclimation and its ergogenic potential for athletic populations have largely not included female cohorts. As a result, there remains a lack of understanding of heat acclimation performance outcomes in the female athlete. Therefore, the overall aim of this thesis was to close this knowledge gap by examining emerging methods of heat acclimation, with a focus on the female athlete's response.

1.4 Summary of individual chapters

Chapter 2 provides a review of the literature to date as it pertains to acute and chronic responses to heat stress, with the effects of sex considered throughout. First, the literature review outlines the principles of human thermoregulation and considers how known effects of sex may affect the acute physiological response to, and subsequently performance

during, exercise-heat stress. Next, the literature review considers the consequences of these sex differences on the adaptive processes necessary to improve exercise capacity in the heat. Finally, the literature review explores different methods and aspects of heat acclimation; namely short-term heat acclimation, post-exercise heating and decay of adaptations. The review also considers how the physiological adaptations to heat acclimation may be ergogenic for performance in cool or temperate ambient conditions. All factors and methods are considered through the lens of the female athlete.

Chapter 3 details the general methods applied across the series of studies contained in this thesis. Specifically, equipment and methods utilised across the experimental chapters are described and critically evaluated as appropriate.

Chapter 4 investigates performance responses to exercise heat stress and associated physiological adaptations following short-term *vs* traditional active heat acclimation in a female cohort.

Chapter 5 investigates whether a female cohort exhibits meaningful exercise performance improvements in cool conditions following either short-term or traditional active heat acclimation.

Chapter 6 investigates the efficacy of 3 and 7 weeks of intermittent post-exercise sauna bathing integrated into normal training to: i) induce and subsequently advance the heat acclimation phenotype, as observed during exercise heat stress, and ii) improve objective markers of temperate exercise performance, relative to an ecologically valid control group.

Chapter 7 compares the responses and adaptations of female *vs* male athletes to 3 weeks of intermittent post-exercise sauna bathing integrated into normal training, as pertaining to: i) the heat acclimation phenotype, as observed during exercise-heat stress, and ii) objective markers of temperate exercise performance, relative to an ecologically valid control group.

Chapter 8 reports the decay of adaptations from 3 weeks of intermittent post-exercise sauna bathing.

Chapter 9 discusses the individual and collective findings of the experimental chapters, including key findings, potential mechanisms and limitations. This chapter also outlines perspectives for practical applications and future research directions, and closes with concluding comments on the thesis.

2. REVIEW OF THE LITERATURE

2.1 Overview

The aim of this literature review is to provide background information on heat acclimation, with a particular focus on sex differences, to highlight areas where the current research and recommendations are lacking and to explore possible avenues to close these knowledge gaps. To do this, this review will first provide a basic summary of the responses to exercise-heat stress and its detrimental effect on exercise performance. This is followed by a description of the physiological adaptations associated with heat acclimation and the mechanisms that underpin them, predominantly addressing those relevant to the variables measured in the studies contained herein. Current heat acclimation recommendations related to modality, type, intensity and protocol duration are discussed, citing the benefits and challenges of each design. Information on the decay of the adaptations from these protocols is also presented. Finally, this review provides current evidence and theories regarding heat acclimation as ergogenic for exercise performance in cool or temperate conditions, and what characteristics might facilitate or limit the capacity of a heat acclimation protocol to be ergogenic. The application of heat acclimation for females is central to the purpose of a number of the studies contained within this thesis. Thus, the aforementioned themes will be examined through the lens of the female athlete and female physiology, and specifically, exploring what is known (and not known) about how sex modifies the response and adaptation to heat stress.

2.2 Human thermoregulation

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

Radiation: heat is exchanged between the body and surrounding objects via electromagnetic waves. The rays of the sun heating the skin 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 vaporised. The body's ability to lose heat via evaporation is dependent on both the temperature and water carrying capacity (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. As ambient temperatures approach, or even exceed core body temperatures, the body's ability to lose heat via conduction, convection and radiation (i.e. dry heat exchange) are minimised (Hardy et al., 1938). The body must then rely on its ability to lose heat through evaporation.

2.2.1 Exercise-heat stress

2.2.1.1 Heat production

Heat exchange is not only influenced by the surrounding environment, but also by the heat stored in the body itself. Approximately 80% of the energy expended by skeletal muscle is released as heat, which accounts for between 70% (at rest) and up to 90% (during exercise) of total metabolic heat production (Cheung, 2010b). Additionally, the type of external work done affects heat storage. Active, concentric work is accounted for with a negative value, whilst 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 exchange is balanced, the equation will equal zero and body temperatures will remain the same. This steady thermal state is known as compensable heat stress (Cheung et al., 2000). If the equation is positive and the body cannot adequately dissipate metabolic heat, body temperatures will rise. This is known as uncompensable heat stress and will be a primary focus of this review.

Each pathway involved in the heat storage equation is affected by both the body's thermoregulatory mechanisms and extrinsic, environmental factors. Physiological and behavioural temperature regulation typically do not operate in a dichotomous "on/off" manner, but instead employ a graded response system whereby the response is proportional to the disturbance, often with a threshold for response initiation (Sawka & Wenger, 1988). Furthermore, factors such as fitness, acclimation status, anthropometry and

circulating sex hormone levels modify these thresholds and the extent of the response. This integrative system was summarised by Havenith (2001) and is depicted in **Figure 2.1**.

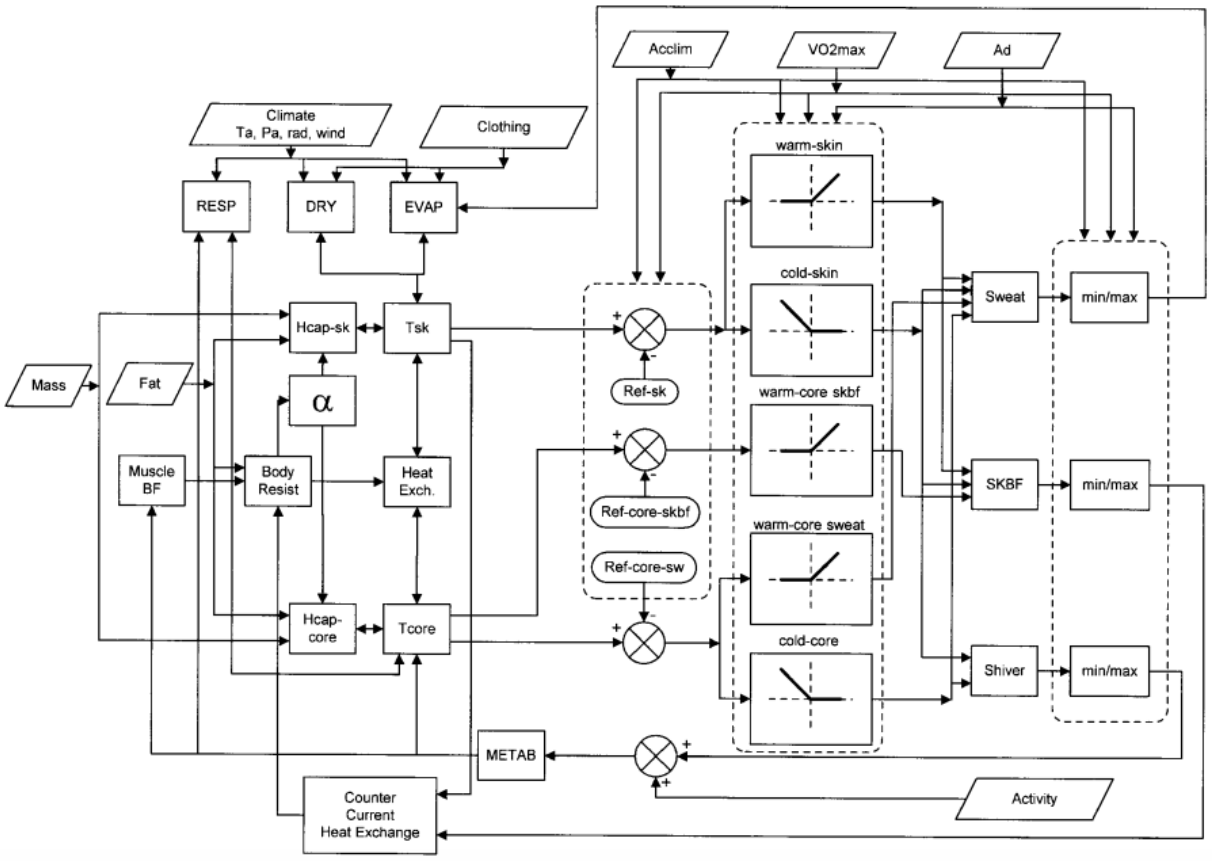


Figure 2.1 Schematic representation of the thermoregulatory control system and modifying factors, originally proposed by Gagge et al. (1986) and modified by Lotens (1993), then by Havenith (2001). Taken from Havenith (2001). T_a, ambient temperature; P_a, humidity or vapour pressure; rad, additional radiation between environment and skin; wind, wind speed; T_{sk}, skin temperature; T_{body}; body temperature; T_{core}, core temperature; METAB, metabolism; Hcap, heat capacity; sk, skin; BF, blood flow; SKBF, skin blood flow; Ref, reference temperature (set-point); shiver, shivering metabolic rate; RESP, respiratory heat loss; Dry, dry (convective + radiative) heat loss; EVAP, evaporative heat loss; α, relative size of skin compartment; Acclim, acclimatory status; $\dot{V}O_{2max}$, maximal oxygen consumption, A_d, body surface area

2.2.1.2 Physiological mechanisms of heat loss

Blood warmed by the heat produced at the muscle is transported to the skin, which allows for heat dissipation via dry heat loss when ambient temperatures are low enough and when sweating has not yet occurred. A greater core-to-skin temperature gradient is advantageous during exercise, as it lessens the demand for skin blood flow by allowing ambient and skin temperatures to efficiently cool the warm blood from the core (Cheuvront et al., 2003; Sawka et al., 2011). Interestingly, a higher internal body temperature (typically

thought to have negative effects on all areas of thermoregulation) also increases this gradient (Sawka et al., 2011). Although internal body temperature is the primary driver of skin blood flow in cooler conditions, exercise in high ambient temperatures, and thus with an elevated skin temperature, results in a substantially greater skin blood flow for a given core body temperature (Nadel, 1985). Elevated skin temperatures trigger cutaneous vasodilation directly via nitric oxide (NO) acting on the vascular smooth muscle (Johnson & Kellogg, 2010) and indirectly through reflexive feedback from the thermoregulatory control centre (located in the preoptic anterior hypothalamus; Boulant, 1996).

Once sweating begins, the primary role of skin blood flow is to deliver heat from the core to the periphery, where it can be dissipated via evaporation of sweat (Taylor, 2014). Thus, the two mechanisms form an important integrative system for heat loss during exercise-heat stress. The addition of sweating to this system allows heat dissipation via evaporation, which as previously noted, is the only avenue for heat loss when ambient temperatures surpass skin and core temperatures (Cheung, 2010b). Depending on the sport or activity, sex, physical size and training status of the athlete, sweat rate typically ranges from 0.5-2 $L \cdot h^{-1}$ (Sawka et al., 2007), although sweat rates ≥3 $L \cdot h^{-1}$ have been recorded in hot environments, at high exercise intensities and/or in athletes of large physical size (Barnes et al., 2019). 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 (Shin & Lee, 2014). Warming the skin also increases sweat rate, as it stimulates local thermal neurons and also because the greater demand for skin blood flow makes more plasma readily available for sweating (Charkoudian, 2003).

Finally, some heat is exchanged through respiration (e.g. evaporation of water vapour in exhaled air, convective heat gain when inspired air is warmer than the body; Cain et al., 1990), however, further discussion of this topic is beyond the scope of the current thesis.

2.2.1.3 Behavioural thermoregulation

In addition to physiological mechanisms of heat loss, humans also modify their behaviour (consciously or subconsciously) to thermoregulate. Humans thermally behave in order to avoid discomfort, and in more severe cases, to avoid thermal injury (Gonzalez-Alonso et al., 1999b). Examples of thermal behaviour include seeking shade, adding/removing clothing, modifying pace during exercise and/or even stopping exercise. These thermal behaviours are preceded by changes in perception of thermal comfort and thermal sensation (Flouris & Cheung, 2009). With initial changes in ambient conditions from

what is perceived as “comfortable” to “uncomfortable”, humans initiate behavioural modifications in an anticipatory manner to defend against changes in skin and core body temperatures (Gagge, 1967). With continued exposure to an uncompensable environment, changes to core body and skin temperatures also influence thermal behaviour; both providing key inputs for control of actions (Flouris & Cheung, 2009; Schlader et al., 2009). Thus, physiological and perceptual changes are linked (Winslow et al., 1937). Further changes in skin and core body temperatures manifest as perceptual responses, and eventually lead to behavioural modifications in a positive feedback loop (Flouris & Cheung, 2009).

During exercise-heat stress, elevated skin and core body temperatures, heart rate and skin wettedness (Vargas et al., 2018) lead to greater discomfort. This discomfort influences thermal behaviours in the form of modifications to speed, power output and pacing strategy (Schlader et al., 2011b; Racinais et al., 2015b). Therefore, thermal behaviour is an integrative aspect of thermoregulation that works in tandem with other thermoeffectors (Schlader et al., 2018) and plays a central role in self-paced exercise performance in the heat.

2.2.1.4 The physiological challenge of exercise-heat stress

The consequence of encountering environmental and metabolic conditions that incite heat stress is a physiological heat *strain* to the body. This strain is multi-faceted, with resulting limitations to exercise capacity due to interactions between and independent of constraints related to the thermoregulatory-mediated cardiovascular challenges, shifts in muscle metabolism and neural function (Nybo et al., 2014). Moreover, the relative contribution of these factors to heat strain are exercise intensity- and modality-dependent (Nybo et al., 2014). The cardiovascular challenges associated with prolonged endurance exercise under ambient-heat stress, which are often associated with dehydration, have been thoroughly discussed and recently reviewed (Trangmar & Gonzalez-Alonso, 2019). Briefly, hyperthermia during fixed-intensity exercise in a euhydrated state is typically characterised by an increased heart rate and cardiac output and a slightly reduced arterial pressure. This is a direct result of a myriad of interactive factors, including: i) the cardiovascular effort required to supply oxygen to the working muscles that typically exhibit a heightened metabolism under heat stress (Febbraio et al., 1994a), ii) the additive challenge of directing blood to the cutaneous vasculature for heat dissipation (Rowell, 1974), iii) compromised venous return due to blood pooling in the venous beds of the legs during upright exercise (Johnson et al., 1974), and iv) intrinsic cardiac factors (i.e. increased activity by heat-

sensitive pacemaker cells and increased sympatho-adrenal activity; Berlyne et al., 1974) reducing ventricular filling time and consequentially stroke volume by 7-8% (Trinity et al., 2010). To add to the cardiovascular challenge of hyperthermic exercise, prolonged exercise-heat stress is often accompanied by progressive dehydration via excessive sweat loss and inadequate fluid replacement. Dehydration and hyperthermia interact to exhibit a unique cardiovascular profile (demonstrated in **Figure 2.2**) that can be characterised by accelerated cardiovascular drift during prolonged whole-body exercise (Gonzalez-Alonso et al., 1997). Stroke volume and cardiac output become markedly reduced, concomitant with a proportional rise in heart rate (Gonzalez-Alonso et al., 1997, 1998; Nybo et al., 2014) and arterio-venous oxygen difference (Rowell et al., 1966; Rowell, 1974; Gonzalez-Alonso, 1998). This can be attributed to the reductions in ventricular filling resulting from the loss of whole-body water and reduced venous return, as well as reductions in diastolic filling time resulting from intrinsic increases in heart rate (Gonzalez-Alonso et al., 1997; Fritzsche et al., 1999; Watanabe et al., 2020). Consequently, blood flow to both exercising muscle and non-exercising tissues (including the skin) is progressively reduced with prolonged dehydrating, hyperthermic exercise (Gonzalez-Alonso et al., 1998); thus compromising key thermoregulatory mechanisms. Overall, hypohydration (the hydration state reached as an outcome of a dehydrating process) and high ambient temperatures additively impair heat loss and result in a lower $\dot{V}O_{2max}$ and a higher heart rate during exercise-heat stress. The higher submaximal heart rate is indicative of working at a higher $\%\dot{V}O_{2max}$ for an equivalent workload as compared to cooler conditions (Sawka et al., 1985; Wingo et al., 2005).

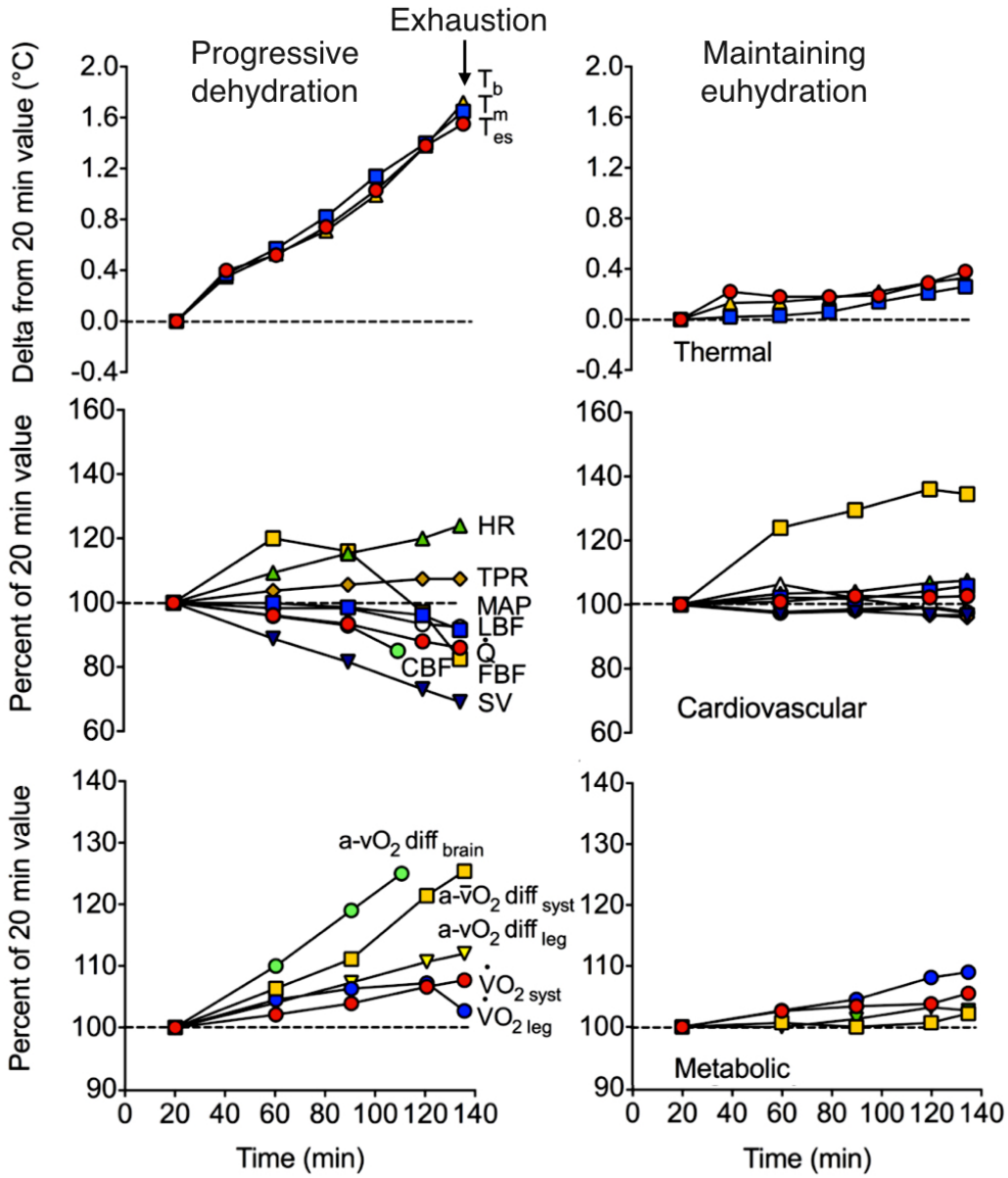


Figure 2.2 Effects of progressive dehydration and maintenance of euhydration by fluid ingestion on thermal cardiovascular and metabolic responses to prolonged submaximal exercise in the heat. Figure drawn by Trangmar & Gonzalez-Alonso (2019) from mean data reported by González-Alonso et al. (1998, 1999) in 40°C, 19%RH and Trangmar et al. (2015) in 35°C, 50%RH. HR, heart rate; SV, stroke volume; $\dot{Q}$, cardiac output; MAP, mean arterial pressure; LBF leg blood flow; TPR, total peripheral resistance; FBF, forearm blood flow; CBF, cerebral blood flow; a-vO_2diff_{brain}, arterio-venous oxygen content differences across the brain; a-vO_2diff_{syst}, systemic arterio-mixed venous oxygen content differences; a-vO_2diff_{leg}, arterio-venous oxygen content differences across the exercising leg; $\dot{V}O_{2syst}$, systemic oxygen uptake; $\dot{V}O_{2leg}$, leg oxygen uptake; T_b, blood (femoral) temperature; T_m, muscle (vastus lateralis) temperature; T_{es}, oesophageal temperature

Concomitant (and somewhat contributory) to cardiovascular strain, exercise-heat stress has a substantial impact on the endocrine system and metabolism (Sawka et al., 1985; Young et al., 1985). Higher cortisol and adrenaline levels whilst exercising in the heat stimulate greater sympathetically mediated carbohydrate metabolism and muscle glycogen

utilisation (Fink et al., 1975; Hargreaves et al., 1996). Additionally, pyruvate kinase, the rate limiting enzyme in glycolysis (Gupta & Bamezai, 2010), is highly heat sensitive and may become impaired as muscle temperatures rise (Herman & Lee, 2009c, a, b). At muscle temperatures of ~40°C, mitochondrial selective permeability also becomes compromised (Brooks et al., 1971), allowing protons to leak back into the intramembrane space and therefore reducing the number of protons moving through the electron transport chain to produce adenosine triphosphate (ATP) (Parkin et al., 1999). The ensuing result is a shift towards anaerobic metabolism (Febbraio et al., 1994b) and eventual oxygen debt (marked by lactate accumulation). The effects of heat stress on metabolism are more prevalent at higher temperatures (e.g. more so at 40°C as opposed to 35°C) and at higher exercise intensities (e.g. at ~80%$\dot{V}O_{2max}$ as opposed to at ~70%$\dot{V}O_{2max}$; Maunder et al., 2019).

High skin, core body and muscle temperatures also independently influence neural factors of exercise performance. Inhibitory signals in response to afferent feedback from hot muscles and internal organs (Gonzalez-Alonso et al., 1999b) likely contribute to an accelerated onset of central fatigue during exercise-heat stress (Nybo & Nielsen, 2001). Compounded by the greater reduction in cerebral blood flow during exercise in the heat as compared to temperate conditions (Rasmussen et al., 2010; Nybo et al., 2014), neural and cerebrovascular factors add additional layers of physiological strain. However, further discussion of these factors is beyond the scope of this review.

Overall, the multi-factorial elements of physiological strain experienced during exercise-heat stress force the attenuation of power-output during self-paced trials (Periard et al., 2011; Périard & Racinais, 2015), earlier fatigue at a fixed workload (Arngrimsson et al., 2003; Arngrimsson et al., 2004) and a reduction in $\dot{V}O_{2max}$ (Sawka et al., 1985; Gonzalez-Alonso & Calbet, 2003).

2.2.1.5 Deleterious effects on endurance exercise performance

The physiological strain associated with exercise-heat stress is known to initiate an earlier onset of fatigue and cause greater detriments to performance with every degree increase in ambient temperature from “optimal” conditions (~10°C; Galloway & Maughan, 1997). Maximal aerobic capacity has been reported to be degraded by 8-21% in the heat (32-49°C) as compared to cooler conditions (~13°C; Lorenzo et al., 2010; James et al., 2017) and 4-18% as compared to temperate conditions (21-25°C; Sawka et al., 1985; Lafrenz et al., 2008; Arngrimsson et al., 2003, 2004). In male cohorts, time trial (TT) performance in the heat (35-40°C) is degraded by ~2% in short trials (4-km TT; Altareki et al., 2009) and up to 17% following longer exercise periods (15-min TT immediately following

30-min at 50% $\dot{V}O_{2peak}$; Ely et al., 2010), when compared to TT performance in cool (13°) and temperate (21°C) conditions, respectively. Most studies examining TT performance decrements in the heat show a degradation within this range (i.e. 6-12%; Tatterson et al., 2000; Tucker et al., 2004; Tyler et al., 2008; Périard et al., 2011; Peiffer & Abbiss, 2011; Racinais et al., 2014; James 2017). Evidently, these impairments are exercise duration-dependant, with longer events such as the marathon typically showing the greatest decrements and sprint-style events being either unaffected or even benefitting from the heat (Guy et al., 2015). Impairments are also training status- and heat severity-dependent (Ely et al., 2007; **Figure 2.3**). Trials employing a time-to-exhaustion (TTE) type protocol have recorded greater detriments of 19-47% in the heat (30-44°C) as compared to cooler (10-23°C) conditions (Dill et al., 1931; MacDougall et al., 1974; Galloway & Maughan, 1997; Morris et al., 1998; Gonzalez-Alonso & Calbet, 2003). It should be noted however, that the reliability of a self-paced time trial is greater than that of a TTE trial (Hopkins et al., 2001; Laursen et al., 2007). Additionally, self-paced time trials allow for thermal behaviour − an important aspect of performance and thermoregulation (Schlader et al., 2011a; Corbett et al., 2018b). Therefore, the larger performance decrements reported with TTE trials may not as accurately reflect the heat-mediated impairments in performance that an athlete is likely to experience in competition, as compared to a time trial.

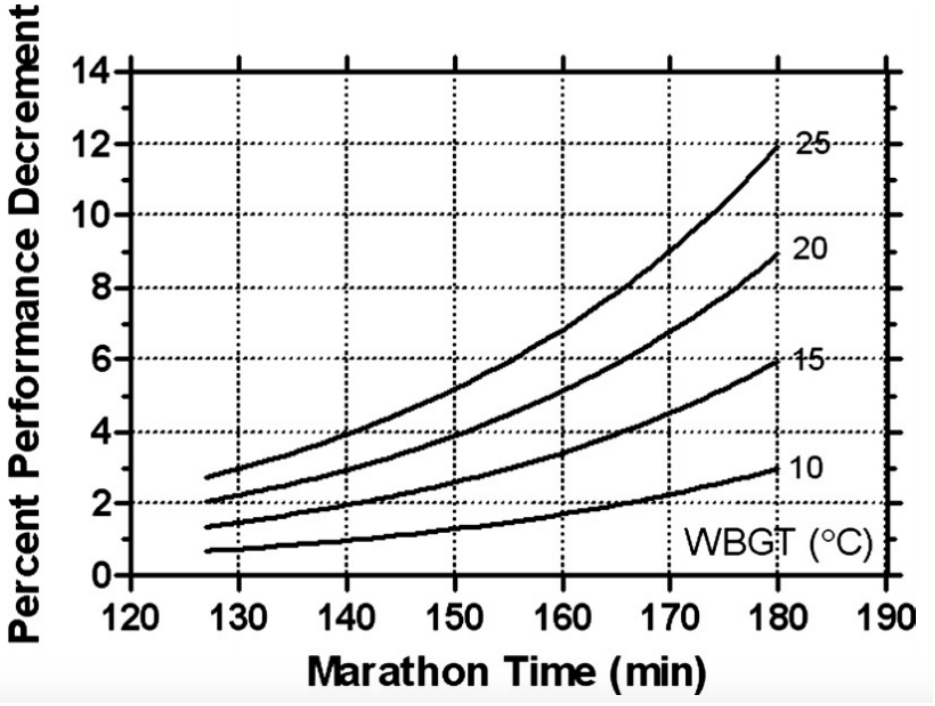


Figure 2.3 The impact of weather on marathon running performance across a range of finishing times. As wet bulb globe temperature (WBGT) increases from 10 to 25°C (dry bulb temperature 8-22°C), elite competitors slow by ~2% (2–3 min), whilst 3-h finishers slow by almost 10% (18 min). Taken from Ely et al. (2007).

2.2.2 Consideration of the effects of sex on thermoregulation

The menstrual cycle and its effects on exercise-heat stress and thermoregulation are not fully understood (or predictable within or between individuals, as described below), so it is common to test female participants with a regular menstrual cycle during the "low-hormone", early-follicular phase (Kolka et al., 1994). Consequently, less-to-little is known about thermoregulatory responses in different phases of the menstrual cycle, or responses of those with irregular cycles (i.e. oligomenhorreic, or commonly for endurance athletes, amenorrheic; Dale et al., 1979) or using different forms of hormonal contraceptives (see **Section 2.2.2.2**). Importantly, both endogenous and exogenous fluctuations in sex hormones (mainly oestrogen and progesterone, for the purpose of this review) are known to affect thermal, cardiovascular, sudomotor, vasomotor, fluid-regulatory and metabolic processes essential in the ability to thermoregulate during exercise-heat stress (Charkoudian & Stachenfeld, 2014). Moreover, exploring the effects of sex on thermoregulation requires consideration of morphological differences, as females are smaller at a population level and typically have a greater body fat percentage. This raises the question of whether size and body composition, and their influence on the thermal physics of heat production and heat loss, should be considered a sex difference *per se*.

2.2.2.1 Endogenous hormones and the menstrual cycle

Fluctuations in endogenous sex hormones cause a 0.2-0.4°C elevation in core body temperatures during the luteal phase of the menstrual cycle (Kleitman & Ramsaroop, 1948; Giersch et al., 2020). In normally menstruating women, oestrogen rises during the follicular phase, and there is a vasodilation-mediated lowering of resting body temperatures (Stephenson & Kolka, 1999; Kim et al., 2008). Following ovulation, progesterone steadily increases, peaking midway through the luteal phase and more rapidly declining in the days preceding menstruation (Charkoudian et al., 1999). Progesterone has direct effects on neurons of the hypothalamus, which stimulate an increase in core body temperatures during the luteal phase of the cycle (Charkoudian & Stachenfeld, 2016). The extent of these fluctuations is highly variable between menstrual cycles and between individuals, as demonstrated in **Figure 2.4**.

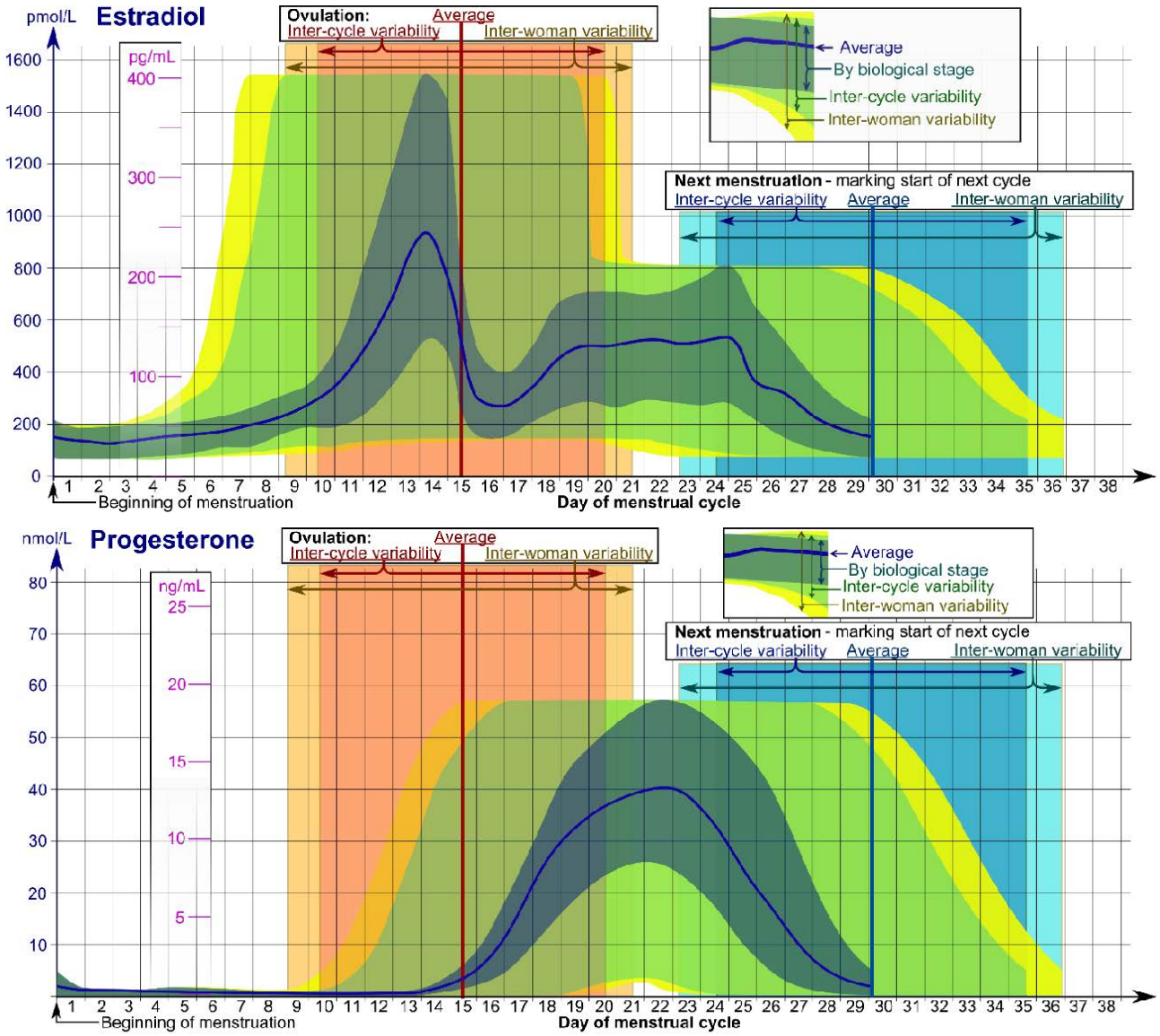


Figure 2.4 Circulating estradiol and progesterone average reference values with 95% prediction intervals for inter-cycle and inter-woman variability. Diagrams taken from Haggstrom (2014), produced with data from Stricker et al. (2006).

Given the influence of female sex hormones on thermoregulatory processes (as outlined above), untrained and recreationally trained eumenhorreic females ($\dot{V}O_{2max}$ 42-45 $mL \cdot kg^{-1} \cdot min^{-1}$) have been observed to exhibit differences in core body temperature and thermoregulatory measures during exercise in the heat across the menstrual cycle (Pivarnik et al., 1992; Kolka et al., 1994; Stephenson & Kolka, 1999; Tenaglia et al., 1999; Kolka et al., 2000; Janse de Jonge et al., 2012). However, high aerobic fitness attenuates phase-related differences in core body temperatures and thermoregulatory responses during exercise (Dale et al., 1979a; Bullen et al., 1984; Kuwahara et al., 2005a; Kuwahara et al., 2005b). In these well-trained ($\dot{V}O_{2max}$ 49-57 $mL \cdot kg^{-1} \cdot min^{-1}$) female cohorts, phase-related differences in resting core body temperature effectively disappear during exercise-heat

stress in dry (≤45% relative humidity; RH) heat (Sunderland & Nevill, 2003; Kuwahara et al., 2005a; Lei et al., 2017). Trained females' enhanced thermoeffector function (i.e. sweating and skin blood flow; Kuwahara et al., 2005) together with a greater sweat rate in the luteal phase (Lei et al., 2017) likely facilitates heat loss to narrow initial resting core temperature differences. Nonetheless, humid heat impairs evaporative heat loss, and thus higher resting core body temperatures in the luteal phase persist throughout exercise in hot-humid conditions (65-81%RH) − even in a well-trained cohort (Avellini et al., 1980a; Lei et al., 2017). Taken together, these data indicate that effects of the menstrual cycle on core body temperature during exercise-heat stress are most apparent in untrained cohorts and/or in humid conditions. Conversely, these effects appear to be considerably attenuated in trained females exercising in hot-dry conditions.

2.2.2.2 Hormonal contraceptives

Approximately half of elite level female athletes use hormonal contraceptives (Martin et al., 2018; **Figure 2.5**). Hormonal contraceptives include oral contraceptive pills and contraceptive patches, injections, implantable rods, intrauterine devices (IUDs; sometimes called "coils") or vaginal rings. Oral contraceptive pills provide varying (i.e. hormonal and placebo pills) and intermittent (once daily) endogenous hormone dosages, whereas most other hormonal contraceptives provide continuous hormone release. There appears to be a ~0.2°C "*quasi*-phase" related shift in resting core body temperature that persists despite oral contraceptive use. This includes both active (i.e. hormonal) *vs* inactive (i.e. placebo) pill phase (Grucza et al., 1993; Charkoudian & Johnson, 1997; Martin & Buono, 1997; Rogers & Baker, 1997; Tenaglia et al., 1999; Sunderland & Nevill, 2003), as well as the during the first *vs* third week of active pill taking (Lei et al., 2019). Female athletes have also reported taking two or more oral contraceptive packs consecutively (i.e. skipping placebo pills) to avoid menses at the time of competition (Findlay et al., 2020). The effects of this behaviour on exercise-heat stress has not been investigated. Monophasic (equal hormone concentration in all active pills), combined ethinyloestradiol and progestin (synthetic oestrogen and progesterone, respectively) hormonal contraceptive pills tend to elicit less pronounced physiological effects than triphasic (increasing hormone concentration for three active-pill weeks) or progestin-only pills (Stachenfeld et al., 2000; Burrows & Peters, 2007). Specifically, progestin-only pills have been observed to illicit higher core body temperatures and sweating onset thresholds than a combined ethinyloestradiol and progestin pill, possibly because they skew the normal ratio of oestrogen and progesterone (Stachenfeld et al., 2000). Moreover, different pill brands have varying dosages (ranging 0.1-1.5 mg progestin

and 0.02-0.05 mg oestradiol; Burrows & Peters, 2007) and use different synthetic forms of progestin, each with a different associated androgenicity (Sims & Heather, 2018). Likewise, other hormonal contraceptives use different synthetic forms of progestin and can be progestin-only (contraceptive injections, implantable rods and IUDs) or combined ethinyloestradiol and progestin (contraceptive patches or vaginal rings).

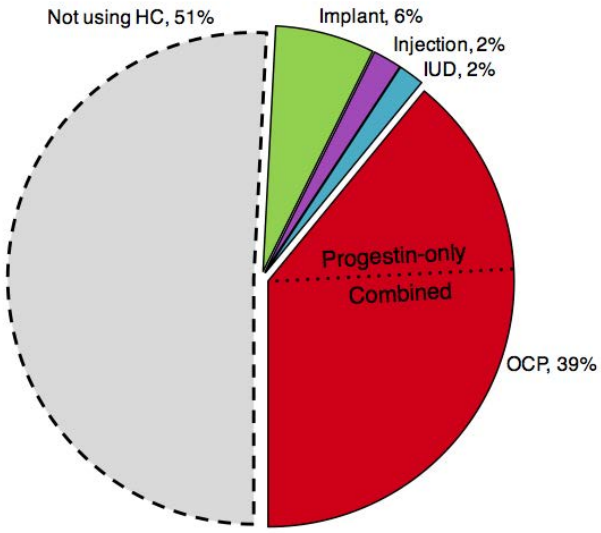


Figure 2.5 Survey results of hormonal contraceptive use in 430 elite female athletes (national, international and professional level). Produced with data from Martin et al. (2018). HC, hormonal contraceptive; IUD, intra-uterine device; OCP, oral contraceptive pill.

In part because of the number of possible variations in endogenous and exogenous hormonal statuses amongst female participants, some authors advise careful consideration of menstrual cycle and oral contraceptive use during study design (Sims & Heather, 2018; Wenner & Stachenfeld, 2020). Alternatively, other authors suggest a more randomised approach for studies not implicitly investigating the effects of the menstrual cycle or hormonal contraceptives to preserve ecological validity for application of the female population as a whole (Stanhewicz & Wong, 2020). The current thesis takes the approach of the latter. The following sections will therefore cover relevant aspects of the effects (or lack thereof) of the menstrual cycle and hormonal contraceptives, so as to provide a level of context and understanding necessary for the studies in the current thesis (which include females randomly sampled irrespective of menstrual cycle phase or hormonal contraceptive use). This information is also important for the latter sections of this review that consider how acute responses impact the adaptive capacity of the female athlete population.

2.2.2.3 Anthropometry

The heat specific capacity of skeletal muscle is ~45% greater than that of adipose tissue (Stolwijk, 1971). Therefore, females' typically higher body fat percentage presents a disadvantage in terms of heat storage during exercise-heat stress (Corbett et al., 2020). Additionally, the amount of heat required to achieve a given change in temperature is inversely related to tissue mass. Hence, males, who are typically larger, will require a greater metabolic heat production to achieve a given change in core body temperature as compared to smaller females (Jay & Cramer, 2015). However, a lighter body mass requires less energy (and consequently less heat production) for a given workload during load-bearing exercise (i.e. walking or running). Body size differences also impact body surface area, where heat exchange between the skin and the environment occurs. The ratio of body surface area-to-body mass is directly related to overall body size and determines the volume of tissue whereby heat can be stored relative to the area whereby heat can be lost (or gained; Corbett et al., 2020).

Taken together, these morphological differences between sexes at a population level (though perhaps not inherent *sex differences* as there are common individual exceptions) and their associated effects on the thermal physics of heat exchange can exacerbate or attenuate physiological sex differences according to environmental conditions, exercise intensity and/or exercise modality. For example, Shapiro and colleagues (1980) observed that females had an attenuated increase in rectal temperature whilst walking on a treadmill in hot-wet conditions (35-37°C, 80-90%RH) but a greater increase in rectal temperature in hot-dry conditions (49-54°C, 10-20%RH), as compared to males. The authors suggested that the greater surface-area-to-mass ratio in females, coupled with a lower, more "efficient" sweat rate, allowed for greater heat dissipation without sacrificing excessive body water stores in humid conditions. Conversely, the greater surface-area-to-mass ratio was *disadvantageous* in hot-dry conditions when ambient temperatures exceeded skin temperatures because of the greater relative area for heat gain. Using direct calorimetry, the gold-standard measure of the whole-body heat loss response, Gagnon and Kenny (2012) showed that there were no sex differences in evaporative heat loss, skin blood flow or rise in oesophageal temperature when male and female participants cycled at matched metabolic heat productions of 200 and 250 $W \cdot m^{-2}$ (external work rates of ~67-71 and ~92-96 W, respectively) in hot-dry conditions (40°C, 24%RH). Importantly, there *were* sex differences in evaporative heat loss at 300 $W \cdot m^{-2}$ (corresponding to ~68 and ~55 $\%\dot{V}O_{2max}$ in the females and males studied, respectively) in hot-dry conditions. Thus, females have a comparatively lower capacity for evaporative heat loss at higher workloads in dry heat, independent of morphological differences. This is important because athletes often work and train at or

above these intensities. Thus, typically smaller female athletes face a distinct disadvantage in hot-dry conditions because of their lower capacity for evaporative heat loss.

2.2.2.4 Thermoeffectors

The sex difference that Gagnon and Kenny (2012) reported in evaporative capacity at higher exercise intensities is directly related to a lower sudomotor activity in females, not skin blood flow, as evidenced by: i) lower regional and total sweat rates compared to males, yet ii) similar skin blood flow onset thresholds and sensitivity (i.e. skin blood flow at a given core body temperature) between sexes (Gagnon & Kenny, 2012). Furthermore, sex differences in sweat rate at higher requirements of heat loss are driven by differences in sweat output per gland and not the density of active sweat glands in the unacclimated stated (Inoue et al., 2005; Ichinose-Kuwahara et al., 2010) or sweat onset threshold (Gagnon & Kenny, 2012). Gagnon and Kenny postulated that the mechanism(s) responsible for reduced sweat output per gland in females may be due to differences in the size of the sweat gland (Sato & Sato, 1983), cholinergic sensitivity (Madeira et al., 2010) and/or acetylcholine handling at the sudomotor junction. However, the role of acetylcholine handling was ruled out in a subsequent study using microdialysis of acetylcholine and methylcholine (Gagnon et al., 2013). The authors instead deduced that sex differences were at the effector organ, and specifically, the maximal sweating capacity of the sweat gland itself. Regardless of the mechanism, this lower sweat rate compromises females' ability to lose heat in hot-dry environments (Frye et al., 1982; Sawka et al., 1983b). However, in humid environments, females may be more "efficient sweaters", preventing unnecessary fluid loss when secreted sweat has not yet been evaporated (Morimoto et al., 1967; Avellini et al., 1980b; Shapiro et al., 1980). Avellini et al. (1980b) suggest that this sweat suppression is a consequence of females being more sensitive to wetted skin. Furthermore, females in an unacclimated state exhibit a sweat rate which, whilst lower overall, is more evenly distributed across their body surface area as compared to males (Havenith et al., 2008). These observations considered, whilst the lower sweat rate in females is typically framed as an impairment, the environment should be carefully considered. A reduced sweat rate may actually serve as an advantage in endurance events undertaken in humid-heat conditions by conserving unnecessary fluid-loss.

It should be noted that Gagnon and colleagues (2012, 2013) tested female participants during the follicular phase of their menstrual cycle (i.e. within the first and tenth day of their cycle). However, it has been established that there is a ~0.2°C shift in onset thresholds for both thermoeffectors during the luteal phase, in line with increases in resting

core body temperature (Hessemer & Bruck, 1985; Grucza et al., 1993; Stephenson & Kolka, 1993; Kolka & Stephenson, 1997; Charkoudian & Johnson, 1999; Stachenfeld et al., 2000). Some authors have also observed greater sweat rates in females in the luteal phase during exercise-heat stress (Hessemer & Bruck, 1985; Garcia et al., 2006). However, when sweat rates are normalised by changes in core body temperature (an index of sudomotor sensitivity), there seems to be little variation between phases of the menstrual cycle (Kolka & Stephenson, 1989; Frascarolo et al., 1992; Charkoudian & Johnson, 1999; Lei et al., 2017; Notley et al., 2019a).

Cyclical hormone fluctuations may have a profound effect on the vasculature and vasomotor activity in addition to centrally mediated shifts in skin blood flow onset thresholds. Oestrogen enhances vasodilation via indirect activation of endothelial nitric-oxide synthase (eNOS; Orshal & Khalil, 2004). Briefly, eNOS mediates skin blood flow during exercise-heat stress (Fujii et al., 2016) by synthesising NO, a powerful vasodilator, and relaxing the smooth muscle of the endothelium (Orshal & Khalil, 2004). Oestrogen also stimulates vasodilation through NO-independent mechanisms (Stanhewicz et al., 2014; Novella et al., 2019). Thus, the sustained elevations of oestrogen during the luteal phase have been reported to cause a profound increase in skin blood flow during exercise-heat stress (i.e. 40-50% higher than early follicular; Kolka & Stephenson, 1997; **Figure 2.6**).

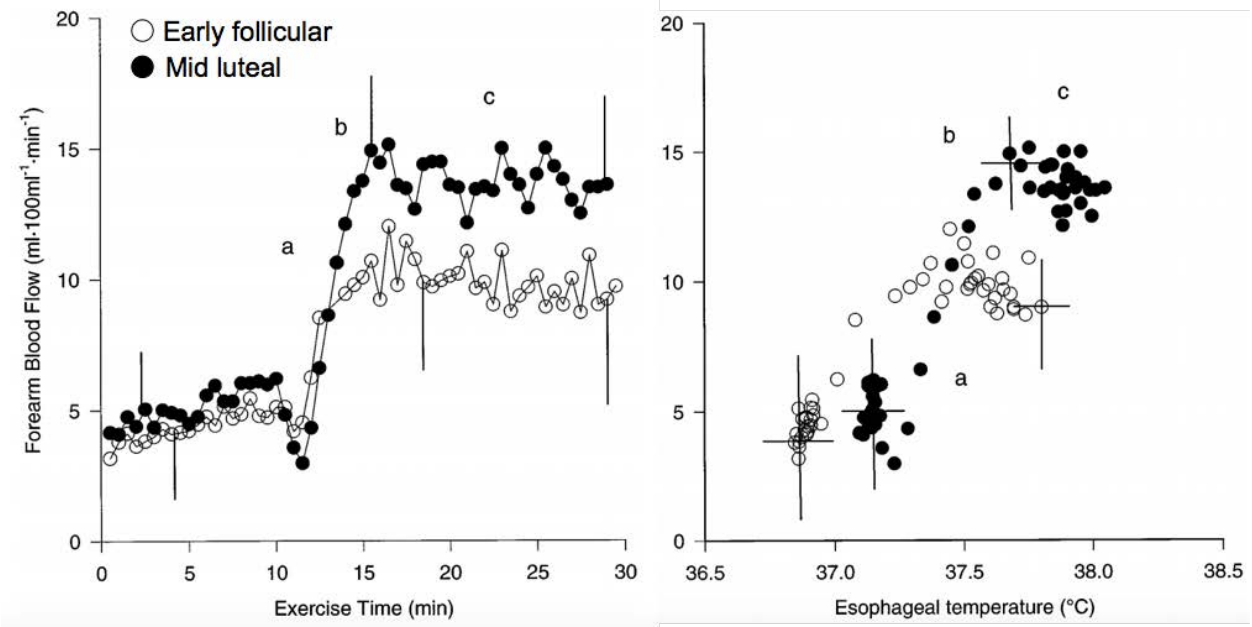


Figure 2.6 Forearm blood flow during exercise at 80% peak aerobic power in the heat (35°C, 22%RH) in the early-follicular (*open circles*) and mid-luteal phase (*filled circles*). Mid-luteal *vs* early-follicular (p<0.05): [a] Exercise transient; [b] attenuation of forearm blood flow increase; [c] levelling off or plateau of forearm blood flow. Taken from Kolka & Stephenson (1997).

Other studies have not observed differences in forearm blood flow or sweating during exercise-heat stress across the menstrual cycle (Lei et al., 2017; Notley et al., 2019a). However, both Notley et al. (2019a) and Lei et al. (2017) observed that phase-related differences in resting core body temperatures “disappeared” during exercise in hot-dry conditions. Since metabolic heat production was matched between phases and there were no phase-related differences in thermoeffector function (and thus dry or evaporative heat loss) in both studies, neither Notley et al. (2019a) nor Lei et al. (2017) could identify the mechanism(s) that resulted in similar exercising core body temperatures despite different resting core body temperatures. Therefore, it is likely that incremental (and undetected) increases in sweat rate and/or skin blood flow in the luteal phase as observed in previous literature (Hessemer & Bruck, 1985; Stephenson & Kolka, 1985; Kolka & Stephenson, 1997; Garcia et al., 2006) additively enhanced heat loss to diminish the phase-related, ~0.2°C difference in resting core body temperature. Nonetheless, the fact that any differences were too small to significantly influence dry or evaporative heat loss and heat storage in both of these independent studies (Lei et al., 2017; Notley et al., 2019a) indicates that phase-related differences in thermoeffector function may have a lesser influence on thermoregulation and whole-body heat loss and/or storage than historically believed. On top of this, Lei et al. (2017) highlighted that athletes appear to effectively moderate the additional thermal challenge of the luteal phase (or *quasi*-luteal phase, as discussed below) with thermal behaviour (i.e. pacing) during self-paced exercise (Wright et al., 2002; Lei et al., 2017, 2019; **Figure 2.7**). Overall, whilst menstrual cycle phase may have some effect on independent mechanisms of thermoregulation, the impact on whole-body physiological responses to exercise-heat stress are perhaps less pronounced than previously thought, especially for trained athletes (Kuwahara et al., 2005a; Lei et al., 2017).

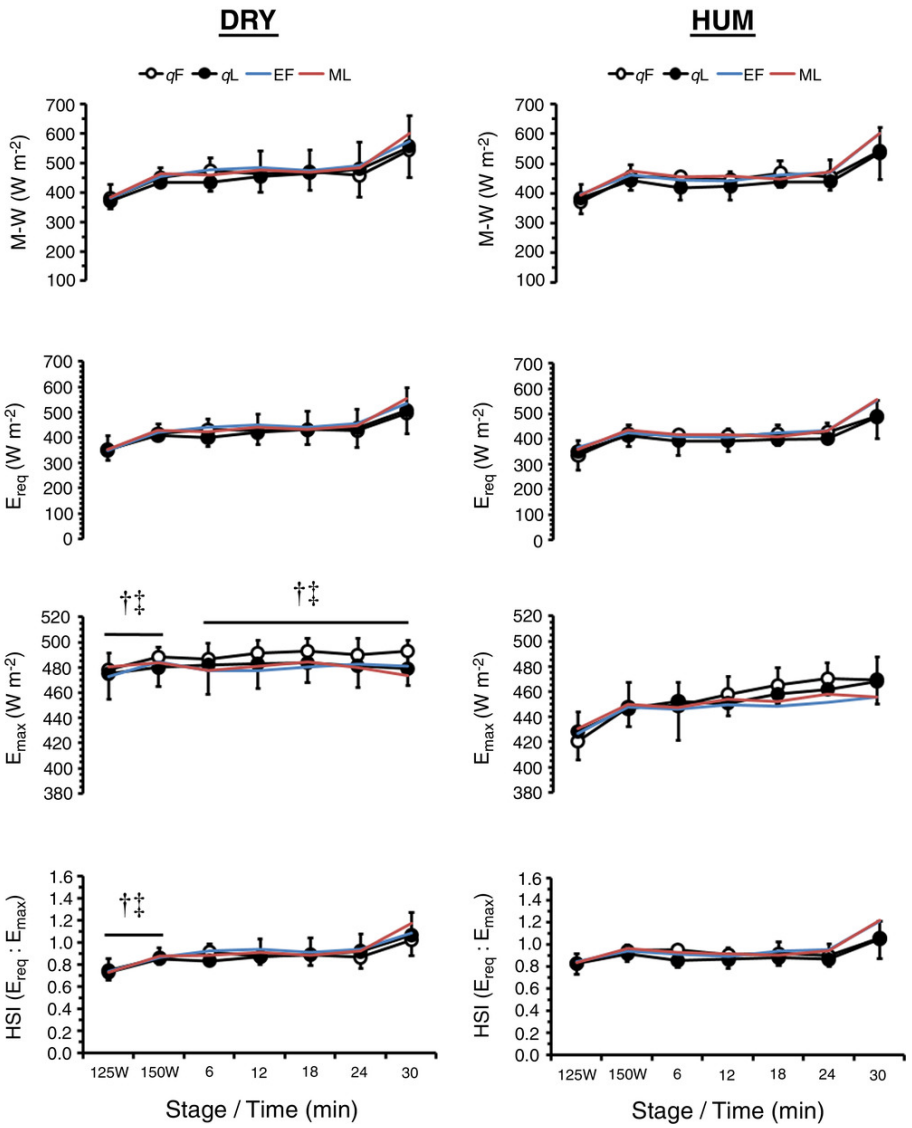


Figure 2.7 Mean±SD rate of metabolic heat production (metabolic rate−external work), required evaporative cooling for heat balance (E_{req}), maximal evaporative capacity of the environment (E_{max}) and heat stress index (HSI; <1 considered compensable) during exercise in dry (DRY) and humid (HUM) heat in oral contraceptive users during the quasi-follicular (days 3-5 of active-pill taking; *q*F) and quasi-follicular phase (days 18-20 of active-pill taking; *q*L) and normally menstruating women in the early follicular (days 3-6 since menses onset; EF) and mid-luteal (days 18-21 since menses onset; ML). No within-environment differences between phases or between oral contraceptive users *vs* non-users. †Significant difference between corresponding *q*F−HUM value. ‡Significant difference between corresponding *q*L−HUM value. Taken from Lei et al. (2019), with mean early-follicular (EF) and mid-luteal (ML) values from Lei et al. (2017).

Additional within-sex differences are apparent when comparing between groups of oral contraceptive pill users and non-users. Oral contraceptive pill users have been observed to exhibit higher resting core body temperatures (0.2-0.5°C) in comparison to non-users (Charkoudian & Johnson, 1997; Rogers & Baker, 1997; Stachenfeld et al., 2000; Sunderland & Nevill, 2003; Minahan et al., 2017), though not when comparing between highly trained ($\dot{V}O_{2max}$ ~56 mL·kg^{-1}·min^{-1}) cohorts of users *vs* non-users (Lei et al., 2019). However, this

difference in resting core body temperature between oral contraceptive pill users and non-users, when it is reported, appears to narrow and subsequently disappear with exercise-heat stress (Tenaglia et al., 1999; Stachenfeld et al., 2000; Sunderland & Nevill, 2003; Minahan et al., 2017). Additionally, oral contraceptive use may modify thermoeffector function. Firstly, with regards to sweating, oral contraceptive pill users exhibit an elevated threshold for sweat onset in comparison to non-users (Charkoudian & Johnson, 1997; Rogers & Baker, 1997; Sunderland & Nevill, 2003; Minahan et al., 2017; Lei et al., 2019), especially when using progestin-only pills (Stachenfeld et al., 2000). Oral contraceptive users have also sometimes (Grucza et al., 1993; Lei et al., 2019) but not always (Tenaglia et al., 1999; Stachenfeld et al., 2000; Sunderland & Nevill, 2003) been reported to exhibit a reduced sudomotor sensitivity and overall lower sweat rate, as compared to non-users. However, any differences in sweat onset, sensitivity and rate are admittedly small and have negligible impact on evaporative heat loss when using a combined oestrogen and progestin pill (Lei et al., 2019). Second, oral contraceptive use has been reported to increase the onset threshold of skin blood flow during passive heating (whereby skin blood flow is initiated at a higher core body temperature) as compared to normally menstruating women (Houghton et al., 2005; Charkoudian et al., 2017). However, these differences have not been observed during either fixed-intensity or self-paced exercise-heat stress (Minahan et al., 2017; Lei et al., 2019). Within oral contraceptive users, studies have reported greater skin blood flow during passive heating (Charkoudian & Johnson, 1997; Lei et al., 2019), but not during exercise-heat stress (Lei et al., 2019), in the *quasi*-luteal phase of oral contraceptive use compared to the *quasi*-follicular phase. Taken together, the use of oral contraceptives *per se* appears to have only minor effects on skin blood flow during exercise-heat stress overall, though some *quasi*-phase-related differences like those of a “normal” menstrual cycle may be a residual effect of the menstrual cycle that is not fully suppressed by exogenous hormones. Nonetheless, any differences in thermoeffector function between *quasi*-phases or as compared to non-oral contraceptive pill users do not translate to significant differences in heat loss and/or heat storage during exercise-heat stress (Lei et al., 2019; **Figure 2.7**).

Finally, training-related amenorrhea could modify the thermoeffector response to exercise-heat stress because of the lower oestrogen levels associated with amenorrhea (Frye (Frye et al., 1982; O'Donnell et al., 2011). Indeed, training-related amenorrhea has been observed to impair endothelium-dependent dilation of the brachial artery (Hoch et al., 2003; Rickenlund et al., 2005). Furthermore, greater menstrual disturbance (i.e. amenorrhea *vs* oligomenorrhea) is associated with greater vascular dysfunction (Rickenlund et al., 2005). However, Frye and colleagues (1982) observed that amenorrheic women and normally menstruating women (in both the follicular and luteal phase) exhibited similar body temperatures (rectal and skin), heart rate and sweating during treadmill walking in hot-dry

conditions (48°C, 15%RH), where effects of vascular impairments should have been most apparent. Nonetheless, further investigation is warranted before fully ruling out any impact of amenorrhea on thermoregulation during exercise-heat stress, given the low intensity of exercise employed by Frye et al. (1982).

The between- and within-sex differences (and similarities) in thermoeffector function discussed throughout this section are important to consider for the series of studies in this thesis, given the inclusion of a random sample of female participants irrespective of sex hormone milieu (as discussed in **Section 2.2.2.2**).

2.2.2.5 Cardiovascular

The cardiovascular profiles of exercise-heat stress and dehydration are largely modelled off male cohorts (e.g. the profiles detailed in **Figure 2.2**). Consequently, the following sex differences regarding the cardiovascular system prompt reconsideration of the effects of both exercise-heat stress and dehydration as independent and combined stressors. Like with other physiological variables, sex differences in cardiac structure and function can be partially attributed to anthropometrics; namely females' typically smaller body size on a population level. For example, in absolute terms, males have larger hearts and stroke volumes, though this is arguably rendered insignificant by normalising to body size (Ogawa et al., 1992). However, neither body size nor body surface area explain the differences observed in left ventricle mass (Morales et al., 1992; Gardin et al., 1995), with a ~31% greater mass observed in male compared to female highly-trained athletes (Pelliccia et al., 1996). A host of other factors could underpin cardiovascular sex differences, including blood vessel size (and the subsequent implications for blood pressure and Ohm's Law), blood volume, distribution of cardiac output, calcium handling, catecholamine response and hormones associated with either sex (i.e. oestrogen, progesterone and testosterone; Parker et al., 2010). Moreover, these factors may jointly and independently influence acute cardiovascular responses to the heat in female athletes.

Females typically have a lower blood volume than males, even when controlling for body mass (Wiebe et al., 1998). The redistribution of blood volume to the skin during exercise-heat stress therefore represents a relatively greater shift in females than in males (Senay, 1973; Paolone et al., 1978). Additionally, females expend an additional ~5% of total exercising $\dot{V}O_2$ on the respiratory muscles during exercise in temperate conditions as compared to males, because of females' greater work of breathing (related to smaller airways, even when normalised for body surface area; Dominelli et al., 2015). Moreover, females have been observed to exhibit a blunted splanchnic vasoconstriction response (to

head-up tilt; Jarvis et al., 2010). Thus, females' greater oxygen (and therefore blood) demand from the respiratory muscles as well as their blunted splanchnic vasoconstriction (nullifying an important compensatory response to exercise-heat stress) would be expected to jointly and independently exacerbate the issue of overall lower blood volume reserve in females.

Males typically exhibit a relatively greater increase in blood pressure than females during exercise (Ogawa et al., 1992; Wheatley et al., 2014) and passive heat stress (Meendering et al., 2005). This is a consequence of greater sympathetically mediated vasoconstriction of the peripheral vasculature in males (Frey & Hoffler, 1988; Dutra et al., 2013). The comparatively lower sympathetic activation of the vasculature in females has implications for conditions when venous return is imperative, such as when orthostatic and thermal stressors are combined (Kenney et al., 2014). Indeed, females have been observed to experience a greater cardiovascular strain, as indicated by a greater elevation in heart rate, under severe passive heat stress (i.e., 90°C sauna; Jezova et al., 1994). Sympathetic baroreflex sensitivity is greater in the follicular phase as compared to the luteal phase, and greater in the active-pill phase of hormonal contraceptive use as compared to the placebo phase (Minson et al., 2000a, b). However, this does not appear to translate to differences in overall blood pressure during exercise-heat stress, which appears to be similar across menstrual cycle phases and when using oral contraceptives (Horvath & Drinkwater, 1982; Minahan et al., 2017).

When dehydration is superimposed onto exercise-heat stress, cardiovascular sex differences may be exacerbated. Females' lower stroke volume during exercise (Wheatley et al., 2014) represents a reduced reserve to cope with decreases in stroke volume during hypohydrated exercise (Gonzalez-Alonso et al., 1997, 1998). Hypohydrated exercise in the heat also tends to increase the degree of ventricular twist compared to euhydrated exercise, in an attempt to oppose the falling end-diastolic volume (Travers et al., 2020a; Watanabe et al., 2020). However, female hearts already exhibit greater ventricular twist in response to reductions in preload (i.e. lower-body negative pressure; Williams et al., 2016), again possibly limiting their functional reserve to oppose reductions in end-diastolic volume (as can occur with heat stress and/or dehydration). With regards to oxygen delivery and extraction, trained females exhibit a lower haemoglobin concentration compared to males, but a greater arterio-venous oxygen difference during exercise at a given $\%\dot{V}O_{2max}$ or heart rate (Ogawa et al., 1992; Wiebe et al., 1998). Furthermore, oral contraceptive users exhibit a higher arterio-venous oxygen difference at a given $\%\dot{V}O_{2max}$ compared to non-users (Gordon et al., 2018). Together, this information has implications for hypohydrated exercise in the heat, where peripheral oxygen extraction is elevated to compensate for the reduction in blood flow to the active muscles (Gonzalez-Alonso, 1998). Thus, females may present a reduced peripheral

reserve to cope with reductions in oxygen delivery when hypohydrated. Overall, these peripheral and central between- and within-sex differences in the cardiovasculature may modify the complex response to acute exercise-heat stress and often-ensuing progressive dehydration.

2.2.2.6 Body fluid regulation

The primary sex difference in fluid-regulatory hormone response is that males exhibit a greater ADH response to changes in plasma osmolality and a lower osmotic threshold for initiation of this ADH response (i.e. sensitivity) than normally menstruating women in the follicular phase (Stachenfeld et al., 2001). However, males' typically higher sweat rates (see **Section 2.2.2.4**) and higher incidence of hypernatremia (plasma sodium concentration of ≥135 $mmol \cdot L^{-1}$ and ≥145 $mmol \cdot L^{-1}$, respectively; Eijsvogels et al., 2013) are also integrative to fluid-regulation during exercise-heat stress.

In addition to sex-differences in fluid-regulation, fluctuations in female sex hormones across the menstrual cycle have numerous effects on the fluid-regulatory hormone response during exercise-heat stress (Wenner & Stachenfeld, 2012). At rest, oestrogen is associated with increased ADH synthesis (Stachenfeld & Keefe, 2002) whereas progesterone is associated with increased aldosterone release (Szmuilowicz et al., 2006). However, recent data indicates that exercise may further elevate this progesterone-mediated, comparatively greater renin-angiotensin-aldosterone system activity in the luteal phase (Nose et al., 2020). Furthermore, thirst onset and ADH release have been observed to occur at a lower plasma osmolality in the luteal phase as compared to the follicular phase (Vokes et al., 1988). Thus, it is especially necessary to consider responses across the menstrual cycle during exercise-heat stress, especially when fluid is not adequately replaced (Stachenfeld et al., 1999).

Lastly, oral contraceptive use is known to modify the fluid-regulatory response. Primarily, oral contraceptive use impacts fluid regulation via a lower osmotic threshold for ADH release and thirst stimulation (Stachenfeld et al., 1999; Stachenfeld, 2008). This means that fluid-retention and -replacement responses are initiated at earlier stages of hypohydration. There has been some variability reported in the magnitude of this difference across oral contraceptive users, which may be due to varying estradiol content across oral contraceptive pill brands (Stachenfeld et al., 1999). The impact of other forms of hormonal contraception (e.g., contraceptive implant, contraceptive patch, intrauterine devices and contraceptive injections) on body fluid-regulation at rest or during exercise has not yet been investigated.

2.2.2.7 Metabolism

Substrate metabolism during exercise (i.e. oxidation and relative contribution of carbohydrates and fats, and to a lesser extent, protein) is affected by sex (Horton & Braun, 2004). At submaximal exercise intensity levels, females rely more on fat oxidation for fuel than males (Horton et al., 1998; Romijn et al., 2000). This is because of sex differences in fatty acid and lactate metabolism capacities (Soler-Argilaga & Heimberg, 1976; Tarnopolsky et al., 1990; Tarnopolsky, 2008; Montero et al., 2018) and because glycogen oxidation and adrenaline levels are lower in females (Horton et al., 1998; Davis et al., 2000; Horton et al., 2006). Thus, females exhibit a lower respiratory exchange ratio at ventilatory thresholds during exercise (Yasuda et al., 2006). It has recently been highlighted by Maunder et al. (2019) that a shift towards greater carbohydrate utilisation during exercise-heat stress is both heat- and exercise-intensity-dependent. However, this was in a male cohort. Given the sex differences in substrate utilisation described above, it is unknown whether females would exhibit similar heat and intensity “thresholds” for a shift to carbohydrate metabolism as Maunder et al. (2019) observed in males.

Notably, carbohydrate availability is not the limiting factor in fatigue during *prolonged* exercise-heat stress. Parkin et al. (1999) observed higher muscle glycogen stores at the point of fatigue in the heat (40°C, <50%RH) in comparison to temperate (20°C) conditions in endurance-trained males. This indicates that other factors (likely cardiovascular) limit the duration of exercise in the heat before glycogen can be depleted enough to make metabolism a limiting factor (at least in male cohorts; Parkin et al., 1999).

Oestrogen in particular appears to influence the “preference” for fat oxidation in females (D'Eon et al., 2002; Devries et al., 2005). It could therefore be hypothesised that the menstrual cycle would impact fat oxidation. However, after reviewing the conflicting evidence, Oosthuyse & Bosch (2010) propose that this is more dependent on the *concentrations* of the oestrogen and progesterone (and their ratio) during the cycle (of which there is marked individual variation) than strictly the menstrual cycle phase. For example, studies reporting longer times to exhaustion and time trial performance (both largely influenced by fat oxidation and glycogen sparing at submaximal intensities) in the luteal phase also reported that participants exhibited high oestrogen-to-progesterone ratios in the luteal phase (Jurkowski et al., 1981; Nicklas et al., 1989; Oosthuyse et al., 2005). It is somewhat surprising then, that the exogenous hormone levels associated with oral contraceptive use do not modify substrate utilisation during exercise in temperate conditions (Vaiksaar et al., 2011; Isacco et al., 2012), nor do they affect submaximal blood lactate concentration in hot conditions (Minahan et al., 2017).

Overall, the effects of sex and hormone status on fuel utilisation may have carryover effects for exercise-heat stress, which increases skeletal muscle metabolism and relative carbohydrate oxidation rates (Febbraio et al., 1994b). Though glycogen depletion may not be a limiting factor *per se* (Parkin et al., 1999), increased metabolism during exercise-heat stress does increase oxygen (and subsequently blood flow) demand at the muscle, exacerbating cardiovascular strain.

2.2.2.8 Exercise performance

In hot ambient temperatures, $\dot{V}O_{2max}$ is reduced to a similar extent in both females and males (Arngrimsson et al., 2004). It has also been reported that in females, neither the menstrual cycle nor monophasic oral or injectable contraceptives modify $\dot{V}O_{2max}$ under heat stress (Grucza et al., 1993; Janse de Jonge, 2003; Armstrong et al., 2005), although reductions in $\dot{V}O_{2max}$ have sometimes been observed in oral contraceptive users in temperate conditions (Casazza et al., 2002; Lebrun et al., 2003; Joyce et al., 2013). Modifications to $\dot{V}O_{2max}$ in the heat are important, as pacing during time trials appears to be regulated within a narrow range of an athletes' environmental condition-specific $\%\dot{V}O_{2max}$ (Périard & Racinais, 2015). Population data from a number of American and European marathons show that heat-mediated decrements to pacing are similar between males and females (El Helou et al., 2012). However, the heat-mediated performance decrements during shorter self-paced time trials in the heat have not been quantified in a female cohort.

Greater fat oxidation rates in the luteal phase could facilitate performance improvements during prolonged exercise (i.e. marathon performance; Greenhall et al., 2020) in cooler conditions. However, in the context of exercise-heat stress, the higher resting core body temperatures in the luteal phase may limit prolonged exercise in the heat because of a reduced capacity for heat storage (if a core temperature limit for exercise is assumed; Janse de Jonge, 2003). Indeed, Tenaglia and colleagues (1999) observed a 20% decrease in exercise tolerance time during the mid-luteal phase in comparison to the early-follicular phase in hot conditions (40°C, 30% RH). Participants reached their "critical temperature threshold for heat tolerance" earlier, concomitant with higher resting core body temperatures. These findings were similar to Janse de Jonge et al. (2012), who observed a higher rate of core body temperature change, higher perceived exertion and earlier fatigue during fixed-intensity exercise in the heat (32°C, 60% RH) during the luteal phase; with effects of a higher core body temperature more pronounced with the longer duration of the tolerance tests (≥60 minutes). Importantly, exercise time-to-exhaustion in this study was similar between menstrual cycle phases in temperate conditions (20°C, 45%RH). Therefore, the

heat was more detrimental to performance in the luteal phase (i.e. ~13% lower in hot than temperate trials) than in the follicular phase (~9% lower).

In contrast to time-to-exhaustion trials, menstrual cycle does not appear to affect performance in either self-paced time trials (Lei et al., 2017) or a sport-specific tests (i.e. high-intensity intermittent running; Sunderland & Nevill, 2003). The tests employed by Sunderland et al. (2003) and Lei et al. (2017) allowed for at least some degree of self-pacing, and therefore allowed for thermal behaviour to aid thermoregulation (Schlader et al., 2011a). Thermal behaviour via pacing is not possible during time-to-exhaustion trials at a fixed intensity and may explain why effects of the menstrual cycle were observed in time-to-exhaustion, but not self-paced trials. Alternatively, the difference in results may be attributed to the different training status of participants, as increased aerobic capacity has been shown to minimise hormonal effects on thermoregulatory mechanisms (Kuwahara et al., 2005a). Indeed, participants in studies employing time-to-exhaustion (Tenaglia et al., 1999; Janse de Jonge et al., 2012) had a lower $\dot{V}O_{2max}$ than the participants in studies employing self-paced performance assessments (Sunderland & Nevill, 2003; Lei et al., 2017; mean $\dot{V}O_{2max}$ 40-44 *vs* 51-57 $mL \cdot kg^{-1} \cdot min^{-1}$ in time-to-exhaustion and self-paced studies, respectively).

A meta-analysis by Elliott-Sale et al. (2020) determined that whilst oral contraceptive use likely has a detrimental effect on exercise performance, these effects are generally trivial. These findings are paralleled by data investigating exercise-heat stress, as Tenaglia et al. (1999) observed similar exercise tolerance times in the heat (40°C, 30% RH) between normally menstruating women and oral contraceptive users. Likewise, Sunderland and Nevill (2003) reported that oral contraceptive users and non-users ran a similar distance in an intermittent sprint test in the heat (31°C, 23%RH), although oral contraceptive users ran further during their *quasi*-follicular phase than their *quasi*-luteal phase, concomitant with lower resting rectal temperatures. However, Lei et al. (2019) did not observe any differences in cycling time trial performance between oral contraceptive users' *quasi* phases in either dry or humid heat (34°C, 42%RH and 29°C, 83%RH, respectively). Therefore, the literature to date indicates that any differences between menstrual cycle phases or hormonal contraceptive use, as indicated from mechanistic or fixed-workload trials (Janse de Jonge, 2003; Charkoudian & Stachenfeld, 2014), do not translate to meaningful performance outcomes in the heat. In the context of the most recent opposing viewpoints on the topic of controlling (or not controlling) for menstrual cycle and oral contraceptive use in physiology research (Stanhewicz & Wong, 2020; Wenner & Stachenfeld, 2020; see **Section 2.2.2.2**), it appears both ecologically valid and methodologically sound to include female participants irrespective of endogenous/exogenous hormone status when investigating performance outcomes in the heat, especially in trained participants.

2.3 The heat acclimation phenotype

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 via two broad methods: acclimation and acclimatisation. Acclimation is often a laboratory-based method, performed in an environmental chamber where conditions such as temperature, humidity and altitude can be artificially manipulated and controlled. Acclimatisation is performed in natural conditions, where these factors cannot be as precisely controlled (Armstrong & Maresh, 1991). Active heat acclimation (exercise combined with hot environmental conditions) has been observed to induce the greatest adaptations, as it drives body temperature increases (core and skin) and provokes substantial sweat loss (Buono et al., 2009a). This combination of stressors also stimulates a unique upregulation of genes that cannot be stimulated by exercise or passive heat individually (Kodesh et al., 2011). However, passive heat acclimation interventions (i.e. heating independent of exercise) are becoming increasingly popular because of their accessible and practical nature (Heathcote et al., 2018). Further discussion of the types of heat acclimation protocols that elicit these adaptations and modifying factors can be found in later sections.

Historically, the primary aim of heat acclimation interventions has been to induce physiological adaptations (the so-called, “heat acclimation phenotype”) to better cope with the challenge of heat stress (Sawka et al., 2011). The expected functional and physiological adaptations associated with the heat acclimation phenotype are presented in **Table 2.1**. However, the path to attaining these adaptations may be affected by sex. By collectively interpreting data from Hertig & Sargent (1963), Wyndham et al. (1965) and Weinman et al. (1967), Burse (1979) concluded that females acclimate at a slower rate, and that fewer females than males are able to tolerate the acclimation protocols employed. Since Burse’s report over 40 years ago, more research has been conducted to elucidate the specifics of these sex differences, however many questions remain. In the following sections, the mechanisms facilitating the physiological adaptations most relevant to the measures in the current thesis, as well as modifying effects of sex on these adaptations, will be discussed.

Table 2.1 Summary of functional and physiological adaptations observed during heat stress following heat acclimation. Taken from Sawka et al. (2011).

Functional		*Physiological, continued*	
Maximal aerobic power	Increased	Muscle and plasma lactate	Lowered
Submaximal aerobic performance	Improved	Skeletal muscle force generation	Increased
Thermal comfort	Improved	Thirst	Improved
Physiological		Electrolyte losses	Reduced
Core temperature	Reduced	Total body water	Increased
Rest (temperate)		Plasma volume	Increased
Exercise		Cardiac output	Better sustained
Skin temperature	Reduced	Heart rate	Lowered
Sweating	Improved	Stroke volume	Increased
Earlier onset		Blood pressure	Better defended
Higher rate		Myocardial compliance	Increased
Skin blood flow	Improved	Myocardial efficiency	Increased
Earlier onset		Cardio-protection	Improved
Higher rate		Heat shock proteins	Increased
Muscle glycogen	Spared	Cellular thermal tolerance	Increased
Lactate threshold	Increased	Whole-body metabolic rate	Lowered

2.3.1 Body temperatures

Thermoregulatory response is estimated to be 90% influenced by core body temperatures (mean skin temperature influences the remaining 10%; Nadel et al., 1971). However, in a hot environment, skin temperatures can deviate greatly from that in temperate conditions, and skin temperature can therefore have a greater influence on thermoregulatory responses (Sawka et al., 2011). Rising core and skin temperatures are a central factor in heat injury and declining performance in the heat, making thermal adaptations a primary target when implementing heat acclimation (Nielsen et al., 1993). Thermal heat acclimation adaptations are observed as reduced core body temperatures at rest (Buono et al., 1998), and reduced exercising core (Eichna et al., 1950) and skin temperatures (Lorenzo et al., 2010). Whilst there is no one “true” core body temperature, it is usually inferred via rectal, oesophageal or intestinal temperature (see **Section 3.9.3.1** in General Methods for further discussion of measurement sites). Lowered resting core body temperatures are likely mediated through neural adaptations to the thermoregulatory control centre, located within the preoptic area of the anterior hypothalamus, that shifts the thermoregulatory “set-point” (Hessemer et al., 1986). Lowered skin and core body temperatures during exercise are mediated through a host of integrative factors. Similar to resting core body temperature reductions, the temperature threshold for reflex activation of the sympathetic nerves

controlling thermoeffectors to initiate heat loss mechanisms is shifted to a lower core body temperature (Charkoudian, 2010), alongside a greater sympathetic excitability in response to a given stressor (i.e. heat and/or exercise; Horowitz 2001). Heat acclimation also reduces the metabolic heat produced by the active skeletal muscle (Rivas et al., 2017), which in turn reduces the requirement for heat loss. These mechanisms occur in addition to a lower core body temperature pre-exercise, thereby increasing available heat storage before reaching a given core temperature.

Of note, other factors aside from acclimation can influence core body temperature's "set-point". These include menstrual cycle, pyrogens, circadian rhythm and training status (Gisolfi & Wenger, 1984; Cabanac, 2006; Sawka et al., 2011), which collectively illustrates that the "set-point" is actually more of a dependent range for each individual than a single fixed temperature. Females' endogenous (Kolka & Stephenson, 1997) or exogenous (Grucza et al., 1993) hormone-mediated fluctuations in the thermoregulatory set-point may cause variations in thermal load during a heat acclimation session in different phases or *quasi*-phases of the menstrual cycle or hormonal contraceptive use, respectively. Furthermore, the biphasic shift in resting temperature across the menstrual cycle could confound quantification of thermal adaptations if pre- and post-intervention measurements are not in the same menstrual cycle phase. Whilst male cohorts have exhibited improvements adaptations to resting and exercising body temperatures after completing only a short-term heat acclimation protocol (i.e. <7 days; Garrett et al., 2011; see **Section 2.4.3: Time course**), females may require a longer, ~10-day heat acclimation protocol to similarly alleviate thermoregulatory strain (Mee et al., 2015b). Indeed, studies using short-term heat acclimation in female cohorts (Sunderland et al., 2008; Mee et al., 2015b; Garrett et al., 2019) have failed to detect a change in resting rectal temperature (with the exception of Mee et al., 2018, who implemented 20 min of sauna suit use in addition to 90-min of heat acclimation) as has been observed in males (Buono et al. 1998; Brazaitis & Skurvydas, 2010; Neal et al., 2016a; James et al., 2017). From these studies, it would appear that <7 days of heat acclimation is insufficient to lower the "set-point" of resting core body temperature in females.

2.3.2 Cardiovascular support

Improved cardiovascular support and stability are primary mechanisms in reducing physiological strain during exercise-heat stress following heat acclimation (Nielsen et al., 1993). Although cardiovascular changes are sometimes discussed as independent factors, like other integrative mechanisms, changes in markers of cardiovascular function are largely

facilitated via interactions with other physiological adaptations, as illustrated in **Figure 2.8** and described throughout this and following sections.

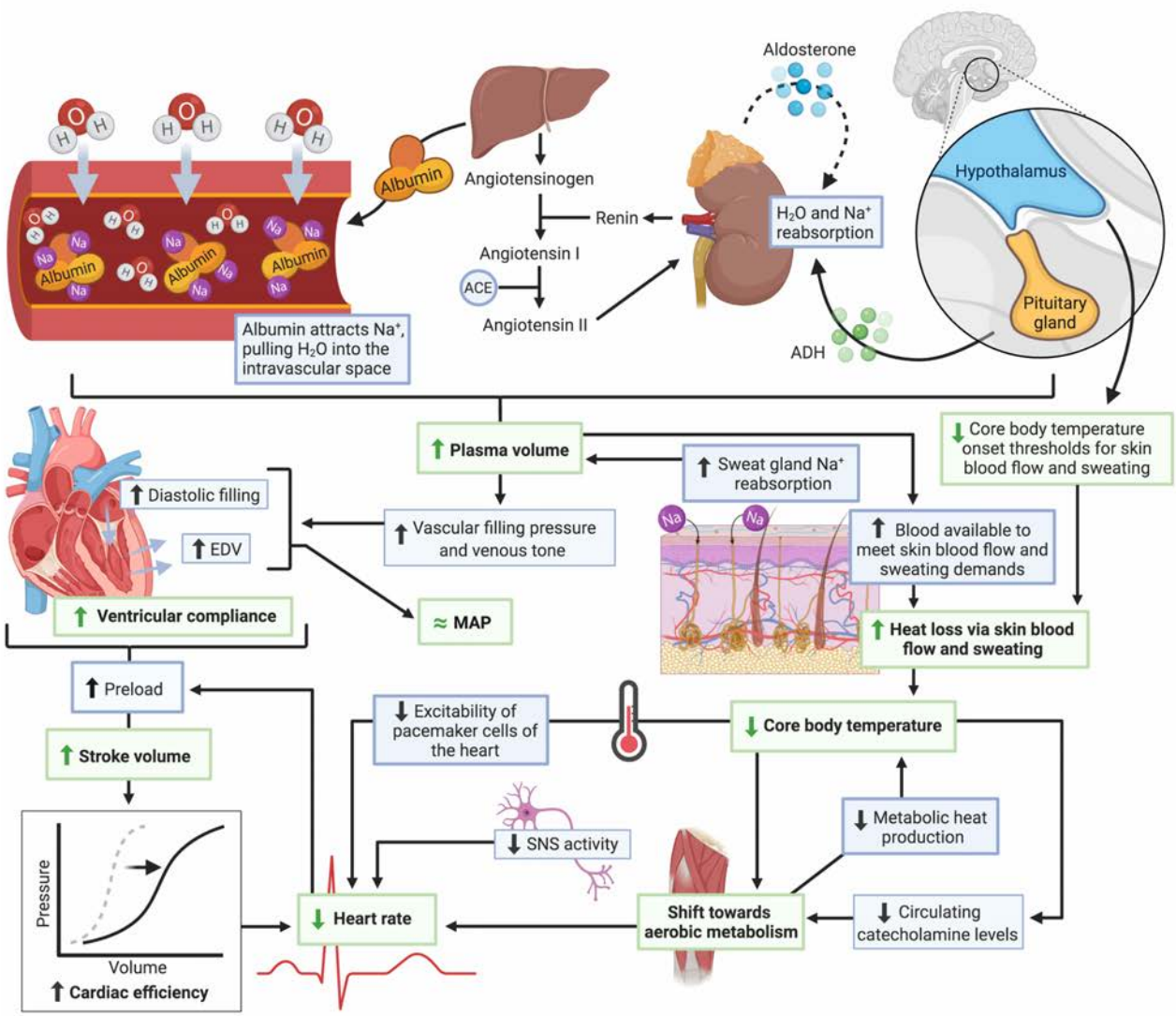


Figure 2.8 Cardiovascular adaptations to heat acclimation are developed independently as well as inter-relatedly with other physiological adaptations. Created with BioRender.com. ≈ indicates that mean arterial pressure (MAP) is better defended. ACE, angiotensin-converting enzyme; ADH, anti-diuretic hormone; EDV, end-diastolic volume; SNS, sympathetic nervous system

Reductions in resting and exercising heart rate following heat acclimation have been attributed to plasma volume expansion during heat acclimation (Buchheit et al., 2011; Racinais et al., 2012). Heat acclimation results in the expansion of total body water, extracellular fluid volumes and plasma volume (at least when thermal stimulus is maintained across a heat acclimation protocol; Patterson et al., 2004b) through two main mechanisms. The first is through an elevated anti-diuretic hormone (ADH) and aldosterone secretion in response to exercise-heat stress, leading to renal retention of water and electrolytes (mainly sodium; Montain et al., 1997; Francesconi, 1988; Luetkemeier et al.,1994), extracellular fluid expansion and overall greater total body water (Patterson et al., 2004; **Figure 2.9**). The

second involves increased plasma albumin, which changes oncotic pressure and draws fluid into the intravascular space (Bass et al., 1955; Senay et al., 1976; Nielsen et al., 1993). Such albumin-mediated changes are increasingly observed as heat acclimation progresses (Patterson et al., 2004b). Plasma volume expansion is likely exercise-intensity dependent (Senay et al., 1980) and therefore a large range (-1.9 to +16%) has been reported in response to various heat acclimation protocols (Tyler et al., 2016). Overall, plasma volume expansion offers cardiovascular support by increasing vascular filling pressure and improving venous tone (Wood & Bass, 1960), thus contributing to an increased stroke volume and better maintenance of blood pressure (Senay et al., 1976). Additionally, more blood is available to meet demands of skin and muscle blood flow during exercise-heat stress, reducing the cardiovascular work required for a given external workload (Nielsen et al., 1993).

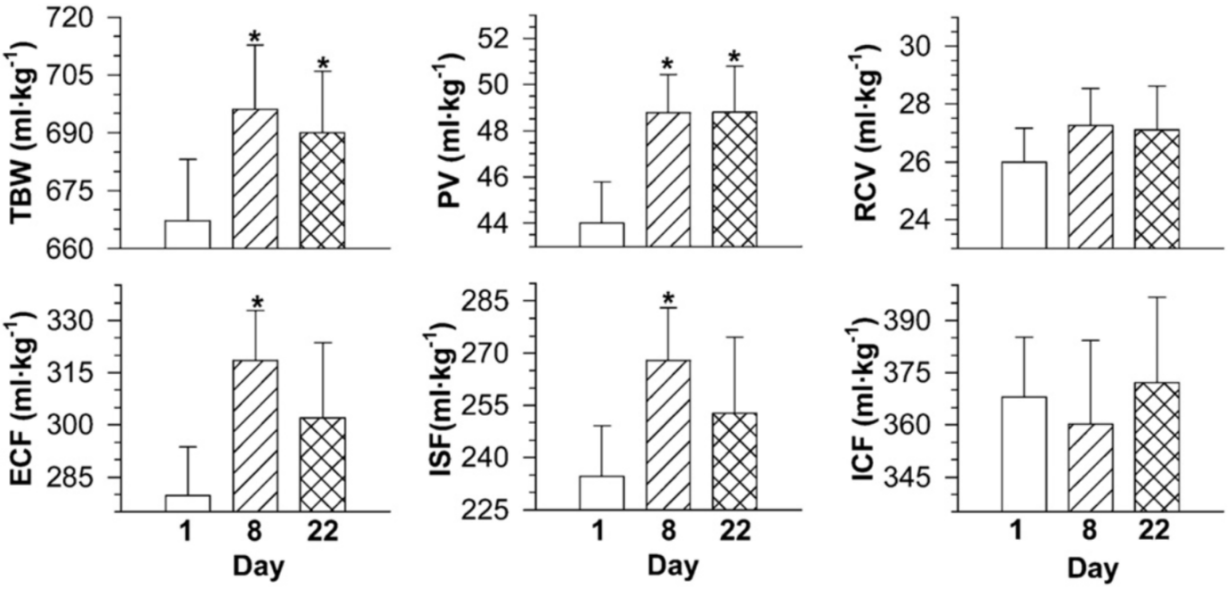


Figure 2.9 Resting total body water (TBW), plasma volume (PV), red cell volume (RCV), extracellular fluid (ECF), interstitial fluid (ISF) and intracellular fluid (ICF) prior to and following ~three-weeks heat acclimation (90 min controlled hyperthermia, core temperature: 38.5°C; ambient conditions: 40°C, 52%RH). Upper panels *n*=12, lower panels *n*=8. Data derived using Evans blue dye dilution technique (PV) and radioisotope-tracer methods. Data are presented as mean ± standard error. *Significantly different from Day 1 (*p*=0.05). Taken from Patterson et al. (2004b).

Other desirable functional alterations during the cardiac cycle likely contribute to an increase in stroke volume following heat acclimation. Wilson et al. (2020) observed that males exposed to 12 days of passive heat acclimation exhibited greater “suction” during left ventricle filling (evidenced by increased early mitral annular velocity at rest). Trachsel et al. (2020) observed that short-term passive heat acclimation (7 days of 60-min isothermic hot water immersion) also increased atrial contribution to left-ventricle filling during passive

heating to a ~1.2°C increase in oesophageal temperature. These studies are supported by rodent models (Horowitz et al., 1986), whereby isolated rat hearts showed a rightward shift in the pressure-volume curves following heat acclimation, meaning volume during ventricular filling was greater for a given filling pressure. This shift was observed alongside a similar myocardial O_2 consumption. Collectively, these results indicate greater cardiac efficiency. Structurally, Wilson et al. (2020) also observed that passive heat acclimation increased left atrial volume and left ventricle end-diastolic volume at rest. Likewise, Parsons et al. (2020) observed that 5 days of active heat acclimation increased stroke volume, early-diastolic filling velocity, left atrial volume and inferior vena cava diameter at rest in males. These functional and structural adaptations are indicative of improved ventricular compliance, culminating in increased preload, and facilitating the increases in stroke volume and cardiac output that have been documented during exercise-heat stress as a result of heat acclimation (Wyndham et al., 1976; Nielsen et al., 1993; **Figure 2.10**). Whilst changes in end-diastolic volume and stroke volume are often implied to be closely linked with plasma volume expansion, interestingly, Wilson et al. (2020) reported that none of the functional or structural cardiac changes were correlated with plasma volume changes following passive heat acclimation.

One study by Travers et al. (2020a) examined cardiac responses during exercise-heat stress (33°C, 50%RH) following 10 days of active heat acclimation (90-min at controlled heart rate corresponding to 65%$\dot{V}O_{2max}$ in 40°C, 40%RH). The authors did not observe any of the structural or functional cardiac adaptations described above (Parsons et al., 2020; Trachsel et al., 2020; Wilson et al., 2020) either at rest or during exercise-heat stress, except for an increase in stroke volume and better maintenance of end-diastolic volume by 180 min exercise. However, participants studied by Travers et al. (2020a) did not show any of the classic cardiovascular or thermal markers of heat acclimation during the pre- *vs* post-acclimation trials (reduced core body temperatures, skin temperatures and heart rate or increased plasma volume). Therefore, these participants did not appear to heat-acclimate completely or effectively, which may explain inconsistencies between their results and others.

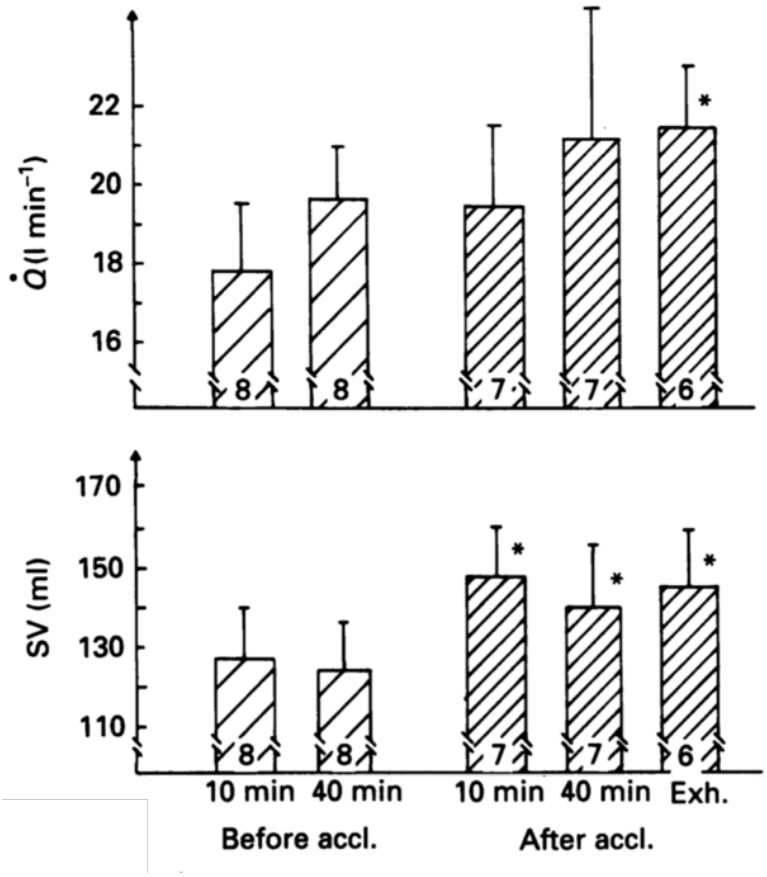


Figure 2.10 Cardiac output ($\dot{Q}$) and stroke volume (SV) measured at 10 min exercise, 40 min exercise and just before exhaustion (Exh), before (*open hatching*) and after 9-12 days heat acclimation (40°C, 10%RH; *close hatching*). Measurements at 40 min before acclimation were taken just before exhaustion. Number of measurements indicated for each time-point. Data is presented as mean ± standard error. *Significantly different from final measurement before acclimation (p<0.05). Taken from Nielsen et al. (1993).

Heat acclimation also modulates autonomic responses that contribute to elevations in heart rate during exercise-heat stress in an unacclimated state. The initial phase of heat acclimation induction is characterised by a decreased effector organ output-to-autonomic signal ratio (Horowitz & Meiri, 1993; Horowitz et al., 1996; Horowitz, 1998). This extends to the myocardium (i.e. the effector organ) and contributes to reductions in exercising heart rate following heat acclimation. Moreover, heat acclimation reduces sympathetic nervous activity and catecholamine levels during exercise-heat stress, which also underpins the reduction in heart rate with heat acclimation (Berlyne et al., 1974; Hodge et al., 2013).

Cardiovascular adaptations have traditionally been thought to be one of the most rapidly developing heat acclimation adaptations (with heart rate reductions plateauing by ~6 days of heat acclimation; Périard et al., 2015). However, Mee et al. (2015b) observed that, similar to thermoregulatory adaptations, females did not develop cardiovascular stability when using a 90-min daily, 5-day isothermic heat acclimation protocol. Conversely, the male

cohort did show the expected reduction in exercising heart rate. The cardio-protective tendencies of oestrogen may contribute to females' "resistance" to the cardiovascular adaptations following heat acclimation (Shinohara et al., 2004). Although Sunderland et al. (2008) *did* observe reductions in exercising heart rate in females following a 4-day high-intensity (i.e. intermittent sprinting) heat acclimation protocol (30°C), this reduction was similar to that observed in a control group exercising in temperate conditions (18°C). Given that plasma volume expansion is greater with increased exercise-intensity (Senay et al., 1980), reductions in heart rate following this high-intensity protocol were likely exercise-mediated and not heat acclimation-mediated *per se*.

One study by Horstman & Christensen (1982) observed an opposite sex difference in the context of cardiovascular adaptations. The authors reported increased stroke volume following an 11-day heat acclimation protocol in trained females but not in trained males, and concluded that females displayed a superior magnitude of cardiovascular adaptations as compared to males. This is interesting, considering that females have previously been shown not to increase stroke volume with exercise training when males did (Spina et al. 1993). Thus, closer examination of central cardiovascular adaptations to heat acclimation in both sexes is warranted.

Female sex hormones affect the fluid-regulatory hormone responses that could influence plasma volume expansion (Wenner & Stachenfeld, 2012). Males exhibit a greater ADH response to changes in plasma osmolality and a lower osmotic threshold for initiation of this ADH response (i.e. sensitivity) than normally menstruating women (Stachenfeld et al., 2001). Nonetheless, female cohorts have been observed to experience plasma volume expansion following 5-6 days of active heat acclimation (Perrotta et al., 2018; Pethick et al., 2018; Garrett et al., 2019). However, the female cohorts studied by Perrotta et al. (2018) and Garrett et al. (2019) were all oral contraceptive users (Pethick et al. did not specify), who have suppressed endogenous female sex hormone levels (Fleischman et al., 2010). This is important, because oral contraceptive use lowers females' osmotic threshold for ADH release (Stachenfeld et al., 1999). Taken together, it is possible that Garrett et al. (2019), Perrotta et al. (2018) and even Pethick et al. (2018) (given the high prevalence of oral contraceptive use in elite athletes; Martin et al., 2018) observed plasma volume expansion following 5-6 days of active heat acclimation as a result of hormonal contraceptive use. Whether plasma volume expansion would be observed in a cohort of eumenhorreic females after 5-6 days of heat acclimation is unknown.

Finally, sex differences have been observed in changes in blood pressure associated with heat acclimation. Morimoto et al. (1967) reported that in males, systolic blood pressure stayed similar during exercise-heat stress across days 1-6 of heat acclimation, but diastolic blood pressure dropped dramatically as heat acclimation progressed. Conversely, systolic

blood pressure appeared to increase after 4-5 heat exposures in females with only a slight decrease in exercising diastolic blood pressure. Data from Morimoto et al. (1967), taken together with the above studies investigating plasma volume and cardiovascular adaptations in female cohorts, provide evidence for sex differences in a number of cardiovascular adaptations attained during the induction of heat acclimation.

2.3.3 Sweating

Sweating adaptations to heat acclimation were first recorded by Dill and colleagues in 1933. Since then, sudomotor adaptations have become categorised as one of the "hallmark" adaptations of heat acclimation (Sawka et al., 2011). Sudomotor adaptations are important, as they contribute to the ~11% increase in evaporative heat loss following heat acclimation (Poirier et al., 2015). Increases in sweat loss for a given exercise intensity and duration are brought about by three main mechanisms, as first collectively described by Wyndham (1967): i) reduced core body temperature threshold for sweating onset, ii) increased sensitivity of sweat output for a given core body temperature, and iii) elevated maximum capacity for sweat output. The shift in temperature threshold for sweat onset is proportionate to an individual's change in resting body temperatures (Patterson et al., 2004a). This indicates that a shift in temperature threshold for sweat onset is an adaptation of the central nervous system, which was recently confirmed by Barry et al. (2020). Thus, elevated brain temperatures during acclimation are probably necessary for initiating temperature threshold shifts (Ogawa et al., 1988). Conversely, adaptations related to *sensitivity* (i.e. rate of sweat output for a given change in body temperature) are peripheral adaptations, meaning that adaptations occur at the effector organ. Researchers have observed improvements in post-junctional sensitivity to acetylcholine (Buono et al., 2009b; Buono et al., 2009a; Lorenzo & Minson, 2010) and methacholine (Collins et al., 1966; Inoue et al., 1999; Pearson et al., 2017) following heat acclimation. Interestingly, it appears that sympathetic activation of the sweat glands during heat acclimation is necessary for these peripheral adaptations to occur (Buono et al., 2009a). Heat acclimation may also make sweat glands more resistant to hidromeiosis (i.e. the "clogging" of sweat glands on wetted skin; Fox et al., 1964; Candas et al., 1980). Additionally, morphological adaptations to the eccrine sweat glands following heat acclimation have been observed in primates (Sato et al., 1990), and has not been ruled out as the mechanism allowing for increased maximum sweat output per gland in humans. A final proposed mechanism facilitating a greater sweat rate following heat acclimation is an increase in the density of active sweat glands. However, this mechanism has been repeatedly refuted based on evidence gathered from male cohorts only (Inoue et al., 1999;

Lee et al., 2010; Poirier et al., 2016). Collectively, these central and peripheral adaptations are represented in **Figure 2.11**.

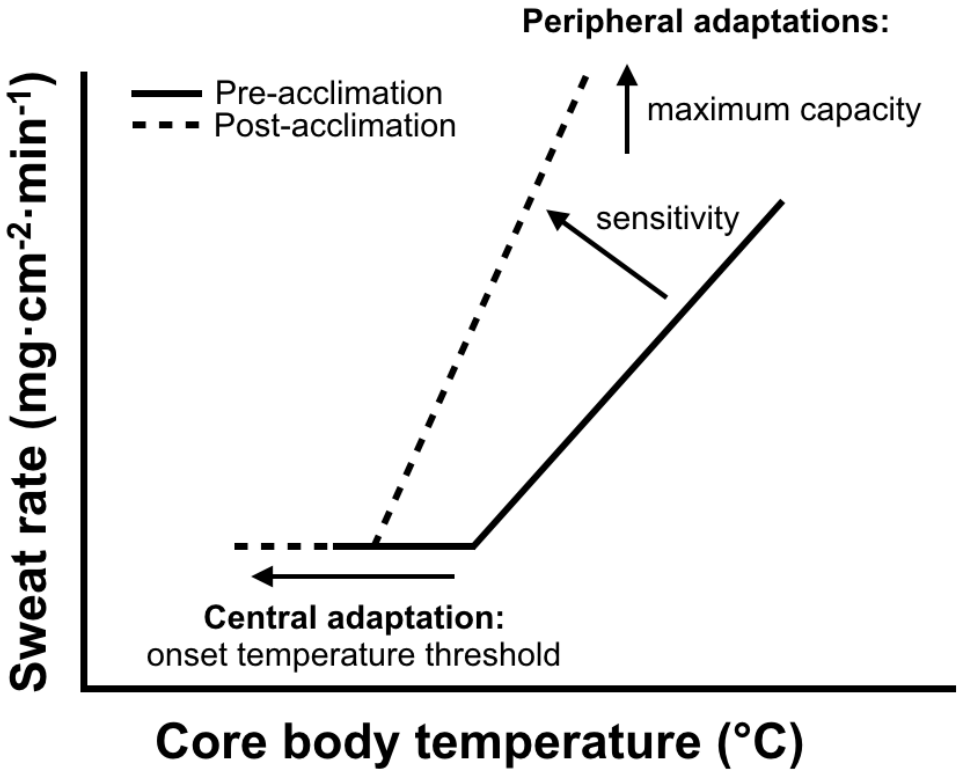


Figure 2.11 Schematic representation of central and peripheral adaptation pre- (*solid line*) and post-heat acclimation (*dashed line*). Following heat acclimation, there is a shift of sweat onset to a lower core body temperature, whilst the sensitivity (represented by the slope) and maximal sweating capacity are increased. Adapted from Taylor (2014) and Nadel et al. (1971b).

Adaptations to the sweat glands may be specific to where on the body they are located. Heat acclimation had previously been thought to promote a greater increase in sweat rate on the limbs, where the increased surface area-to-mass ratio is advantageous for heat dissipation (Höfler, 1968; Shvartz et al., 1979; Regan et al., 1996). However, due to methodological concerns with previous studies (e.g. sample size and technique), Patterson et al. (2004a) revisited this hypothesis and reported that the percent increase in local sweat rate following heat acclimation (40°C, 60%RH) was no different between the chest and forearm, which were both greater than the thigh. Conversely, when employing acclimation to dry heat (45°C, 20%RH; which may be preferential for sudomotor adaptations; Tebeck et al., 2019), Smith and Havenith (2019) reported that heat acclimation *did* promote a greater relative sweat rate at the periphery, specifically on the upper-body. Smith and Havenith (2019) contend that the inconsistencies between their data and that of Patterson et al. (2004a) were merely due to differences in data analysis. Namely, Smith and Havenith reported that it is not the absolute percent increase that changes, but the relative

contribution of the limbs of the upper body to whole-body sweat rate that is disproportionately increased relative to the rest of the body (**Figure 2.12**).

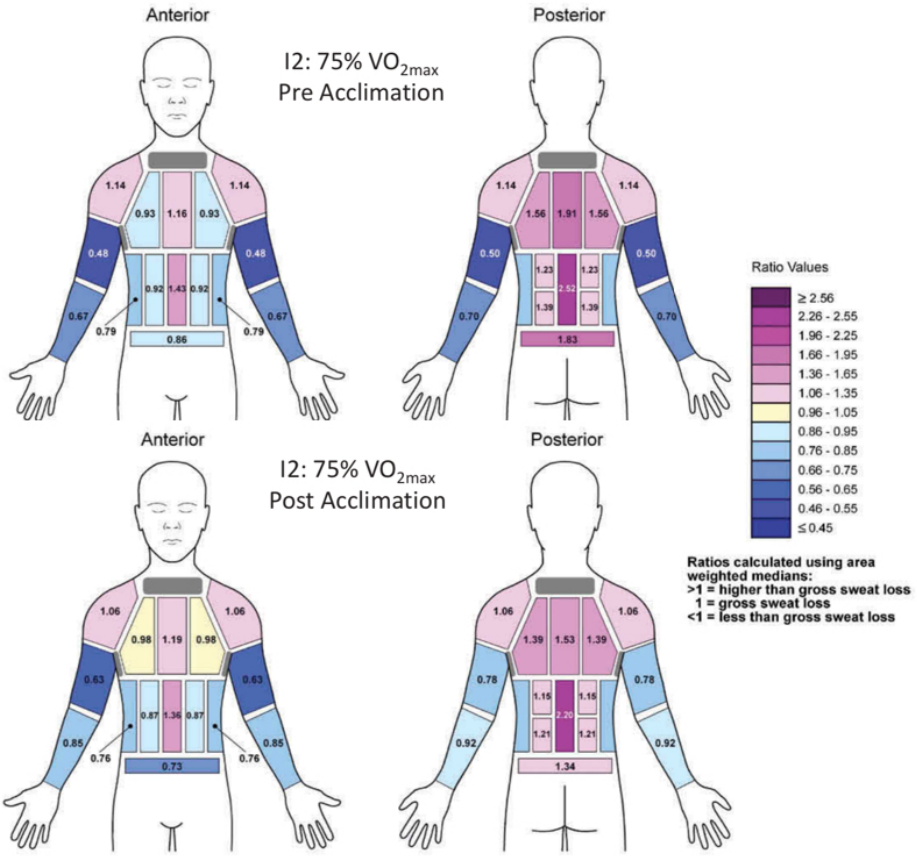


Figure 2.12 The weighted ratio of the regional contribution to total sweat rate (i.e. regional sweating rate; RSR) during exercise in the temperate conditions (26°C, 47%RH) pre- and post 6 days hot-dry heat acclimation (45°C, 20%RH). At 75%$\dot{V}O_{2max}$, RSR is decreased at the lateral upper back and increased at the anterior and posterior upper arm and the posterior lower arm following acclimation in males (p<0.05). The remaining sites had a similar weighted RSR ratios pre- and post-acclimation. Taken from Smith and Havenith (2019).

Alongside adaptations related to rate, sensitivity and capacity of sweat output, acclimation alters sweat composition through: i) decreases the concentration of sodium and chloride ions in sweat (Dill et al., 1938; Klous et al., 2020), and ii) active sodium reabsorption through the sweat gland (Kirby & Convertino, 1986). The benefits of adaptations to sweat composition are two-fold, firstly in maintaining plasma electrolyte content (and thus maintaining plasma volume; Sawka et al., 1996) and second in diluting sweat (and thus allowing it to evaporate more readily; Taylor, 2014). These adaptations are rapid; with decreases in sweat sodium observed after only 2 days of heat acclimation, and a continued linear decrease until at least 7 days of heat acclimation (Buono et al., 2018).

Sweat *rate* adaptations, on the other hand, have historically been considered a more gradual adaptation than the comparatively rapid adaptive markers of heart rate and body

temperatures (at least in males; Armstrong & Maresh, 1991; Cotter et al., 1997; Périard et al., 2015). However, sweat rate adaptations may be accelerated to occur within ~7 days when using isothermic (core body temperature maintained ~38.5°C; see **Section 2.4.1.2**) heat acclimation (Patterson et al., 2004b; Gibson et al., 2015c). Most studies have failed to detect a so-called "short-term" improvement in sweat rate in female cohorts (Sunderland et al., 2008; Pethick et al., 2018), even when using isothermic heat acclimation (Garrett et al., 2019). Yet, when directly compared to males using an isothermic heat acclimation protocol, Mee and colleagues reported that females exhibited increases in sweat rate after only 5 days with no additional benefits after 10 days, whereas males required 10 days of heat acclimation to exhibit an increased sweat rate from pre-acclimation. The authors suggested that a comparatively lower maximal sweat response to acetylcholine in females than males was a possible mechanism for this greater sweat rate (Gagnon et al., 2013). Notably, Mee et al. (2015b) was the only heat acclimation study in females to normalise sweating by body surface area and to specify that the change in body mass (used to estimate sweat rate) was measured to 0.01 kg. The latter point is important, given that the female cohort with a body surface area ~1.6 m^2 (estimated based on mean height and weight) increased sweat rate by ~428 $g \cdot h^{-1} \cdot m^{-2}$ post-acclimation. This equates to a ~0.1 kg absolute increase in sweat loss during their 30-min test. Other studies (Sunderland et al., 2008; Pethick et al., 2018; Garrett et al., 2019) did not specify their measurement precision, but measuring to ≥0.1 kg may result in a type II error in females, who demonstrate a lower absolute sweat loss (due to their smaller body size) and sweat rate (Gagnon & Kenny, 2012) as compared to males.

Frye and Kamon (1981) observed that 8-9 days of acclimation to dry heat (48°C, 14%RH) increased sweat rates (normalised by body surface area) in male and female cohorts matched for fitness (~55 $mL \cdot kg^{-1} \cdot min^{-1}$). Interestingly, whilst males exhibited higher sweat rates than females at pre-acclimation, this difference disappeared post-acclimation. Employing hot-wet conditions during heat acclimation may result in suboptimal sweat rate adaptations in males (Tebeck et al., 2019). Likewise, studies employing 8-11 exposures to humid heat stress (34-36°C, ≥75%) have observed that females either did not increase exercising sweat rate or did not increase exercising sweat rate to the same extent as males (Wyndham et al., 1965; Weinman et al., 1967; Avellini et al., 1980a; Buono et al., 2010). From these data, it may be postulated that humid-heat acclimation limits sudomotor adaptations in females even more so than in males. However, Buono et al. (2010) observed an interesting sex difference. Like in previous investigations, their study showed that females did not increase sweating during exercise-heat stress after acclimation to humid heat. However, both females and males exhibited improved sweat gland *function* (tested independent of exercise-heat stress using pilocarpine-induced sweating) following 8 days of acclimation to humid heat (35°C, 75%RH). Thus, females likely make peripheral adaptations

at the sweat gland regardless of acclimation condition (i.e. hot-wet or hot-dry), even though unnecessary increases in sweat rate are suppressed in females during exercise in humid heat post-acclimation. Future studies should not use exercise in hot-wet conditions to assess changes in sweat rate as a qualifying marker of heat acclimation in females.

There have been few investigations specifically investigating the role of hormonal contraceptives on thermoeffector adaptations following heat acclimation. One study by Armstrong and colleagues (2005) reported that none of the three cohorts of females (eumenhorreic, combined oestradiol-progestin oral contraceptive users and depot medroxyprogesterone acetate contraceptive injection) increased sweat rate during exercise-heat stress following 7-8 weeks of 3 weekly 90-min heat acclimation bouts in 36-37°C, ~35%RH (supplemented by three weekly outdoor training sessions in ~-4°C). Furthermore, only oral contraceptive users reduced sweat onset threshold. This reduction resulted in a ~0.6°C lower sweat onset threshold in the oral contraceptive group than the contraceptive injection group at post-acclimation. This may raise concerns about the effects of other progestin-only contraceptive methods, such as IUDs and the contraceptive implant, on thermoeffector adaptive capacity following heat acclimation. Since that by Armstrong et al., (2005), no further research has examined whether the contraceptive patch, implant or IUD differentially impact exercise in the heat or capacity for heat acclimation. Differences are difficult to infer from the limited literature, as both central and peripheral thermoregulatory processes respond differently to synthetic progestin compared to endogenous progesterone (Charkoudian & Stachenfeld, 2014). More research is needed in this area to understand such differences.

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2.3.4 Skin blood flow

Similar to sudomotor adaptations, heat acclimation results in both centrally mediated shifts in core body temperature threshold for the onset of skin blood flow, as well as increased cholinergic-sensitivity of the response at the periphery (Roberts et al., 1977; Armstrong & Kenney, 1993; Lorenzo & Minson, 2010; Notley et al., 2018). However, unlike at the sweat gland, heat acclimation is not sufficient to drive structural changes in the cutaneous vasculature. This is evidenced by similar maximal skin blood flow responses following 10 days of active (Lorenzo & Minson, 2010) or following 8 weeks of passive heat acclimation (Brunt et al., 2016a).

Changes in core body temperature onset threshold for initiation of skin blood flow have been observed following passive heat acclimation in a female cohort (Bailey et al.,

2016), though skin blood flow changes following active heat acclimation have not been directly compared between males and females. However, females may have an augmented adaptive response to functional vascular adaptations following heat acclimation. Brunt and colleagues (2016a) propose that heat-mediated endothelial adaptations are attained in a NO-dependent manner. This is important because Brazaitis et al. (2019) showed that normally menstruating women in the follicular phase responded to severe passive heat stress (lower body hot water immersion until rectal temperature 39.5°C, approximately ~54 min) with increased levels of oestrogen and progesterone. This presents a unique pathway for which females may improve vascular outcomes following heat acclimation, given oestrogen's marked effects on increasing NO and endothelium-dependent vasodilation (Orshal & Khalil, 2004). Alternatively, it is possible that this instead presents a redundant adaptation for females, as circulating oestrogen attenuates females' reliance on eNOS for vasodilation (Stanhewicz et al., 2014).

2.3.5 Metabolism and skeletal muscle adaptations

Heat acclimation promotes a preferential shift in fuel metabolism, attenuating carbohydrate utilisation for a given exercise intensity during exercise-heat stress (Febbraio et al., 1994a). This is associated with a lower respiratory exchange ratio (RER; Febbraio et al., 1994a; Young et al., 1985), lower muscle and plasma lactate (most likely due to changes in metabolic factors and not changes related to blood flow distribution; Young et al., 1985) and glycogen sparing of up to 50% (King et al., 1985; Young et al., 1985; Kirwan et al., 1987; Febbraio et al., 1994a)(**Figure 2.13**). Overall, these improvements manifest as more holistic markers of exercise performance in the heat, such as improved power at 4 $mmol \cdot L^{-1}$ blood lactate concentration ($[La^-]$; Lorenzo et al., 2010).

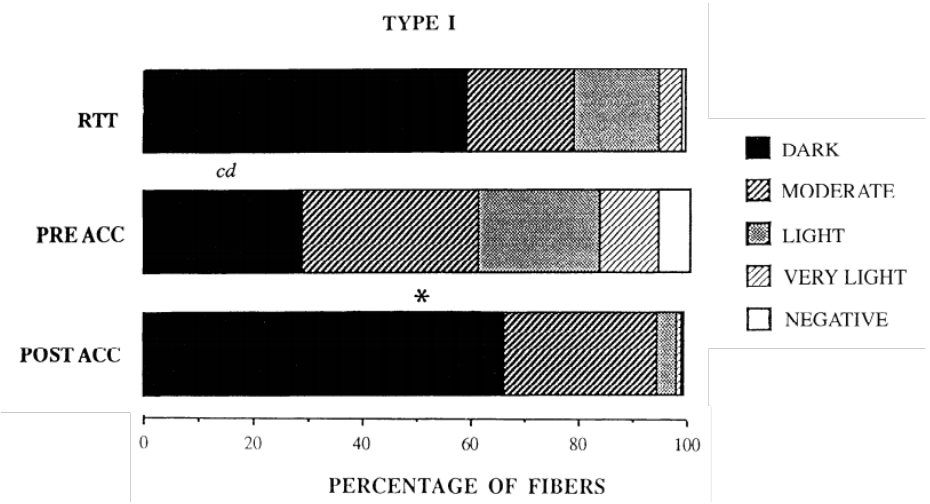


Figure 2.13 Histochemical estimation of glycogen content in muscle section stained with periodic adic-Schiff reagent in type I fibres after 40 min at 70% maximal oxygen consumption in temperate conditions (20°C, 20%RH; RTT) or in the heat (40°C, 20%RH) pre- (PRE ACC) or post- (POST ACC) 7-days heat acclimation. "Darkness" of stained fibres indicates glycogen content. Data are mean ± standard error. [c] Significant difference in percentage of dark-stained fibres PRE ACC *vs* RTT (p<0.05); [d] Significant difference in percentage of dark-stained fibres PRE ACC *vs* POST ACC (p<0.05); *Significant difference in mean rating of fibres PRE ACC *vs* POST ACC (p<0.05). Taken from (Febbraio et al., 1994a).

The mechanisms underpinning these improvements have received considerable attention in recent years, and appear to be multifactorial. Firstly, studies investigating passive heating have shown that heat independently triggers responses stimulating mitochondrial biogenesis (i.e. PGC-1α upregulation) and aerobic respiration (Hafen et al., 2018; Ihsan et al., 2020), as a downstream effect of increased heat shock protein (HSP) synthesis (see **Section 2.3.6**; Ohno et al., 2015; Ihsan et al., 2020). These adaptations facilitate energetic efficiency in the myocytes to minimise heat production (Horowitz & Kodesh, 2010), which are likely to be at least in-part responsible for the reduction in metabolic heat production (and thus body temperatures) during exercise-heat stress following heat acclimation (Rivas et al., 2017). Importantly, Ihsan et al. (2020) reported that an acute bout of *whole-body* passive heating (60 min in an environmental chamber at 44-50°C, 50%RH) increased mRNA signalling mitochondrial biogenesis, aerobic respiration and HSP up-regulation; but an acute bout of *localised* passive heating (60 min of single leg in a water-perfused suit at ~50°C) was not sufficient to observe any such increases. However, Hafen et al. (2018) applied localised single leg heating for a longer duration (2 h using daily diathermy with muscle temperatures ~40°C) for 6 days and observed an increase in muscle PGC-1α, aerobic respiration capacity and HSPs. Based on these data, it is somewhat surprising that Mang et al. (2020) observed an increase in HSP72, but no increases in skeletal muscle PGC-1α or markers of improved aerobic respiration following 10 days of active heat acclimation (2x45 min bouts of walking at 30-40% maximal velocity, 3% grade in

~42°C, 30-50%RH). However, Hafen et al. (2018) studied a sedentary cohort, who may have required a lesser stimulus for adaptation than more active participants; whereas Mang et al. (2020) studied regularly active participants (>150 weekly minutes of moderate-to-vigorous intensity exercise, $\dot{V}O_{2max}$ ~51 $mL \cdot kg^{-1} \cdot min^{-1}$). Indeed, Mang et al. (2020) reported that increases in markers of mitochondrial biogenesis were negatively correlated with $\dot{V}O_{2max}$. Therefore, 10 days of active heat acclimation may not increase mitochondrial density and function in a well-trained cohort. Conversely, participants studied by Ihsan et al. (2020) were recreationally trained (2-4 h weekly endurance and/or resistance training activity) and showed skeletal muscle signalling for mitochondrial biogenesis and aerobic respiration following an acute bout of passive heating. Nonetheless, the acute responses observed by Ihsan et al. (2020) do not necessarily signify a chronic adaptive response. Overall, whether the differences in muscle mitochondrial findings result from differences in participants' training status or mode of heating (i.e. active *vs* passive) is unknown, but has possible implications for performance outcomes following heat acclimation (e.g. speed at $4 mmol \cdot L^{-1}$ $[La^-]$).

Alongside possible adaptations to the mitochondria, post-acclimation reductions in circulating catecholamines during exercise-heat stress likely help facilitate the improvements in the metabolic markers described above (Nielsen et al., 1993; Febbraio et al., 1994a). Additionally, attenuation of the heat-mediated decrements in neural drive contributes to improvements in exercise involving prolonged, repeated muscle contractions (Racinais et al., 2017a).

Localised passive heating and the ensuing hyperaemia and elevated shear stress are also likely to be sufficient for inducing microvascular changes (Green et al., 2010). Acutely, Kuhlenhoelter et al. (2016) reported that a single bout of either lower- or whole-body heating increased expression of mRNA involved in skeletal muscle angiogenesis (e.g. vascular endothelial growth factor, amongst others). With chronic local heating, Kim et al. (2020) observed that 5 weekly bouts of 90 min of single-leg heating (via water-perfused suit) prevented a decline in muscle capillary density, which was observed in the control leg, after both a 4- and 8-week intervention in sedentary participants. Moreover, Hesketh et al. (2019) observed that a whole-body passive heating intervention (3 weekly bouts of 40-50 min in an environmental chamber at 40°C, 40%RH for 6 weeks) increased capillary density by 21%, with a similar absolute increase as following a time-matched moderate-intensity (~65%$\dot{V}O_{2peak}$) cycling intervention. Collectively, these findings are important because a larger capillary network allows for a longer mean transit time and greater surface area for gas, substrate, and metabolite exchange between the blood and skeletal muscle (Saltin et al., 1986). One limitation of these studies is that they were all in sedentary participants, thus angiogenic effects of a passive or active heat acclimation intervention may not be as

pronounced in trained athletes, who already have a greater capillary density than sedentary people (Kuzon et al., 1990).

The majority of research on metabolic and skeletal muscle adaptations with heat acclimation has been in male participants or mostly male cohorts. Females' relatively greater reliance on fatty acid metabolism for fuel (Tarnopolsky et al., 1990), lower muscle glycogen levels (Horton et al., 2006) and elevated levels of adrenaline when exposed to heat stress (Jezova et al., 1994), could be postulated to translate to sex differences in the metabolic response to heat acclimation. However, 6 days of active heat acclimation (100 min walking in 49°C, 20%RH) lowered exercising metabolic rate similarly (-5%) in females and males (Sawka et al., 1983a). Likewise, Mang et al. (2020) also reported that sex did not affect markers of mitochondrial biogenesis or function following active heat acclimation (6 females, 7 males). These data are in line with temperate exercise training outcomes, as females and males appear to show similar trainability of metabolic thresholds (i.e., speed at 4 $mmol \cdot L^{-1}$; Joyner, 2017). Therefore, although relatively few studies have included sufficient numbers of female participants, data thus far does support sex as a potential independent factor affecting mechanisms of metabolic and skeletal muscle adaptations.

Most of the investigations described in this section (especially those investigating the *mechanisms* of metabolic improvements) have used passive heating to isolate the effects of heating independent of exercise. However, exercise in-and-of itself is a whole-body thermal stimulus, which includes heating of the active muscles (Saltin & Hermansen, 1966). Therefore, it is unknown: i) in what capacity the thermal stressors of exercise and ambient heat stress during a traditional active heat acclimation protocol combine to additively enhance (or even impede) the underlying mechanisms of metabolic adaptations observed following heat acclimation, and ii) if the mechanisms underpinning these adaptations are similar in response to passive and active heat acclimation.

2.3.6 Cellular thermal tolerance

Heat shock proteins are the molecular response to heat stress, which drive thermotolerance at the cellular level (Moseley, 1997; Horowitz, 1998; Amorim et al., 2015). Heat shock proteins are named according to their molecular weight, and HSP72 and HSP90 are most often associated with the response to heat stress. Further, HSPs can be divided into intracellular HSPs (iHSPs) and extracellular HSPs (eHSPs). Heat stress appears to have a greater impact on iHSPs than eHSPs (Tyler et al., 2016). Intracellular HSPs are activated by heat stress (amongst 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 (Moseley,

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). Thermo-tolerance may explain why some marathon runners, among other endurance athletes, are able to tolerate internal temperatures of up to 41.5°C, far past clinical heat stroke thresholds (Maron et al., 1977; Racinais et al., 2019b). 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, 1994).

Heat shock protein 72, specifically, is found in the nucleus and cytosol of the cell, and is responsible for protein folding and cytoprotection (Mayer & Bukau, 2005). Other members of the HSP70 family found in the mitochondria have the additional role of molecular chaperone (Mayer & Bukau, 2005). Transcription of HSP72 mRNA requires sustained elevations in core body temperature at or above 38.5°C (Gibson et al., 2014; Gibson et al., 2016). Following transcription and eventual synthesis of HSPs, some HSPs are released from the cells in exosomes, where they then become extracellular HSPs (Lancaster & Febbraio, 2005). These eHSPs trigger an immune response and cytokine release (Asea, 2005), which, combined with their chaperoning function, has earned HSPs the name of “chaperokines” (Asea, 2003). Acute heat stress causes an upregulation of iHSPs proportional to an individual’s increase in core body temperature (Magalhães et al., 2010), as well as an increase in eHSPs. As acclimation develops, basal iHSPs are elevated (Magalhães et al., 2010) and the eHSP response to acute heat exposure is reduced (Maloyan et al., 1999).

Not only are HSPs essential for acquiring thermal tolerance, but the HSP response has a central role in achieving the heat acclimation phenotype. In comparison with placebo treatment, Kuennen et al. (2011) observed that suppressing the heat shock response with quercetin increased the inflammatory response to exercise-heat stress, inhibiting acclimation-mediated reductions in core body temperature and delaying cardiovascular adaptations. Moreover, an individual’s heat shock response is related to their ability to effectively reduce core body temperatures following heat acclimation, whereby those who showed greater reductions in HSP “inducibility” *ex vivo* following heat acclimation also showed greater reductions in core body temperatures following heat acclimation (McClung et al., 2008).

Whilst 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%) following 6 weeks of interval training in temperate conditions, females did not experience any significant increase. This difference was linked to

findings in rodent studies, whereby female rodents have been observed to exhibit higher basal levels of HSPs, therefore diminishing the necessity for further production (Paroo et al., 2002a; Voss et al., 2003; Hamilton et al., 2004; Shinohara et al., 2004; Rayner et al., 2008; Bombardier et al., 2009). These authors theorised that oestrogen regulates this process through stimulation of HSP release in the unstressed state to increase basal HSP levels, and thus providing cytoprotection when the cell comes under stress (Voss et al., 2003; Hamilton et al., 2004). For example, female rodents have been observed to exhibit reduced vascular (Otsuka et al., 1996; Hinojosa-Laborde et al., 2000; Rayner et al., 2008) and skeletal muscle damage (Paroo et al., 2002a; Bombardier et al., 2009) following exposure to various stressors, including exercise and heat stress, concomitant with a lower HSP70 response in females. Furthermore, ovariectomised rats had an increased HSP70 skeletal muscle response to exercise that decreased with oestrogen therapy (Paroo et al., 2002a).

In humans, an acute bout of exercise-heat stress (60 min at 60%$\dot{V}O_{2peak}$ in 42°C, 20%RH) upregulated iHSPs in males only (Gillum et al., 2013). Despite the implication of oestrogen's direct role in sex differences in the HSP response, Gillum and colleagues (2013) did not observe any differences in the iHSP72 response to exercise-heat stress across the menstrual cycle, nor any relationship between individuals' oestrogen levels and the iHSP72 response. Furthermore, Chang et al. (1998) increased females' oestrogen levels from ~33 $pg \cdot mL^{-1}$ to ~1,167 $pg \cdot mL^{-1}$ with three days of oestrogen supplementation, and still did not observe any differences in either pre-exercise iHSPs or the heat shock response to 20 min of temperate exercise, as compared to the placebo group. Finally, Mee et al. (2016) reported that there were no sex differences in the transcription of HSP72 mRNA following either acute or chronic (5 and 10 days) exercise-heat stress. Taken together, the relationship between oestrogen in the heat shock response seems to be more complex in humans. Both sexes receive similar signals for genetic upregulation of HSPs in response to exercise-heat stress (Mee et al., 2016), and thus oestrogen's direct role in mediating the heat shock response, if any, is likely further downstream from transcription. Given the key role of HSPs in thermotolerance and in the development of the heat acclimation phenotype, the relationship between oestrogen and HSPs may provide some explanation for the sex differences in responses to acute and chronic heat stress described throughout this review.

2.3.7 Perceptual and behavioural responses

Athletes commonly report higher ratings of perceived exertion (RPE; Borg, 1962) for a given workload during exercise-heat stress as compared to exercise in temperate conditions. This elevated RPE results from the greater discomfort associated with warmer

ambient and skin temperatures, as well as greater perceived effort in the heat due to greater cardiovascular strain (Cheuvront et al., 2010). Naturally, athletes also report greater thermal discomfort and hotter thermal sensation (Toner et al., 1986) during exercise-heat stress, concomitant with higher RPE. Overall, RPE and thermal sensation have an inverse relationship with muscular and aerobic performance (Cabanac, 2006; Nybo et al., 2014). These may be partially overridden however, by external motivation factors, such as head-to-head competition (Corbett et al., 2018b).

Recently, in their meta-analysis, Tyler et al. (2016) stated that whilst RPE was seldom reported (3 of 96 heat acclimation manuscripts analysed), heat acclimation typically reduced RPE by ~1 scale point. Conversely, Armstrong et al. (2005) reported that 8 weeks of combined heat acclimation and training in females (eumenhorreic, oral contraceptive pill users and contraceptive injection users), reduced RPE during exercise-heat stress (90 min walking at 5% grade in 37°C, 37%RH) by ~4 scale points. However, the limited heat acclimation research including females makes it difficult to compare the study by Armstrong et al. (2005) with typical male responses.

With regards to thermal perception, Sunderland et al. (2008) reported an improvement in thermal sensation for females in hot conditions after only 4 days of heat acclimation. Males have sometimes been observed to improve their thermal perception following a short-term (5 day) protocol (Neal et al., 2016a; Neal et al., 2016b), though not always (Gibson et al., 2015c; James et al., 2017b). Overall, these perceptual improvements may stem from the reduction in cardiovascular strain following heat acclimation (Cheuvront et al., 2010), although comfort may also improve with increased experience of performing in the heat. This translates to behavioural modifications, as athletes appear to improve their pacing strategies with heat acclimation (Racinais et al., 2015b). Indeed, Schlader et al. (2010) suggest that self-paced trials in the heat are an adequate index of “thermal behaviour”, as athletes voluntarily adjust their work-rate to manipulate metabolic heat production and thus core body temperatures.

Of note, Vargas et al. (2019) observed that females utilised “thermal behaviour” for cooling during exercise in warm (24°C, 45%RH) conditions more so than males. Namely, females opted to keep their dorsal neck areas cooler (via self-controlled cooling device) than males whilst cycling at similar rates of metabolic heat production and with similar mean and core body temperatures (Vargas et al., 2019). This could indicate an interesting sex difference in the context of pacing, since Schlader et al. (2010) suggested that thermal behaviour (and thus pacing) is preceded by feelings of thermal comfort and/or sensation. Additionally, Lei et al. (2017) observed that thermal sensation differed according to menstrual cycle phase (i.e. higher ratings in the luteal phase) during exercise-heat stress at fixed workloads. However, this difference was eliminated once participants were allowed to

self-pace. Collectively, these data warrant further investigation of females' perceptual responses to exercise-heat stress and heat acclimation, as well as the concomitant effects of perception on self-paced exercise performance.

2.3.8 Endurance exercise performance

Heat acclimation studies in the 1930's and 1940's focused on measuring physiological adaptations assumed to decrease heat injury risk (Dresoti, 1935; Dill et al., 1938; Robinson et al., 1943; Eichna et al., 1945a). Physiological adaptations gained from heat acclimation were later shown to translate to improved exercise performance (i.e. marathon running) in the heat (Adams et al., 1975). Since then, a number of studies have quantified maximal and submaximal performance benefits alongside physiological adaptations following traditional heat acclimation and acclimatisation protocols in male, or mostly male cohorts (King et al., 1985; Sawka et al., 1985; Lorenzo et al., 2010; Castle et al., 2011; Keiser et al., 2015; Racinais et al., 2015b; Lee et al., 2016; Schmit et al., 2017; Sotiridis et al., 2019). On average, heat acclimation improves time trial performance and time-to-exhaustion in the heat by ~7% and ~23%, respectively (Tyler et al., 2016). According to a meta-analysis by Benjamin et al. (2019), the magnitude of performance change following heat acclimation is greatest with time-to-exhaustion trials, followed by time trials, mean power, $\dot{V}O_{2max}$, and then peak power. Eighteen of 19 studies included observed improvements in time-to-exhaustion in the heat following acclimation, all studies observed improvements in time trial performance, five of six observed improved mean power, and seven of ten observed improved $\dot{V}O_{2max}$. Accordingly, 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 heat acclimation, as indexed by an 8% increase in mean power across a 1-h time trial alongside an 8% improvement in $\dot{V}O_{2max}$ and a 5% increase in power at 4 $mmol \cdot L^{-1}$ [La^{-}]. Similarly, Keiser et al. (2015) demonstrated performance improvements in mean (+10%) and maximal (+8%) power output during a 30-min time trial and in $\dot{V}O_{2max}$ (+10%) in the heat following 10 days of heat acclimation (**Figure 2.14**). Both of these studies did not observe any changes in the control group that trained in cool or temperate conditions.

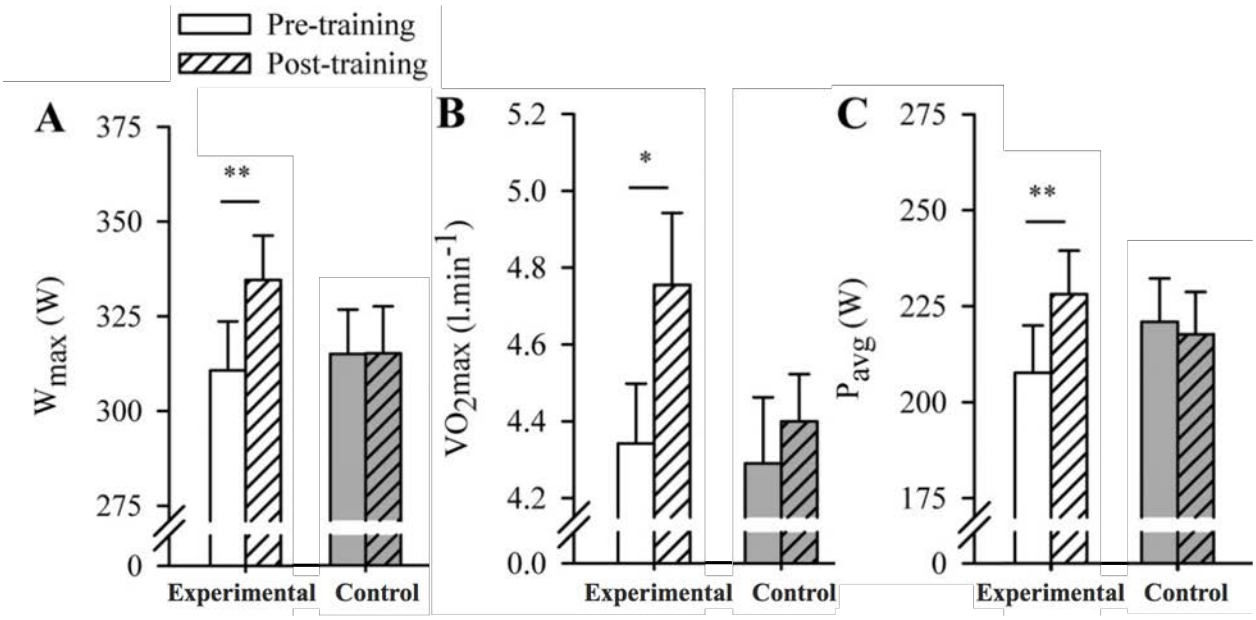


Figure 2.14 Absolute pre- (*open bars*) and post-training (*hatched bars*) performance in 38°C, 30%RH for the experimental (10 days of training in 38°C, 30%RH) and the control group (10 days of training in 18°C, 30%RH). Data are mean ± standard error. Pre- vs post-training values significantly different at *p<0.05 and **p<0.01. Taken from Keiser et al. (2015). W_{max}, maximal power in an incremental test; $\dot{V}O_{2max}$, maximal aerobic capacity; P_{avg}, average power during a 30-min time trial

Performance improvements following heat acclimation extend to pacing, and specifically the ability to maintain power output during prolonged exercise. Following ~2 weeks of training in hot conditions in Qatar, Racinais et al. (2015b) 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 pre-acclimatisation trials in these male athletes (**Figure 2.15**). Although mean power output during the post-acclimatisation hot time trial still remained slightly lower than during the cooler performance, the "drop-off" in power output that was observed in the pre-acclimatisation hot trial was largely prevented during the post-acclimatisation hot trial. Notably, Racinais et al. (2015b) observed that performance improvements were greater following 2 weeks of heat acclimation than following 1 week. Indeed, >7 heat exposures is recommended to maximise exercise performance improvements (Tyler et al., 2016). Further discussion of the efficacy of short-term heat acclimation protocols (<7 days) can be found in **Section 2.4.3.1**.

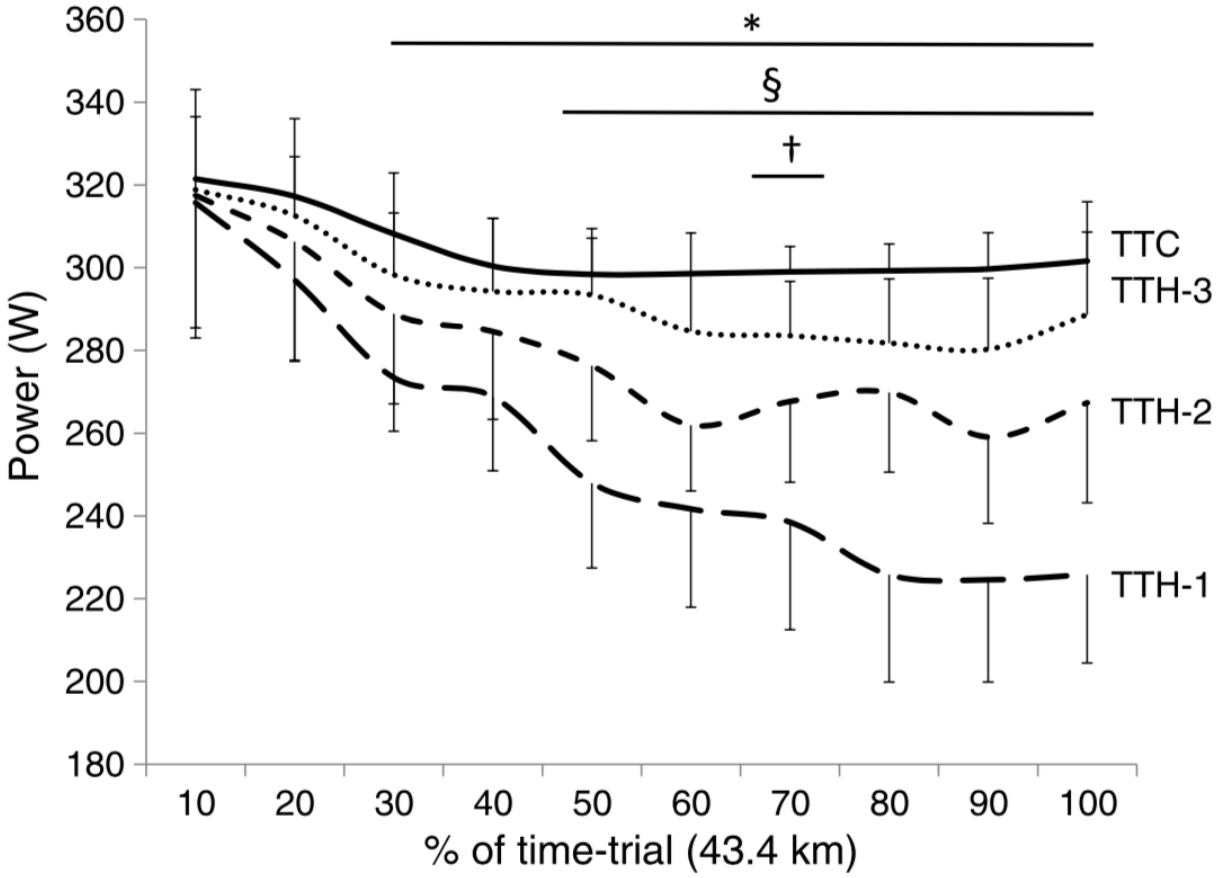


Figure 2.15 Power output during a 43.4-km cycling time trial (TT) in cool conditions (8°C, 30%RH, TTC, *solid line*) and in the heat (36-37°C, 12-16%RH) before (TTH-1, *long dashed line*), following 1-week (TTH-2, *short dashed line*) and 2-weeks (TTH-3, *dotted line*) heat acclimatisation (34°C, 18%RH). Data are mean±SD. *§†TTC was significantly (p<0.05) higher than TTH-1, TTH-2, and TTH-3, respectively. Taken from Racinais et al. (2015b).

Studies that have investigated the physiological adaptations of heat acclimation have included female cohorts (Fein et al., 1975; Fortney & Senay, 1979; Avellini et al., 1980b; Shapiro et al., 1980; Stephens & Hoag, 1981; Horstman & Christensen, 1982; Francesconi et al., 1983; Sawka et al., 1983a; Sawka et al., 1983b; Armstrong et al., 2005; Mee et al., 2015b; Mee et al., 2018). However, self-paced time trial performance following traditional active heat acclimation protocols (8-14 days) has yet to be quantified for females, exposing a knowledge gap in real-world application for females competing in hot conditions. Sex differences described in previous sections, especially differences in cardiovascular (i.e. a limiting factor in time trial performance; Périard et al., 2011) and thermal behaviour (expected to influence pacing) adaptations, indicate that there may also be differences in the time course, underlying factors and/or magnitude of changes to exercise performance in the heat following heat acclimation in a female cohort.

2.4 Heat acclimation administration

Adaptation occurs when a stressor repeatedly provokes homeostatic challenges (Taylor, 2014). Specifically, the heat acclimation phenotype is attained following repeated exposures to heat stress. The definition of what can be considered “heat stress” is broad. The following section will discuss the modifying factors in heat acclimation protocols that have been employed in the literature. An overview of heat acclimation protocols is presented in **Figure 2.16**.

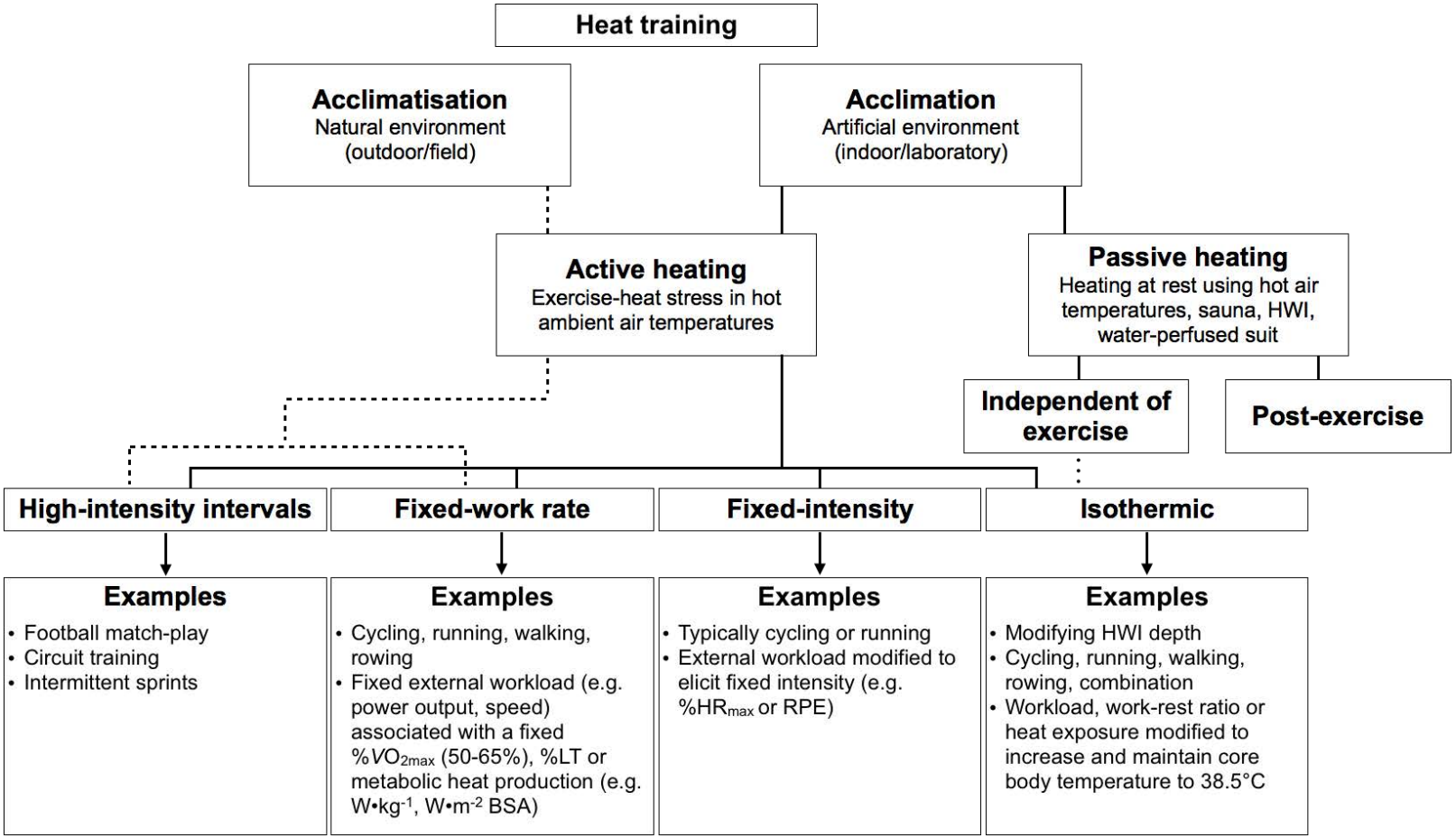


Figure 2.16 Schematic overview of methods for heat acclimation and heat acclimatisation, with examples. Within these methods, various combinations of temperature and humidity are possible. Adapted from Daanen et al. (2018) BSA, body surface area; HR_{max}, maximum heart rate; HWI, hot water immersion; LT, lactate threshold; RPE, ratings of perceived exertion; $\dot{V}O_{2max}$, maximal aerobic capacity

2.4.1 Active heat acclimation

Active heat acclimation combines exercise with high ambient temperatures. The increased metabolic demand initiates a greater thermoregulatory challenge, creating a unique stimulus for adaptation when it is administered chronically (Taylor, 2014). Active heat acclimation likely induces the greatest adaptation and appears to stimulate a unique upregulation of genes that cannot be stimulated with exercise or passive heat individually (Buono et al., 2009a; Kodesh et al., 2011).

Various exercise modalities (including walking, running, cycling and rowing) all appear to effectively induce the heat acclimation phenotype, and thus, the choice of modality should be based on training specificity (Taylor & Cotter, 2006). Recent literature most commonly reports using 60-90 min daily active heat acclimation sessions (Tyler et al., 2016), although shorter (e.g., 30-35 min; Houmard et al., 1990) and longer (e.g., 2 h; Turk & Worsley, 1974) sessions, or even sessions to exhaustion (Nielsen et al., 1993) have previously been reported. Intensity, type and duration of exercise bouts performed under hot ambient temperatures vary, but can be broadly grouped into the recognised protocols described below.

2.4.1.1 Fixed-intensity

Fixed-intensity heat acclimation typically refers to a fixed external workload, corresponding to a $\%\dot{V}O_{2max}$, exercise threshold (e.g. ventilatory thresholds) or power output relative to body size (eliciting a fixed metabolic heat production; i.e. $W \cdot kg^{-1}$). The fixed-intensity method is simple and relatively well-controlled, considering the minimal monitoring equipment needed for practical implementation (Taylor, 2014). Because of the duration of heat acclimation sessions, protocols often employ what would be a low exercise intensity in cool or temperate conditions (50-60%$\dot{V}O_{2max}$; Tyler et al., 2016). Indeed, Lorenzo and colleagues used 90 min heat acclimation sessions cycling at ~50%$\dot{V}O_{2max}$ and observed that 10 days of heat acclimation elicited robust adaptations during exercise-heat stress (i.e. improved time trial performance, $\dot{V}O_{2max}$ and power at 4 $mmol \cdot L^{-1}$ [La^{-}], increased cardiac output and plasma volume and reduced body temperatures and heart rate). In contrast, when Nielsen et al. (1993) employed a moderate workload, equivalent to power at ~70%$\dot{V}O_{2max}$, participants reached exhaustion at only 48 minutes in the initial heat exposure. A criticism of protocols employing fixed external workloads is that the relative intensity of the thermal stimulus is progressively reduced as participants adapt across the protocol. Consequently, the rapid rate of adaptions experienced in the first 3-4 days of heat exposure

begin to plateau (Periard et al., 2015) and plasma volume expansion adaptations are not sustained (Patterson et al., 2004b).

Apart from fixing external workloads, fixed-intensity protocols also include clamping intensity to elicit a fixed heart rate. For example, participants' heart rate during the session can be clamped at an intensity corresponding to that achieved at 50-65%$\dot{V}O_{2max}$ in cool conditions (Keiser et al., 2015; Travers et al., 2020b). Alternatively, particularly in more applied settings, protocols can be clamped at a set *perceived* intensity (i.e. RPE) so that participants' self-regulated external workload and RPE remains clamped (Armstrong et al., 1986; Cheung, 2010a). The benefits of these methods are that monitoring does not involve invasive equipment and the relative cardiovascular or perceptual heat strain is maintained for the duration of the protocol.

2.4.1.2 Isothermic

Whilst controlling heart rate may maintain cardiovascular strain, maintaining a high core body temperature (and thus thermal impulse; Taylor, 2014) has been argued to be the most important driver of adaptation to chronic heat stress (Taylor & Cotter, 2006). This approach, called isothermic heat acclimation, involves manipulating exercise intensity to induce a rapid rise in core temperature up to a target ~38.5°C (known as a "thermoregulatory threshold" for adaptation; Taylor & Cotter, 2006), which is maintained for the duration of the session by modifying exercise intensity and/or work-rest intervals. This endogenous temperature threshold is required to elicit an eHSP response to acute bouts of exercise-heat stress (Gibson et al., 2014), which is important, given the aforementioned critical role of the heat shock response in acquiring thermal tolerance and adaptation (Kuennen et al., 2011).

The isothermic protocol was first proposed by Fox et al. (1963a). However, the protocol itself was under-utilised for decades in favour of a fixed-exercise intensity approach before being adopted again in more recent years (Regan et al., 1996; Cotter et al., 1997; Weller & Harrison, 2001; Patterson et al., 2004b, a; Weller et al., 2007; Garrett et al., 2009; Magalhaes et al., 2010; Magalhães et al., 2010; Garrett et al., 2012; Garrett et al., 2014; Patterson et al., 2014; Gibson et al., 2015a; Gibson et al., 2015c; Mee et al., 2015b; Neal et al., 2016a; Neal et al., 2016b; Rendell et al., 2017; Willmott et al., 2017; Corbett et al., 2018a; Costello et al., 2018; Mee et al., 2018; Notley et al., 2018; Garrett et al., 2019; Sotiridis et al., 2019; Barry et al., 2020; Moss et al., 2020; Ravanelli et al., 2020; Sotiridis et al., 2020a). It has previously been suggested that isothermic heat acclimation affords a more "complete" adaptation (Taylor, 2000; Taylor, 2014), meaning adaptations will plateau closer

to their genetically pre-determined maximum and participants will exhibit the complete heat acclimation phenotype (i.e. attaining *all* hallmark adaptations including thermal, cardiovascular, sudomotor, fluid-regulatory and exercise capacity; Sawka et al., 2011). However, Gibson et al. (2015c) compared an isothermic with a fixed-intensity (50%$\dot{V}O_{2peak}$) protocol and observed similar thermal, cardiovascular and sudomotor adaptations following both 5 and 10 days of acclimation in males. Whilst the isothermic approach was therefore not superior in terms of inducing adaptations, it was *equally* effective for inducing heat acclimation as fixed-intensity heat acclimation, but with less cumulative mechanical load, and thus may be more efficient (Gibson et al., 2015a; Gibson et al., 2015c). Notably, Gibson et al. (2015c) also investigated progressively raising the target core body temperature to 39.0°C for the latter 5 days of a 10-day heat acclimation protocol and reported adaptations analogous to 10 days of 38.5°C isothermic heat acclimation.

The isothermic approach requires core body temperature monitoring, which may not be as feasible in a field-setting. Furthermore, a criticism of this approach is that participants with larger body sizes may receive a greater heat stimulus than their relatively smaller counterparts, given the greater total heat production required to elicit core temperature elevations to the 38.5°C threshold in larger individuals (Jay & Cramer, 2015). This stipulation may be particularly pertinent when applying the isothermic technique (primarily validated in males) to a typically smaller female athlete population.

2.4.1.3 High-intensity intervals

Studies in team sport athletes have successfully used acclimation or acclimatisation protocols with exercise bouts that are more sport-specific than a traditional “fixed” protocol (Sunderland et al., 2008; Petersen et al., 2010; Racinais et al., 2012; Pethick et al., 2018; Gale et al., 2020). Inherent to these protocols are short bouts of high intensity exercise. Sunderland et al. (2008) credit the rapid improvement in exercise performance following only 4 days of heat acclimation to the high-intensity nature of their intermittent sprints protocol. Indeed, this likely allowed for a rapid elevation in core body temperature. Of note, these high-intensity heat acclimation protocols all used ≤7 exposures, possibly limiting the cumulative fatigue of a high-intensity protocol.

2.4.2 Heat exposure frequency

Traditionally, heat acclimation has consisted of consecutive days of exercise-heat exposure, as this facilitates a more rapid induction of adaptation than does non-consecutive exposures (Gill & Sleivert, 2001). However, if rapid induction is not required (e.g. a competition is scheduled months in advance), the consecutive-day model may be suboptimal due to impracticalities for athletes. Indeed, integrating heat acclimation into an athlete's training without disrupting other training objectives can be difficult and has been identified as a potential barrier to widespread use (Chalmers et al., 2014; Périard et al., 2017). Instead, employing heat exposures intermittently may be preferred. However, a classic study from Gill & Sleivert (2001) reported that 10 consecutive days of heat acclimation (30 min at 70%$\dot{V}O_{2peak}$ in 38°C, 70%RH) was superior to 10 intermittent heat exposures over 3 weeks, in that intermittent exposures only induced a (comparatively inferior) reduction in exercising rectal temperature without any other adaptations. Thus, it was suggested that 2-3 days without heat exposure was too long, as adaptations' decay during off-days prevented heat stimulus accumulation. However, other studies using a variety of intermittent protocols have instead shown intermittent heat acclimation (i.e. every 2-3 days) to be effective in inducing heat acclimation. These intermittent protocols have included fixed-intensity active heat acclimation (i.e. walking or continuous calisthenics; Fein et al., 1975; Ormerod et al., 2003), high-intensity interval heat acclimation (i.e. repeated-sprints; Sunderland et al., 2008; Brade et al., 2013; Kelly et al., 2016; Duvnjak-Zaknich et al., 2019), passive heat acclimation (i.e. hot water immersion; Brazaitis & Skurvydas, 2010) and post-exercise passive heating (Scoon et al., 2007). Moreover, Duvnjak-Zaknich et al. (2019) and Fein et al. (1975) compared consecutive-day heat acclimation against intermittent exposures every other or every third day, respectively, and observed similar thermal, cardiovascular and sudomotor adaptations in both consecutive and intermittent groups. Therefore, intermittent heat acclimation presents an attractive alternative for athletes, assuming they have the time to implement it.

2.4.3 Time course

2.4.3.1 Short-term heat acclimation

Most of our knowledge to date regarding heat acclimation is based on studies undertaken with what is classified as "medium-term" heat acclimation, which involves 8-14 heat exposures (Garrett et al., 2011). Short-term heat acclimation (STHA; ≤7 exposures) offers the advantages of a reduced time and cost, which is especially valuable when access

to hot ambient temperatures is limited, or when ability to perform in the heat is required within a rapid time-frame (e.g. military deployment). Garrett and colleagues (2009) first demonstrated the effectiveness of a STHA protocol on physiological markers of acclimation in moderately trained males. In a subsequent study, the authors then showed that STHA induced meaningful performance improvements in highly trained male athletes (Garrett et al., 2012). Following 5 days of heat acclimation (39.5°C, 60% RH), athletes studied by Garrett et al. (2012) improved 2,000-m rowing time trial performance by an average of 4 s (1.5%), with a 10-s reduction at the highest range of improvement. This was a particularly meaningful performance improvement, given that participants in this study 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 et al., 1999) and in the sport of rowing, disproportionally large increases in power output are required to achieve measurable increases in speed, which indicates that these improvements were both meaningful and substantial. These initial observations by Garrett and colleagues have since been replicated and extended by others; all demonstrating that STHA improves performance during exercise-heat stress in male (or mostly male) cohorts (Petersen et al., 2010; Brade et al., 2013; Chen et al., 2013; Best et al., 2014; Costa et al., 2014; Gibson et al., 2015c; Racinais et al., 2015b; Kelly et al., 2016; Zurawlew et al., 2016; James et al., 2017b; Willmott et al., 2017; Gale et al., 2020).

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, since rapid plasma volume expansion partially alleviates the cardiovascular strain experienced during exercise-heat stress. Indeed, Gibson et al. (2015c) reported that 5 days may even be enough to reach full acclimation status when an isothermic protocol is implemented, as they showed no additional physiological improvements from 5 additional days of heat acclimation.

Whilst these positive results point towards the use of the contemporary STHA protocol for its reduced temporal and financial cost, it may not be appropriate for a female cohort. Mee et al. (2015b) were the first to formally compare STHA adaptations between a female and male cohort. The results indicated that although females became partially acclimated (increased sweat rate), they were unable to attain other classic markers of heat acclimation (i.e. reduced heart rate and skin and core body temperatures) after 5 days of isothermic heat acclimation; whilst males did reduce heart rate and skin and core body temperatures. Twenty minutes of sauna suit use in addition to 90 min of isothermic heat acclimation was required to elicit the classic markers of heat acclimation after 5 exposures in females (Mee et al., 2018). Importantly, these findings contrast with current recommendations, as the 2015 consensus statement on training and competing in the heat

recommends that, when using isothermic heat acclimation for at least 60 min per day, heat acclimation adaptations are complete within ~1 week (Racinais et al., 2015a). Therefore, findings from Mee et al., (2015) highlight that female populations performing or working in the heat may not be as acclimated, and thus as prepared, as their male counterparts after following current guidelines to only undertake a short-term, isothermic heat acclimation protocol.

It should be noted that physiological adaptations measured by Mee et al. (2015, 2018) likely underpin, but do not directly represent exercise performance, *per se*. As discussed in previous sections, exercise performance in the heat depends not only physiology, but also on factors such as thermal comfort and modifications to thermal behaviour. For example, Sunderland et al. (2008) observed a 33% improvement in shuttle-run endurance time in female soccer players in the heat following only 4 days of heat acclimation (intermittent-sprint sessions in 30°C, 24%RH), with an improved thermal comfort, but no marked physiological differences from the temperate exercise training group. However, these were team sport athletes who may have a greater capacity for adaptation compared to endurance athletes, who typically spend more time under endogenous heat stress and are thought to be "partially acclimated" (Taylor, 2000). Since the publication of the studies by Mee et al. and Sunderland et al. (and the undertaking of this thesis), two other studies have compared outcomes of STHA in females. Following 5-days sport-specific circuits in the heat (35°C, 36-46%RH; ~38.4°C mean gastro-intestinal temperatures for final 60 min), Pethick et al. (2018) observed a 1.5% increase in the 30–15 Intermittent Fitness Test (a speed-based test to exhaustion) in temperate (20°C) conditions alongside a ~9.5% plasma volume expansion in female athletes. However, no other heat acclimation markers were reported in that study. Garrett et al. (2019) investigated the effects of 5 days of isothermic heat acclimation (~40°C, 60%RH) on heat stress test performance (9 × 5-min bouts of intermittent treadmill running plus 6-s maximal cycle ergometer sprints in 31°C, 50%RH) in female games-players (all oral contraceptive pill users in the active-pill/*quasi*-luteal phase). The authors reported that total work done during cycling sprints tended to increase following STHA, alongside a ~7% expansion in resting plasma volume plus a reduced exercising rectal temperature (though only during the 8th and 9th 5-min exercise bouts, and not at rest) and heart rate (only during the final 5-min bout). Still, neither of these studies quantified *self-paced exercise performance* (i.e. time trial) outcomes in the heat in a female cohort.

2.4.3.2 Long-term heat acclimation

In addition to short- and medium-term heat acclimation, the use of long-term heat acclimation (LTHA) has also been investigated in the literature (Ormerod et al., 2003; Patterson et al., 2004b; Armstrong et al., 2005; Patterson et al., 2014; Stacey et al., 2018; Mikkelsen et al., 2019; Oberholzer et al., 2019; Omassoli et al., 2019; Ronnestad et al., 2020). Long-term protocols are defined as employing >14 heat exposures (Garrett et al., 2011) and are expected to maximise adaptation (Tyler et al., 2016). The mechanisms that underpin physiological markers of adaptation during LTHA may differ from those underpinning short- and medium-term heat acclimation. Horowitz et al. (1993; 1996) have proposed that heat acclimation manifests in a biphasic pattern, whereby the initial phase (i.e. short-, and most of medium-term heat acclimation) is transient and characterised by a decreased effector organ output-to-autonomic signal ratio (**Figure 2.17**). Accordingly, peripheral responses are heightened due to an increased autonomic excitability. During long-term heat acclimation, the effector organ output-to-autonomic signal ratio increases instead, resulting in more stable adaptations and improved physiological efficiency. For example, Levy et al. (1997) showed that one month of heat acclimation increased cardiac work (as indexed by rate-pressure product) for a given myocardial oxygen consumption in rodents. This demonstrated improved myocardial efficiency and thus a stable adaptation of the effector organ with LTHA in these heat-acclimated rats. Although this conceptual model was deduced from rodent data, Stacey et al. (2018) more recently monitored military personnel during a 22-day deployment and long-term heat acclimatisation in Cyprus. The authors reported that exercising heart rate was lowered following 5 days of acclimatisation, and remained lowered for the remainder of the protocol. However, heart rate variability (indexed by LF/HF ratio and MSSD) changed following 1 and 5 days of heat exposure only, indicating a greater sympathetic excitability of the heart and reduced vagal input during STHA, which subsequently dissipated with LTHA. These data support the biphasic model described by Horowitz et al. (1993; 1996) because reductions in heart rate during LTHA were maintained amid the disappearance of changes in heart rate variability (implicating stable adaptations and improved physiological efficiency). Overall, whilst some classic markers of heat acclimation (e.g. reduced heart rate) may not change from STHA to LTHA, the mechanisms that underpin these physiological markers may not necessarily be the same.

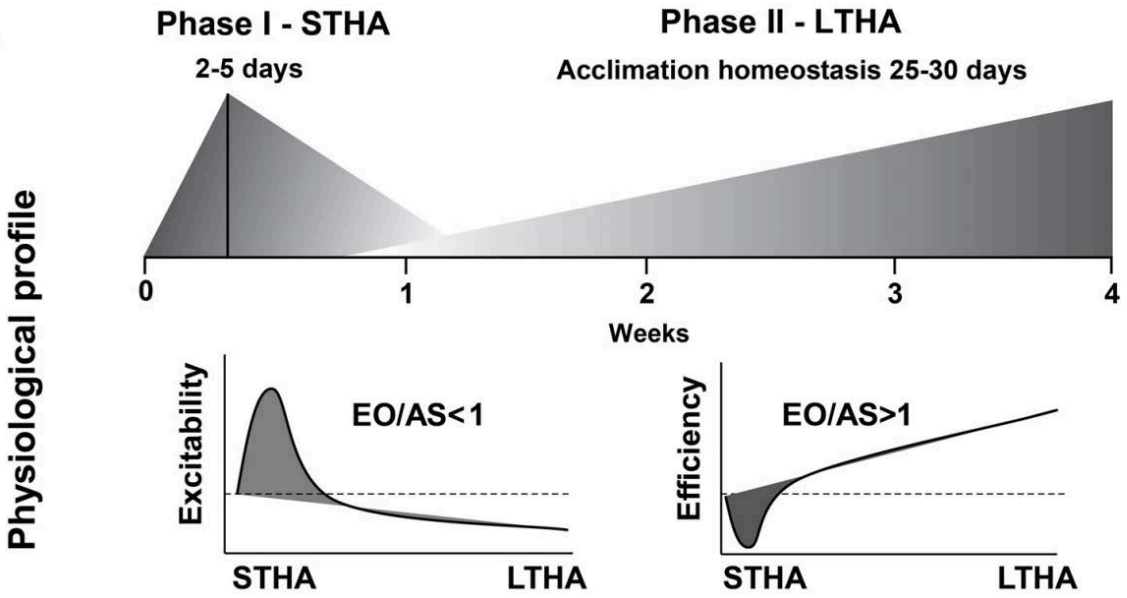


Figure 2.17 Heat acclimation's biphasic physiological profile. At the onset of heat acclimation (short-term heat acclimation; STHA), the autonomic nervous system compensates for impaired cellular performance. Increased efficiency, a signature of long-term heat acclimation (LTHA), diminishes the need for accelerated autonomic excitability. EO/AS, effector organ-to-autonomic signal ratio. Taken from Horowitz (2016).

It is also important to note that the aforementioned "stable adaptations" and "physiological efficiency" require sufficient time to manifest. For example, medium-term heat acclimation has been observed to increase extracellular levels of hypoxia inducible factor 1-alpha (HIF-1α; Lee et al., 2016), which activates downstream targets of erythropoietin (EPO) and EPO receptor when a long-term protocol is employed (Maloyan et al., 2005). Salgado et al. (2014) propose that an increase in HSP90 expression following heat acclimation (as discussed in **Section 2.3.6**) is the mechanism for increases in HIF-1α, since HSP90 stabilises HIF-1α.

2.4.4 Permissive dehydration

Permissive dehydration refers to restricting fluid intake during a heat acclimation session. The rationale for employing permissive dehydration is two-fold; firstly, dehydration elicits a unique cardiovascular and thermoregulatory challenge during exercise-heat stress (Gonzalez-Alonso, 1998). Thus, athletes require a lower mechanical load on the skeletal muscles to achieve target criteria when using an isothermic or controlled-heart rate heat acclimation protocol (Pethick et al., 2019). Second, hypohydrated exercise initiates a greater fluid-regulatory hormone response (i.e. aldosterone and ADH), known to be critical for provoking plasma volume expansion (Francesconi et al., 1983; Brandenberger 1986;

Kenefick et al., 2007; **Figure 2.18**). Indeed, Akerman et al. (2017) observed that performing orthostatically-stressful calisthenics in the heat with dehydration induced a 4% larger plasma volume expansion 24-h post-exercise than euhydrated exercise in either a temperate or hot environment. Although in this study, the rise in rectal temperature during exercise predicted plasma volume expansion when data from all groups was pooled. Nonetheless, when core body (rectal) temperature was controlled using an isothermic heat acclimation protocol, Garrett et al. (2014) observed permissive dehydration to be superior for adaptations related to plasma volume expansion, forearm blood flow and exercising heart rate (**Figure 2.19**).

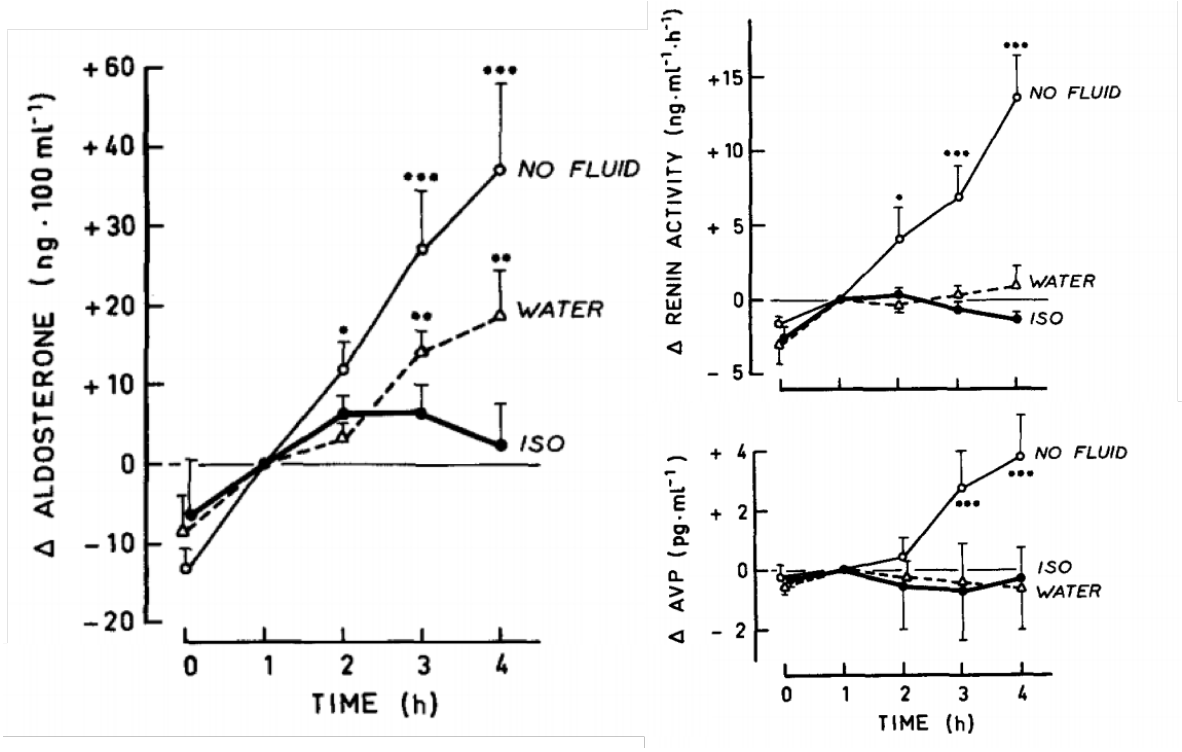


Figure 2.18 Changes in aldosterone, renin and vasopressin (AVP; i.e. anti-diuretic hormone) concentration during 4-hours cycle ergometer exercise at ~85 W in 34°C, consuming no fluid, or water or isotonic fluid (ISO) at 10-min intervals. Significantly different from first hour at *p<0.05, **p<0.01, ***p<0.001. Taken from Brandenberger et al. (1986).

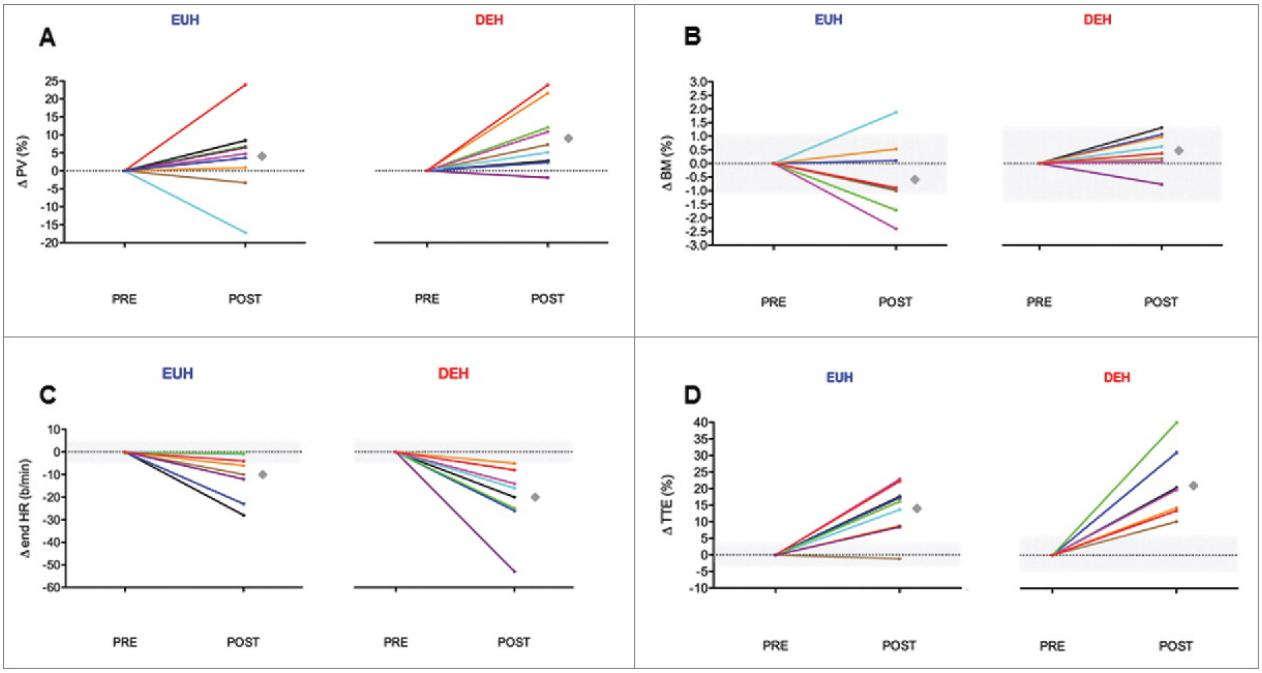


Figure 2.19 Individual changes in resting plasma volume (*A*), resting body mass (*B*), end-exercise heart rate (*C*), and subsequent time-to-exhaustion (TTE; *D*) pre- (PRE) and post- (POST) short-term isothermic heat acclimation undertaken with euhydration (EUH) or dehydration (DEH). Mean values are illustrated as a dark grey diamond. The smallest worthwhile difference is shown as a grey band, where it could be calculated. Taken from Akerman et al. (2016) with data from Garrett et al. (2014).

Contrary to findings by Garrett et al. (2014), Pethick et al. (2019) reported that there were no between-group differences in plasma volume changes following 5 days of heat acclimation (35°C, 55%RH) in either a euhydrated or dehydrated state (1.7-2.0% body mass change) or following 5 days of exercise in temperate conditions (22°C, 37%RH), when cardiovascular strain (i.e. $\%HR_{max}$) was matched. Schleh et al. (2018) and Neal et al. (2016b) also found that implementing a permissive dehydration heat acclimation protocol did not additively enhance any plasma volume, cardiovascular, body temperature, sudomotor or exercise performance benefits of heat acclimation, when using either 4 days of fixed-intensity (i.e. $50\%\dot{V}O_{2max}$) or 5 and 10 days of isothermic heat acclimation, respectively. Thermo-regulatory, cardiovascular, fluid-regulatory and performance outcomes also did not differ when restricting or not restricting fluid intake during a passive heat acclimation protocol (12 sessions of 45°C, 38%RH infrared sauna wearing a sweat suit; Barley et al., 2019). Lastly, Travers et al. (2020b) reported that not only was a permissive dehydration protocol not superior with regards to cardiovascular or plasma volume adaptations, but that 10 days of active heat acclimation (40°C, 40%RH) under progressive dehydration was ineffective in: i) increasing sweat rate, ii) decreasing skin temperatures, and iii) improving power output during a 30-min time trial in hot-wet conditions (35°C, 60%RH); whereas these adaptations were evident following heat acclimation in a euhydrated state at a matched heart rate

(equivalent to 65%$\dot{V}O_{2max}$ in cool conditions). The blunting of adaptations as observed by Travers et al. (2020b) may have been related to dehydration-mediated increases in stress and inflammation biomarkers (i.e. cortisol and interleukin-6) as compared to levels during exercise-heat stress alone (Costello et al., 2018). Chronic exposure to these elevations in stress and inflammatory markers may be excessively stressful and inhibit adaptation (Akerman et al., 2016). Additionally, hyperosmotic hypovolemia has been observed to attenuate vasodilation and sweating (Bittel & Henane, 1975; Kenney et al., 1990). This could perhaps result in suboptimal adaptations, since stimulating the thermoeffectors (and particularly the sudomotor response) facilitates thermoeffector adaptation (Buono et al., 2009a).

Females likely experience greater cardiovascular strain than males when exercising in a hypohydrated state (Sawka et al., 1983b). This could be due to females' lower blood and stroke volume (Wiebe et al., 1998; Wheatley et al., 2014), blunted peripheral vasoconstrictor response to sympathetic activity (Baker et al., 2016) and/or reduced reserve to increase peripheral oxygen extraction (Ogawa et al., 1992; Wiebe et al., 1998), as described in **Section 2.2.2.5**. This may have implications for the level of cardiovascular strain and consequential adaptive response to a permissive dehydration protocol in females. Additionally, females show a reduced osmotic sensitivity for ADH release as compared to males, whereby males show a greater increase in plasma ADH in response to a given change in plasma osmolality (Stachenfeld et al., 2001). Furthermore, females in the luteal phase or taking combined oral contraceptives have a lower osmotic threshold for ADH release than females in the follicular phase and males (Stachenfeld et al., 1999; Stachenfeld et al., 2001). These differences also may have implications for the adaptive response to permissive dehydration in females, given that fluid-conservatory responses intrinsic to plasma volume expansion are dependent on exceeding an "osmotic threshold" (~2% increase in plasma osmotic pressure) of the intravascular fluid (Andreoli et al., 2010; Cheuvront & Kenefick, 2014). Notably, Garrett et al. (2019) has shown a permissive dehydration protocol to be effective in inducing some markers of heat acclimation in female oral contraceptive users. However, a euhydrated *vs* a permissive dehydration protocol has yet to be directly assessed in a female cohort.

2.5 Passive heat acclimation

Active heat acclimation or acclimatisation, whilst effective, requires either exercise in a controlled environmental chamber or travel to a hot outdoor environment. These options carry considerable logistical and accessibility constraints, as well as time and/or financial costs. Moreover, the specificity of sessions needed to induce heat acclimation (i.e. intensity sufficient to drive and maintain core body temperatures ≥38.5°C) may interfere with normal training and/or an athlete's taper (Casadio et al., 2017). Passive heating offers a pragmatic, accessible and cost-efficient alternative. Whilst it had previously been suggested that heat exposure without exercise confers only slight adaptation (Bass et al., 1955), heat acclimation has been successfully achieved using passive heating in environmental chambers, hot-water immersion, sauna baths, "sweat suits" and water-perfused suits, amongst other modalities (**Table 2.2**).

In the absence of heat produced metabolically, ambient air (or water) temperatures must be high enough to present a thermoregulatory challenge sufficient to elevate core temperatures during passive heating. As these temperatures are typically higher than those employed in active heat acclimation protocols (i.e. >45°C; **Table 2.2**), there is a strong thermal stimulus directed at the thermoreceptors of the skin during passive heating, which generate important afferent feedback for optimising heat adaptation (Regan et al., 1996). Additionally, without the same sympathetic drive as experienced during exercise, skin blood flow is maximised or reaches near maximum values when skin temperatures are passively elevated to ~42°C (Barcroft & Edholm, 1943). Indeed, shear rate in the superficial femoral artery during hot-water immersion (30 min at 42°C) is greater than that following 30-min treadmill running at 65-75%HR_{max} in temperate (22-23°C) conditions (Thomas et al., 2016). Taken together, it is clear that passive heating alone is a considerable haemodynamic and thermoregulatory stimulus.

Ko et al. (2020) recently observed that 10 days of either 1-h walking (33°C, 60%RH) plus 1-h water-perfused suit (44°C; peak rectal temperature ~38.5°C) or 2-h water-perfused suit (44°C; peak rectal temperature ~38.5°C) were both superior to 10 days of 1-h walking in the heat (33°C, 60%RH; peak rectal temperature ~37.8°C) for improving whole-body sweat rate and perceptual strain during heat stress. Thus, if passive heating chronically provides a substantial thermoregulatory stimulus whereby core body and skin temperatures are elevated to a similar degree as is recommended during active heat acclimation (i.e. core body temperatures ≥38.5°C; Regan et al., 1996; Taylor & Cotter, 2006), hallmark adaptations will almost certainly be observed (Fox et al., 1963a; Henane & Valatx, 1973; Barry et al., 2020).

For athletes or occupational personnel undertaking heat acclimation, a primary aim is to improve exercise capacity in the heat, not just tolerance to passive heat stress. Indeed, there is sufficient evidence that adaptations attained using passive heating interventions translate to attenuated thermoregulatory and cardiovascular strain during exercise in the heat (**Figure 2.20**), as well as improved exercise capacity in the heat in male cohorts (Fox et al., 1963a; Henane & Bittel, 1975; Bonner et al., 1976; Tyka et al., 2008). To use an early example, Fox et al. (1963a) observed that 14 exposures to 2-h passive heating (combination of steam room, sweat suit and environmental chamber) with oral temperature clamped at either 37.9°C or 38.5°C both resulted in marked reductions in oral temperature (-1.3°C and -1.1°C, respectively) and heart rate (-16 beats·min^{-1} and 27 beats·min^{-1}, respectively) during two 30-min bouts of standardised stepping in hot conditions (40°C, 57%RH) post-acclimation. Likewise, there is substantial evidence supporting plasma volume expansion following passive heat acclimation via environmental chamber (Beaudin et al., 2009; Beaudin et al., 2012; Wilson et al., 2020), hot-water immersion (Bonner et al., 1976) and sauna bathing (Gryka et al., 2014; Drzazga et al., 2020). This plasma expansion is important for the improved cardiovascular stability during exercise-heat stress observed in the initial stages of heat acclimation (Periard et al., 2015).

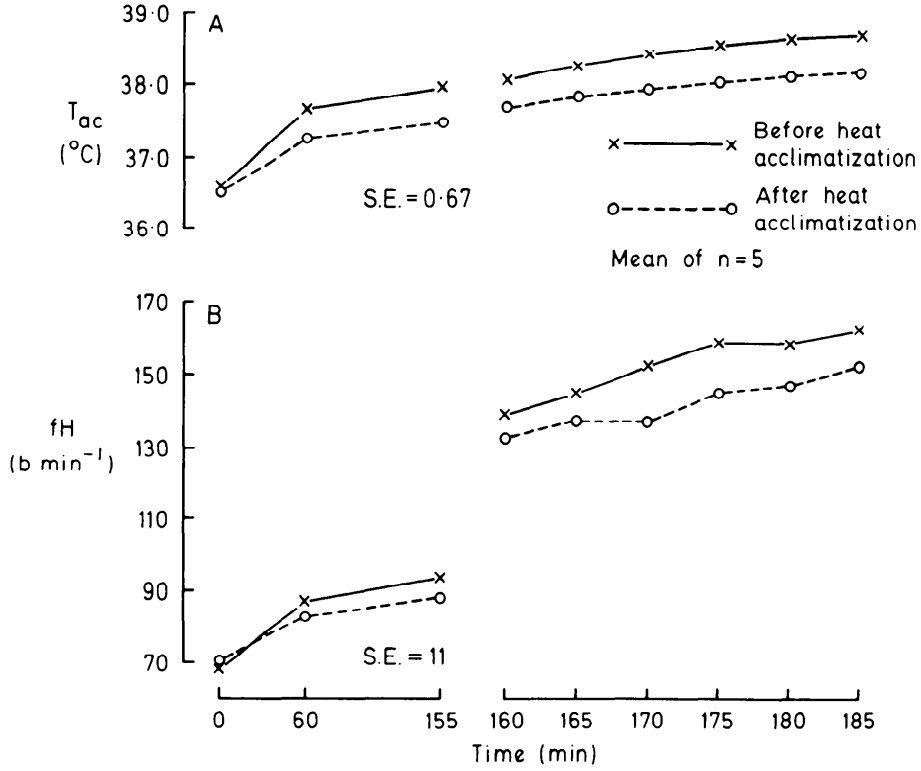


Figure 2.20 Tympanic temperature (T_{ac}) and heart rate (fH) during the heat stress test before (*solid line*) and after (*dashed line*) isothermic heat acclimation (target T_{ac} 38.5°C) achieved via hot-water immersion (41°C water) in a 40°C environmental chamber for 1 h daily for 13 days (excluding weekends). Heat stress test (48°C, 36%RH) consisted of 155

min passive heat exposure plus 25 min cycling at 50 W. Data are mean values with standard error (S.E.) indicated. Taken from Bonner et al. (1976).

Passive heating protocols minimise the issues surrounding mismatched metabolic heat production between sexes during active heat acclimation, since participants are not performing exercise. Rodrigues et al. (2020) observed similar thermoregulatory and cardiovascular responses, plus similar elevations in deep muscle temperature during a single 2-h bout of hot-water immersion (42ºC) to the waist in 15 females and 15 males. These similarities were observed in spite of anthropometric differences (height, body mass and body composition) between sexes. Pilch et al. (2010) investigated two female cohorts (each *n*=10) that undertook seven total sessions every other day of either 30-minute sauna (~80°C, 5-27%RH) or two 20-minute sauna bouts with 5-min break and a cold shower. Both protocols lowered resting rectal temperature as well as peak rectal temperature and heart rate in the 7th sauna session. Others (Brazaitis & Skurvydas, 2010; Brunt et al., 2016b) have also shown hallmark adaptations related to body temperatures, heart rate, sweating and skin blood flow as well as improvements in arterial function (i.e. FMD, arterial compliance, aortic pulse wave velocity, blood pressure) in mixed-sex (4-5F, 6M) sedentary, but otherwise healthy cohorts using 45-60 min of hot-water immersion. Nonetheless, the efficacy of passive heat acclimation to improve physiological responses during *exercise*-heat stress remains to be compared in both sexes.

Outcomes of passive heat acclimation studies in healthy human participants are summarised in **Table 2.2**.

Table 2.2 Passive heat acclimation interventions in healthy humans

Authors, year	Participants	Heat stimulus (modality/duration)	Total sessions & frequency	Outcomes
Environmental Chamber				
Henane & Valatx (1973)	9M	Environmental chamber for 3 h (30 min at 30°C, ~55%RH + ramp to 55°C until tympanic temp 38°C, then maintained in 35°C)	9 sessions (daily)	↓ T_{rec}, sweat onset temp during passive heating, ↑ max sweat rate
Henane & Bittel (1975)	12M	Environmental chamber for 90 min at 45°C, 24%RH	9 sessions (daily)	↑ sweat sensitivity, ↓ end-exercise T_{rec}, body heat storage. No change in resting T_{rec}, exercising sweat rate, T_{sk}
Hessemer et al. (1986)	7M	Environmental chamber for 60 min at 55°C	5 sessions (daily)	↓ resting T_{rec}, sweat and vasodilation onset temp, forearm blood flow during passive heating, ↑ sweat rate. No change in passive heating HR
Shido et al. (1999)	10M, 2F	Environmental chamber for 4 h at 46°C, 20%RH	9-10 sessions (daily)	↓ resting T_{rec}, sweat onset temp, ↑ sweat rate during passive heating. No change in resting HR, blood pressure, albumin, plasma electrolytes
Maruyama et al. (2006)	4M, 4F	Environmental chamber for 4 h at 40°C, 40%RH	9-10 sessions (daily)	↓ resting T_{rec}, HR, blood pressure, ↑ venous and arterial compliance
Beaudin et al. (2009)	PHA: 8M CON: 4M	Environmental chamber for 2 h at 50°C, 20%RH (T_{rec} 38.5-39°C for second hour by modifying plastic sweat suit use)	10 sessions (daily)	↓ resting T_{es}, sweat onset temp, temp thresholds for ventilation, ↑ PV, sweat rate
Beaudin et al. (2012)	9M	Environmental chamber for 2 h at 50°C, 20% RH	10 sessions (daily)	↓ resting T_{es}, sweat onset temp, breathing frequency, tidal volume, mean pulmonary ventilation, ↑ PV
Racinais et al. (2017a)	14M	Environmental chamber for 60 min at 48–50°C, 50%RH	11 sessions (daily)	↑ peak twitch amplitude, MVC

Authors, year	Participants	Heat stimulus (modality/duration)	Total sessions & frequency	Outcomes
Racinais et al. (2017b)	14M	Environmental chamber for 60 min at 48–50°C, 50%RH	11 sessions (daily)	↑ sustained voluntary contraction, activation and torque production during a prolonged contraction in hyperthermia
Pallubinsky et al. (2017)	11M	Environmental chamber for 6 h at ~33°C, 22%RH	7 sessions (daily)	↓ GI temp during passive heating, ↓ resting systolic and diastolic and heated systolic blood pressure, skin blood flow, sweat loss. No change in resting GI temp, HR at rest or during passive heating
Hesketh et al. (2019)	10M	Environmental chamber for 40-50 min at 40°C, 40%RH	18 sessions (3x/wk for 6 wks)	↑ aerobic capacity, insulin sensitivity, eNOS content, capillary density, capillary-fibre perimeter exchange index. No change in mitochondrial density, GLUT4.
Wilson et al. (2020)	12M	Environmental chamber for 60 min at 48-50°C, 50%RH	12 sessions (daily)	↑ resting PV, BV, left atrial volume, LVEDV, LV early diastolic velocity. No change in other functional or structural echo measures at rest.
Hot-Water Immersion				
Allan & Wilson (1971)	3M	HWI (depth varied to maintain T_{rec} 38.0-38.5°C) in 40°C for 60 min	15 sessions (weekdays for 3 wks)	↑ sweat rate, ↓ sweat sodium, T_{sk} during immersion
Bonner et al. (1976)	5M	HWI (41°C) in 40°C environmental chamber for 60 min, alternating in and on the side of tub to maintain T_{rec} 38.5°C	13 days excluding weekends	↓ exercise tympanic temp, HR, ↑ PV, sweat rate. No change in resting typanic temp, HR, plasma aldosterone, cortisol, intravascular fluid volume
Brazaitis & Skurvydas (2010)	PHA: 7M, 6F CON: 6M, 6F	HWI (waist) in 44°C for 45 min	7 sessions (every other day for 2 wks)	↓ resting T_{rec}, immersion T_{rec}, HR, PSI, ↑ sweat loss. No change in MVC or twitch

Authors, year	Participants	Heat stimulus (modality/duration)	Total sessions & frequency	Outcomes
Kanikowska et al. (2012)	6M	HWI (chest) in 42°C for 10 min + 90-min warm blanket	9 sessions (daily)	No change in white blood cell concentration, lymphocytes B and T, cortisol, interleukin 6, tumour necrosis factor alpha and natural killer cell activity
Shin et al. (2013)	9M	HWI (waist) in ~42°C for 30 min	10 sessions (every other day for 3 wks)	↓ resting tympanic temp, T_{sk}, ↑ sweat loss
Carter et al. (2014)	10M	HWI (waist) in 40°C for 30 min	24 sessions (3x/wk for 8 wks)	↑ FMD
Bailey et al. (2016)	9F	HWI (chest, arms not immersed) in 42°C for 30 min	24 sessions (3x/wk for 8 wks)	↑ SV, *Q*, FMD, $\dot{V}O_{2max}$, middle cerebral artery velocity, cerebrovascular conductance, sweat sensitivity, ↓ resting GI temp, vasodilation & sweat onset temp
Brunt et al. (2016b)	PHA: 4M, 6F CON: 4M, 6F	HWI (shoulders) in 40.5°C for 25-30 min until T_{rec} 38°C, then 60 min HWI (waist)	36 sessions (4-5x/wk for 8 wks)	↑ FMD, arterial compliance, ↓ aortic PWV, carotid intima media thickness, MAP. No changes in arterial compliance, endothelium-independent dilation
Brunt et al. (2016a)	PHA: 4M, 5F CON: 4M, 5F	HWI (shoulders) in 40.5°C for 25-30 min until T_{rec} 38°C, then 60 min HWI (waist)	36 sessions (4-5x/wk for 8 wks)	↑ sweat rate, CVC, nitric-oxide dependent dilation, ↓ resting and immersion T_{rec}. No changes in max CVC
Lee et al. (2017)	9M	HWI (waist) in 42°C for 30 min	Every other day for 3 wks	↓ resting tympanic temp, mean body temp, immersion sweat onset time, sweat rate
Ravanelli et al. (2020)	7M, 5F	HWI (neck) in 40-41°C for ~30min (until T_{rec} 38.5°C), then HWI (waist) for 60 min	7 sessions (daily)	↑ urine-to-serum osmolality ratio, free water clearance, fractional excretion rate of potassium, incidence of albuminuria. No change in GFR, fractional excretion rate of sodium, chloride, during passive heating

Authors, year	Participants	Heat stimulus (modality/duration)	Total sessions & frequency	Outcomes
Trachsel et al. (2020)	9M, 1F	HWI (neck) in 40-41°C for ~30min (until T_{rec} 38.5°C), then HWI (waist) for 60 min	7 sessions (daily)	↓ resting T_{rec}, ↑ sweat rate, LVEDV, E/A ratio, late atrial contribution. No change in T_{rec}, HR, SV, *Q*, MAP, ESV, ejection fraction, other echo measures of cardiac function during 30-min water-perfused suit (~30°C)
Gendron et al. (2020)	11M, 5F	HWI (neck) in 40-41°C for ~30min (until T_{rec} 38.5°C), then HWI (waist) for 60 min	7 sessions (daily)	No change in HR, markers of HR variability
Barry et al. (2020)	9M, 5F	HWI (neck) in 40-41°C for ~30min (until T_{rec} 38.5°C), then HWI (waist) for 60 min	7 sessions (daily)	↓ resting T_{rec}, immersion T_{rec}, HR, vasodilation & sweat onset temp, ↑ sweat rate, CVC, sympathetic nerve activity. No change in sensitivity of thermoeffectors
Sauna Bathing				
Leppäluoto et al. (1986)	10M	Dry sauna (80°C) for 60 min	14 sessions (twice daily for 7 consecutive days)	↓ resting T_{rec}, HR, serum potassium, sodium, iron
Tyka et al. (2008)	PHA: 11M CON: 11M	Sauna (~90°C, 5-15%RH at chest) for 3x15 min with ~5 min break for cold shower	12 sessions (every 3 days for ~5 wks)	↓ resting and sauna T_{rec} and HR, ↑ sauna sweat rate, and power at lactate turnpoint and TTE in the heat (33°C, 50%HR). No change in (hot) $\dot{V}O_{2max}$, maximal anaerobic power
Pilch et al. (2010)	20F	Sauna (~80°C, 5-27%RH) for 30 min (*n*=10) OR 2x20 min with 5 min break for cold shower (*n*=10)	7 sessions (every other day for 2 wks)	↓ resting T_{rec} and total cholesterol, sauna T_{rec} and HR. No change in resting HR, blood pressure, PV, free fatty acids
Gryka et al. (2014)	16M	Sauna (90°C, 5-16%RH) for 3x15 min, 2 min "cool-down" between bouts	10 sessions (daily or every 2 days)	↑ PV, ↓ total and LDL cholesterol, triacylglycerols. No chronic changes in HDL cholesterol

Authors, year	Participants	Heat stimulus (modality/duration)	Total sessions & frequency	Outcomes
Barley et al. (2019)	20M	Infrared sauna (45°C 38%RH) for 90min wearing plastic sweat suit	12 sessions separated by 1-3 days	↓ resting HR, ↑ Hct during and post- 3h weightloss protocol, faster temperate sled-push performance. No change in resting or temperate exercise GI or tympanic temp
Glazachev et al. (2020)	PHA: 14M CON: 14M	Infrared sauna (65-80°C) for 25-40 min	24 sessions (3x/wk for 4 wks + 2x/wk for 6 wks)	↑ temperate $\dot{V}O_{2max}$, minute ventilation, muscle mass
Drzazga et al. (2020)	10M	Sauna (~90°C, 12%RH at head) for 3x15 min with ~5 min breaks for cold shower	10 sessions (daily, except 2 days rest between session 5 & 6)	↑ PV, ↓ exercising and sauna T_{sk}. No change in $\dot{V}O_{2max}$, peak power
Post-Exercise Heating				
Scoon et al. (2007)	PHA: 6M CON: 6M (cross-over)	Post-exercise sauna (90°C) for ~31 min	12 sessions (4x/wk for 3 wks)	↑ PV, BV, body mass, temperate TTE *vs* CON
Stanley et al. (2015)	7M	Post-exercise sauna (87°C, 11%RH) for 30 min	10 sessions (daily)	↑ PV, HR recovery (i.e. faster) ↓ resting HR
Zurawlew et al. (2016)	PHA: 10M CON: 7M	Post-exercise HWI (neck) in 40°C for 40 min	6 sessions (daily)	↓ resting and exercise T_{rec}, exercise T_{sk}, HR, sweat onset temperature, RPE, ↑ sweat rate, hot 5-K TT performance. No change in sweat rate, temperate TT performance
Zurawlew et al. (2018b)	10M	Post-exercise HWI (neck) in 40°C for 40 min	6 sessions (daily)	↓ resting and exercise T_{rec}, exercise T_{sk}, HR, sweat onset temp, RPE. No change in sweat rate.
Zurawlew et al. (2018a)	16M	Post-exercise HWI (neck) in 40°C for 40 min	6 sessions (daily)	↓ resting and exercising T_{rec}, exercising T_{sk}, HR (in recreationally-trained, not well-trained), sweat onset temp, RPE, O_2 consumption, ↑ sweat rate.
Novak et al. (2018)	1F	Post-exercise sauna for 20 min (conditions not reported)	12 sessions (4x/wk for 3 wks)	↑ TTE, $\dot{V}O_{2max}$, anaerobic threshold, PV, red cell count, agility, lower body power

Authors, year	Participants	Heat stimulus (modality/duration)	Total sessions & frequency	Outcomes
Zurawlew et al. (2019)	13M	Post-exercise HWI (neck) in 40°C for 40 min	6 sessions (daily)	↓ resting and exercise T_{rec}, exercise T_{sk}, HR, sweat onset temp, RPE. No change in total Hbmass, BV, PV, sweat sensitivity, sweat rate
Ko et al. (2020)	6M	Post-exercise (33°C, 60%RH) water-perfused suit (44°C) for 60 min	10 sessions (daily)	↑ sweat rate, ↓ immersion T_{rec}, HR perception of heat strain
Other Passive Heating				
Fox et al. (1963a)	18M	Steam room at 43°C, 100%RH for 8 min + 2 h sweat suit in 37°C with oral temp 37.9°C	12 sessions (2 x 6 daily sessions with one rest day)	↓ resting and exercise T_{rec} and HR
Fox et al. (1963b)	20M	Steam room at 43°C, 100%RH for 8 min + 1-3 h plastic sweat suit in 37°C with oral temp 37.3-38.5°C	12-24 sessions (daily)	↓ resting T_{rec} and HR, immersion sweat onset temp, ↑ immersion forearm blood flow, sweat rate. No change in immersion peak T_{rec} or HR
Fox et al. (1964)	10M	Steam room at 43°C, 100%RH for 8 min + 2 h sweat suit in 37°C with oral temp 37.9°C	15 sessions (daily)	↑ sweat rate. No change in immersion HR
Goto et al. (2011)	8M	Heat- & steam-generating sheet placed on the thigh for 8 h, ~3°C increase in muscle temp	40 sessions (4x/wk for 10 wks)	↑ MVC, muscle size
Hafen et al. (2018)	10M, 10F	Shortwave diathermy for 2 h, ~4°C increase in muscle temp	6 sessions (daily)	↑ PGC-1α, HSP70, HSP90, mitochondrial respiratory capacity

Hafen et al. (2019)	PHA: 6M, 6F CON: 6M, 5F	Shortwave diathermy for 2 h, ~4°C increase in muscle temp	10 sessions (daily)	↑ PGC-1α, HSP70, HSP90. Attenuated muscle atrophy and myofibre respiratory capacity loss following immobilisation
Kim et al. (2020)	10M, 2F	Water-perfused suit (~52°C) on 1 thigh for 90 min	40 sessions (5x/wk for 8 wks)	↑ MVC. No difference in muscle cross-sectional area *vs* control leg
Ko et al. (2020)	7M	Water-perfused suit (44°C) for 2 h	10 sessions (daily)	↓ T_{rec}, HR, perception of heat strain, ↑ sweat rate during passive heating. No change in resting T_{rec}, HR

F, females; M, males; PHA, passive heat acclimation group; CON, control group
BV, blood volume; echo, echocardiography; CVC, cutaneous vascular conductance; E/A ratio, E-wave-to-A-wave ratio; eNOS, endothelial nitric-oxide synthase; ESV, end-systolic volume; FMD, flow-mediated dilation; GFR, globular filtration rate; GI temp; gastro-intestinal temperature (telemetric pill); GLUT4, Glucose transporter type 4; Hbmass, haemoglobin mass; HR, heart rate; HSP, heat shock protein; HWI, hot-water immersion; LV, left ventricle; LVEDV, left ventricle end-diastolic volume; MAP, mean arterial pressure; MVC, maximal voluntary contraction; O_2, oxygen; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PSI, physiological strain index; PV, plasma volume; PWV, pulse-way velocity; *Q*, cardiac output; RPE, ratings of perceived exertion; SV, stroke volume; temp, temperature; T_{es}, oesophageal temperature; T_{rec}, rectal temperature; T_{sk}, skin temperature; TT, time trial; TTE, time-to-exhaustion

2.5.1 Post-exercise sauna bathing

Sauna bathing is a specific passive heating modality of interest for the current thesis. Saunas are arguably one of the most extreme environments available for passive heating, with dry "Finnish sauna" conditions ranging between 80-100°C and 10-20%RH (Casadio et al., 2017). Independent of exercise, sitting passively in a sauna has been observed to elevate skin temperatures up to 40°C in just a few minutes, quickly creating an uncompensable environment. Core body temperatures can be defended against such high ambient temperatures at first (~0.2°C increase in rectal temperature in 15 min; Leppäluoto et al., 1987), but a ~1°C increase in core body temperature has been recorded after just 30-min sauna bathing (Leppäluoto et al., 1986). Associated physiological responses include sweat rates up to 1 $L \cdot h^{-1}$, skin blood flow of up to 7 $L \cdot min^{-1}$, cardiac output up to 10 $L \cdot min^{-1}$ and heart rate up to 150 $beats \cdot min^{-1}$ (Hannuksela & Ellahham, 2001). Also, acute hormonal responses to sauna bathing include a 3- to 6-fold increase in aldosterone (Kosunen et al., 1976; Leppäluoto et al., 1991), a 1.5- to 2-fold increase in ADH (Bussien et al., 1986; Leppäluoto et al., 1991) and a 1.5- to 2-fold increase in renin activity (Kosunen et al., 1976; Leppäluoto et al., 1991). These hormonal responses are important for plasma volume adaptations and are of a similar magnitude to that observed during exercise-heat stress (Francesconi et al., 1983; Montain et al., 1997). Hence, implementing 10-12 sauna sessions following temperate exercise has been observed to promote plasma and blood volume expansion (Scoon et al., 2007; Stanley et al., 2015).

Passive heating immediately post-exercise elevates core body temperature to an even greater degree than without prior exercise (Ridge & Pyke, 1986). Recent investigations into post-exercise hot-water immersion have shown this method induces thermoregulatory adaptations and improved exercise capacity during exercise-heat stress in recreationally- and well-trained participants (Zurawlew et al., 2016; Zurawlew et al., 2018a)when tested at various times of day (Zurawlew et al., 2018b). Furthermore, in rodents, Tamura et al. (2014) observed that 30 min of post-exercise heating (5 $sessions \cdot week^{-1}$ for 3 weeks) was more effective in inducing mitochondrial adaptations (e.g. mitochondrial enzyme activity and aerobic respiratory protein content) than either exercise or heating alone.

A key advantage of employing post-exercise sauna bathing (or any post-exercise heating regime) is that athletes' training remains relatively uninterrupted, allowing for sport- or intensity-specific sessions to be completed in line with an athlete's training programme (Pryor et al., 2019a). In addition, more time is spent at higher core body temperatures (maximising thermal impulse), since body temperatures elevated by exercise are not allowed time to "cool down" before being exposed to heat stress (Heathcote et al., 2019). Whilst

these advantages are certainly attractive, some knowledge gaps remain with regards to using post-exercise passive heating to induce heat acclimation. In the context of a central topic of this thesis, but for a case study of a young female tennis player (Novak et al., 2018), studies of post-exercise heating have been in male cohorts only. Furthermore, though sometimes grouped with other forms of post-exercise heating (e.g. post-exercise hot-water immersion), post-exercise sauna bathing has yet to be proven as a viable method to induce classic markers of heat acclimation or improve tolerance to *exercise*-heat stress in males or females.

2.6 Decay of heat acclimation adaptations

Heat acclimation is a transient phenomenon, in that adaptations will gradually recede if the heat stimulus is not maintained (Armstrong & Maresh, 1991). The so-called, “decay” of heat acclimation is not as rapid as its induction, with some adaptations (i.e. heart rate and core body temperature reductions) observed to be retained (albeit partially) for up to about a month (Weller et al., 2007). However, some degree of decay is often observed by ~14 days from the final heat exposure, especially when STHA is used (Williams et al., 1967; Garrett et al., 2009; Neal et al., 2016b). Some authors have proposed that adaptations are more “stable” or lasting when induced using medium-term heat acclimation (Weller et al., 2007; Daanen et al., 2011; Poirier et al., 2015).

Whilst the state of the heat acclimation decay literature has been periodically reviewed (Pandolf, 1998; Garrett et al., 2011; Daanen et al., 2018), authors repeatedly conclude that there is a great deal of variability in the literature due to inconsistencies and methodological issues. Admittedly, investigations of heat acclimation decay suffer from study design challenges that do not exist when studying heat acclimation induction. To track the daily rate of decay in heat acclimation adaptations would require daily exercise in the heat and thus would effectively continue to heat acclimate participants. Instead, studies typically test participants at 1 or 2 time-points post-heat acclimation. With a meta-analysis approach, Daanen et al. (2018) quantified the rate of decay and its modifying factors (i.e. number of heat exposures and duration, days of decay and severity of heat stress as indexed using wet bulb temperature) using stepwise multiple regression equations. The authors identified the rate of heart rate decay as being relatively stable, in that neither number nor temperature severity of heat exposures modified the ~2.3%·day^{-1} rate of decay. The rate core body temperature decay was not affected by the number of heat exposures. However, greater exposure duration appeared to profoundly attenuate decay of core body temperature adaptations, as increasing exposures from 60 to 90 minutes attenuated the decay in core body temperature adaptations by ~80%, as calculated using regression equations. On

average, decay of core body temperature adaptations progresses at a rate of ~2.6%·day^{-1}. Finally, Daanen et al. (2018) reported that sweat rate adaptations were best maintained when heat acclimation protocols included more exposures at higher ambient temperatures. Although notably, these sweat rate adaptations decay at approximately twice to three times the rate of heart rate and core body temperature adaptations. Data from decay studies included in the analysis by Daanen et al. (2018) are presented in **Figure 2.21**.

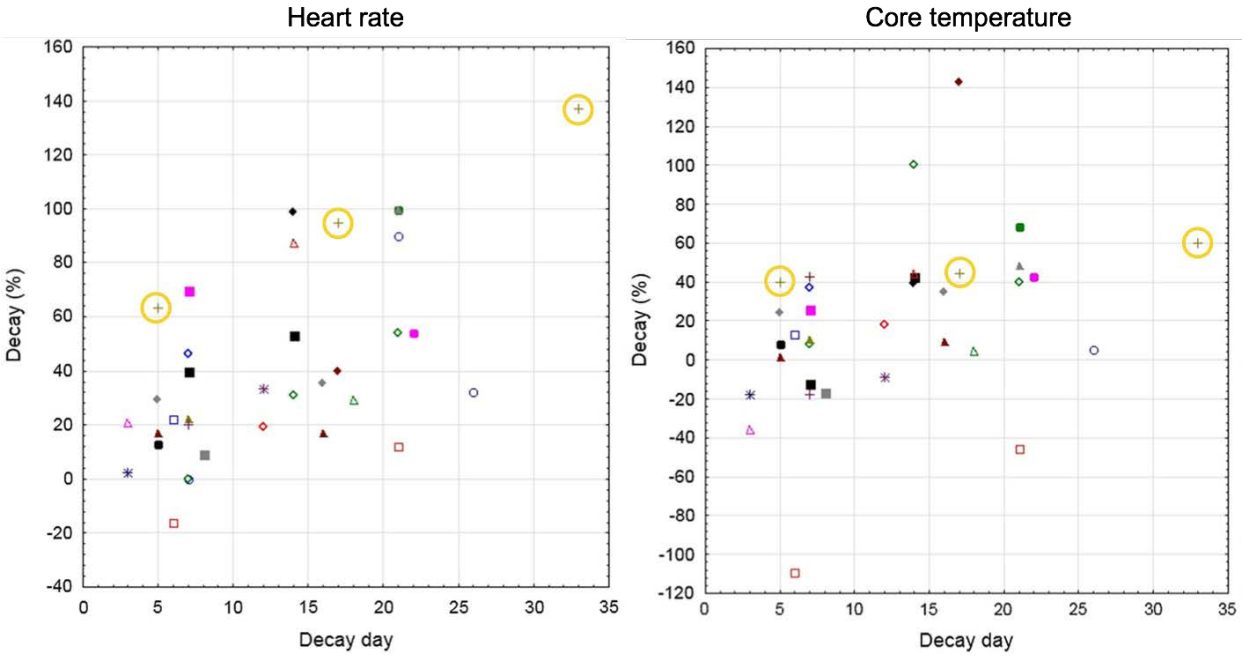


Figure 2.21 Decay (%) in heart rate (*left*) and body core temperature (*right*) adaptations following heat acclimation relative to the number of decay days (i.e. days without heat exposure) for selected studies. Studies that appear more than once in the figure are longitudinal studies in which heat acclimation decay was tested multiple times in a single heat acclimation group. Data from the only study with an exclusively female cohort (Stephens & Hoag, 1981) are circled in yellow. Taken from Daanen et al. (2018).

There are a number of other factors that modify heat acclimation decay. Participants with a higher cardiorespiratory fitness tend to better retain heat acclimation adaptations (Pandolf et al., 1977). Maintaining (temperate) exercise intensity during the decay period also appears to help maintain adaptations (Pryor et al., 2019b). However, heat acclimation induced using intermittent exposures (Duvnjak-Zaknich et al., 2019) and post-exercise hot water immersion (Zurawlew et al., 2019) appear to have similar rates of decay for heart rate and body temperature adaptations as consecutive-day active heat acclimation protocols (i.e. adaptations are mostly retained for at least two weeks). The decay of adaptations induced using post-exercise sauna bathing are yet to be assessed. Additionally, most adaptations (reduced core body temperatures, heart rate and sweat rate) decay at a similar rate following heat acclimation with permissive dehydration as compared to adaptations attained using

heat acclimation with euhydration, at least at one week post-acclimation (Neal et al., 2016b). However, plasma and blood volume expansion may attenuate faster when heat acclimation with permissive dehydration is used (Neal et al., 2016b).

Early studies show that, despite a similar time course for induction, heat adaptation retention is superior following heat acclimation using hot-dry conditions (Bean & Eichna, 1943; Henschel et al., 1943; Robinson et al., 1943; Lind & Bass, 1963) as compared to hot-wet conditions (Eichna et al., 1945b; Stein et al., 1949; Wyndham & Jacobs, 1957; Adam et al., 1960). In the context of practicality for the athlete, maintaining heat acclimation by employing a few weekly "top-up" heat exposures (Casadio et al., 2016; Pryor et al., 2019b), and thus avoiding decay, or implementing a rapid re-acclimation protocol (Weller et al., 2007; Racinais & Periard, 2020) are also viable options. Thus, better understanding and optimisation of heat acclimation decay/retention have a number of practical applications for athletes preparing for competition in the heat. Moreover, more data on heat acclimation decay from emerging passive or post-exercise heating acclimation protocols is needed, particularly with regards to potential sex differences.

Daanen et al. (2018) emphasised in their review that females were underrepresented in heat acclimation decay research to date, since only 21 of 428 participants from the included studies were female (<5%). The female data presented in their review were from two mixed-sex studies (Gill & Sleivert, 2001; Ashley et al., 2015) and only one study investigating females exclusively (Stephens & Hoag, 1981). As indicated in **Figure 2.22**, the exclusively female cohort demonstrated some of the fastest rates of heat acclimation decay for a given number of days without heat exposure, especially regarding heart rate. This could perhaps be related to previously discussed discrepancies in the stability of cardiovascular adaptations induced in female cohorts (**Section 2.3.2**).

2.7 Heat acclimation as an ergogenic aid

Until this point, this review has focused primarily on heat acclimation's efficacy for improving exercise capacity in the heat. However, the complex, integrative and multifaceted adaptations accrued during heat acclimation (**Section 2.3**) have been proposed to benefit exercise performance in "normal" (i.e. temperate or cool) conditions. Thus, amongst some controversy, heat acclimation has been labelled an ergogenic aid (Minson & Cotter, 2016; Nybo & Lundby, 2016). Research to date has documented improvements in muscle strength, torque and size with chronic heating (Goto et al., 2003; Goto et al., 2011; Racinais et al., 2017b; Miles et al., 2019; Nakamura et al., 2019). These results alone satisfy the criteria for being ergogenic for exercise performance (i.e. strength/power), however, in the current section, heat acclimation's ergogenic potential will be discussed in the context of improving

endurance exercise performance. **Table 2.3** presents a summary of studies to date that have investigated endurance exercise performance outcomes in cool or temperate conditions following heat acclimation.

Table 2.3 Heat acclimation interventions investigating endurance exercise performance outcomes in cool or temperate conditions

Authors, year	Participants	Heat stimulus (Modality/Duration)	Total sessions & frequency	Temperate/Cool Testing Conditions	Outcomes (% change)
Control group included					
Shvartz et al., 1977	HA: 21M CON: 5M	3 h bench stepping at 41 W in 30°C WBGT (39°C DBT) (CON: 23°C)	8 sessions (daily)	16°C WBGT (23°C DBT)	↑ $\dot{V}O_{2max}$ in untrained (+23%) and unfit (+23%) only. No change in trained (+3%) or CON (+6%).
Takeno et al., 2001	HA: 5M CON: 5M	Cycling at 60% $\dot{V}O_{2peak}$ for 60 min in 30°C, 50%RH (CON: 20°C, 50%RH)	10 sessions (daily)	25°C, 50% RH	Similar change in $\dot{V}O_{2peak}$ as CON (HA: +6.5%, CON: +8.7%)
Morrison et al., 2002	HA: 10M CON: 10M*	Cycling 90 min at 45%PPO (days 1&7) or self-selected (days 2-6) at 37°C, 50%RH (CON: 20°C, 50%RH)	7 sessions (daily)	"room temperature"	↑ 40-km TT mean power (+0.7%), time (-0.4%)
Creasy et al., 2003	HA: 9M CON: 9M*	Post-exercise sauna (84°C) for ~31 min	15 sessions (across 3 wks)	Not reported	↓ 2-km row TT power 2-days post-sauna (−1.0%), ↑ power 5-days (+0.3%) and 9-days post-sauna (+0.1%)
Hue et al., 2006	HA: 6M CON: 4M	30°C, 80%RH acclimatisation plus swimming in 30°C (CON: no acclimatisation, swimming in 27°C)	8 sessions (daily)	27°C	↓ 400-m swim TT time (-10%) 3 weeks post-acclimation. No change 10 days post-acclimation
Scoon et al., 2007	HA: 6M CON: 6M*	Post-exercise sauna (90°C) for ~31 min	12 sessions (4x/wk for 3 wks)	Not reported	↑ TTE (+33%)
Lorenzo et al., 2010	HA: 10M, 2F CON: 7M, 1F	2x45 min (10min rest) cycling at 50% $\dot{V}O_{2max}$ in 40°C, 30%RH (CON: 13°C, 30%RH)	10 sessions (daily)	13°C, 30%RH	↑ $\dot{V}O_{2max}$ (+4.9%), LT (+5.1%), 60-min cycling TT mean power (+6.2%)

Authors, year	Participants	Heat stimulus (Modality/Duration)	Total sessions & frequency	Temperate/Cool Testing Conditions	Outcomes (% change)
Chen et al., 2013	HA: 7M CON: 7M	25-45 min at 10% below to 10% above VT1 in 38°C, 52%RH (CON: 24°C, 52%RH)	5 sessions (daily)	24°C, 52%RH	No change in $\dot{V}O_{2max}$, TTE
Bradford et al., 2015	HA: 8M CON: 8M*	60 min swimming sessions (mix of continuous-intensity, intervals) in 33°C water (CON: 28°C)	7 sessions (daily)	28°C water	No change in 20-min swim TT
Karlsen et al., 2015	HA: 9M CON: 9M	30-45 min HIIT + 60-120 min <80%HRmax (~20% total training >80%HRmax) in 34°C, 18% (CON: <15°C)	14 sessions (daily)	5-13°C, 23-39%RH	No change in 43.4-km cycling TT, $\dot{V}O_{2max}$, PPO
Keiser et al., 2015	HA: 9M CON: 9M	90 min cycling at 50%HRmax 38°C, 30%RH (CON: 18°C, 30%RH, matched HR)	10 sessions (daily)	18°C, 30%RH	No change in $\dot{V}O_{2max}$, 30-min cycling TT
Chalmers et al., 2016	HA: 21M CON: 20M	190-240 min HIIT (running) in 35°C, 30%RH (CON: 19 °C, 30%RH matched RPE)	4-5 sessions (daily)	19 °C, 30%RH	No change in LT (+1.6%, similar to CON)
Bailey et al., 2016	HA: 9F CON: 9F	HWI (chest, arms not immersed) in 42°C for 30 min, no exercise (CON: cycling at ~70%HRmax for 30 min in 21°C, 45%RH)	24 sessions (3x/wk for 8 wks)	24°C	↑ $\dot{V}O_{2peak}$ (+5.6%), HWI alone similar to exercise intervention
Zurawlew et al., 2016	HA: 10M CON: 7M	40 min post-exercise HWI (neck) in 40°C (CON: 34°C water)	6 sessions (daily)	18°C, 40%RH	No change in 5-km running TT
Gollan et al., 2017	HA: 9M, 3F CON: 7M, 2F	30-min running at 80-115% LT in 30°C, 50%RH (CON: 18°C, 30%RH)	8 sessions (across 28 days)	18°C, 30%RH	"possible increase" in LT, but "unclear difference" from CON

Authors, year	Participants	Heat stimulus (Modality/Duration)	Total sessions & frequency	Temperate/Cool Testing Conditions	Outcomes (% change)
Philp et al., 2017	HA: 6M CON: 6M	50 min cycling at 70%HR reserve in 35°C, 56%RH (CON: 15°C, 81%RH)	5 sessions (daily)	17°C, 58%RH	↑ 30-15 intermittent running (0.5%), but similar to CON
McCleave et al., 2017	HA: 6M, 3F CON: 5M, 3F	90 min treadmill sessions (6 x HIIT + 3 x continuous) in 33°C, 60%RH (CON: 14°C, 55%RH)	9 sessions (3x/wk for 3 wks)	14-20°C, 54%RH	↑ 3-km running TT at 3 wks post-HA (+3.3%). No change immediately post-HA
Mackay et al., 2018	HA: 6M CON: 6M	60-min cycling at 40% $\dot{V}O_{2peak}$ in 33°C, 70%RH (CON: 19°C, 50%RH)	5 sessions (daily)	19°C, 50%RH	Similar ↑ TTE as CON, no change in submax lactate, ↓ $\dot{V}O_{2peak}$
Willmott et al., 2018	HA: 20M CON: 20M	60 min isothermic (T_{rec} 38.5°C) cycling in 45°C, 20% RH (CON: matched workload in 22°C, 40%RH)	10 sessions (consecutive weekdays OR 2x/day every other weekday for 2wks)	22°C, 40% RH	↑ $\dot{V}O_{2max}$ (4.1%), LT (+4.4%). No change in PPO (4.1%), TTE (+13.6%)
Mikkelsen et al., 2019	HA: 12M CON: 12M	60 min cycling at 60% $\dot{V}O_{2max}$ in 35-40°C, 30%RH (CON: ≤15°C)	28 sessions (5x/wk for 5.5 wks)	14°C, 54%RH	↑ 15-km cycling TT power (+6.0%), TT time (5.5%), though changes similar to CON. No change in $\dot{V}O_{2max}$, PPO
Waldron et al., 2019	HA: 12M CON: 10M	60-min cycling at 50% $\dot{V}O_{2max}$ in 38°C, 30%RH (CON: 20°C, 30%RH)	10 sessions (daily)	20°C, 30%RH	↑ $\dot{V}O_{2max}$ 4-days post-HA. ↓ $\dot{V}O_{2max}$ day 5 & 10 of HA, 1-, 2-days post-HA. No change 3-, 5-, 10-days post-HA
Glazachev et al., 2020	HA: 14M CON: 11M	25-40 min passive heating (infrared sauna) in 65-80°C (CON: 40 min elliptical training at 25-55%HRmax in room temp)	24 sessions (3x/wk for 4 wks + 2x/wk for 6 wks)	21–23°C	↑ $\dot{V}O_{2max}$ (12.5%), TTE (15.1%)

Authors, year	Participants	Heat stimulus (Modality/Duration)	Total sessions & frequency	Temperate/Cool Testing Conditions	Outcomes (% change)
Waldron et al., 2020	HA: 9M CON: 10M	60-min cycling at 55% $\dot{V}O_{2max}$ in 36°C, 40%RH (CON: 17°C, 30%RH)	5 sessions (daily)	~17 ◦C	↑ $\dot{V}O_{2max}$ (+5.4%), PPO (6.4%), VT (+2.3% of $\dot{V}O_{2max}$)
Ronnestad et al., 2020	HA: 11M CON: 10M	60 min cycling at 45%LT in 38°C, 65%RH (CON: 16°C, 25%RH)	25 sessions (5x/wk for 5 wks)	16-19°C	↑ $\dot{V}O_{2max}$ (+4.6%), 15-min cycling TT power (+6.1%), PPO (+2.3%) but similar to CON. No change in submax lactate
No control group					
Nadel et al., 1974	HA_{DRY}: 3M HA_{WET}: 3M	60 min cycling at 50% $\dot{V}O_{2max}$ in 45°C, ~25%RH (HA_{DRY}) or 36°C, ~90%RH (HA_{WET})	10 sessions (daily)	23.5°C	↑ $\dot{V}O_{2max}$ (+5.8%)
Fortney et al., 1979	9F	90 min cycling at 40% $\dot{V}O_{2max}$ in 45°C, 30%RH	21 (daily for 2 wks + every other day for 2 wks)	Not reported	No change in $\dot{V}O_{2max}$
Sawka et al., 1985	13M	2 h walking at 40-50% $\dot{V}O_{2max}$ in 49°C, 30%RH	9 sessions (daily)	21°C, 30%RH	↑ $\dot{V}O_{2max}$ (+3.5%) and PPO (+4.4%)
Pivarnik et al., 1986	16M	90 min cycling at 60% $\dot{V}O_{2max}$ in 20°C (first 3 wks) and 40°C, 30-35%RH (fourth wk)	24 sessions (6x/wk for 4 wks)	20°C	↑ $\dot{V}O_{2max}$ (+12.4%)
Racinais et al., 2011	15M	2 x low-intensity (60 min), 1 x walking (60 min), 4 x soccer-specific sessions (70-95min) in ~35°C WBGT	7 sessions (across 8 days)	22°C, 57%RH	↑ Yo-Yo intermittent run test (7%)
Buchheit et al., 2012	8M	~70 min football-specific sessions in 29-33°C, 37−50%RH	10 sessions (across 14 days)	23°C, 57%RH	↑ Yo-Yo intermittent run test after 5 (+25%) and 10 sessions (+44%)
Heled et al., 2012	8M	2 h walking at ~30% $\dot{V}O_{2max}$ in 40°C, 40%RH	12 sessions (daily)	24°C, 40%RH	↑ LT. No change in $\dot{V}O_{2max}$ (+0.2%)

Authors, year	Participants	Heat stimulus (Modality/Duration)	Total sessions & frequency	Temperate/Cool Testing Conditions	Outcomes (% change)
Molloy et al., 2013	HA: 9M (young), 7M (middle-aged)	30 min running at 75% $\dot{V}O_{2max}$ in 35°C, 35%RH	14 sessions (within 16 days)	22-24°C	↑ $\dot{V}O_{2max}$ in young only (+2.5%)
Neal et al., 2016a	10M	90 min isothermic (38.5°C T_{rec}) cycling with HA_{DEH} in 40°C, 50%RH	5 sessions (daily)	22 °C, 60%RH	↑ PPO (1.8%), LT (6.3%), 20km TT mean power (2.0%). No change in $\dot{V}O_{2max}$
Neal et al., 2016b	HA_{EUH}: 8M HA_{DEH}: 8M*	8 x 90 min isothermic (38.5°C T_{rec}) cycling + 3 x 60 min cycling at 35%PPO in 40°C, 50%RH (with HA_{DEH} or HA_{EUH})	11 sessions (daily)	22°C, 55%RH	↑ PPO (4.6%). No change in $\dot{V}O_{2max}$, LT
Perrotta et al., 2018	10F	60 min hot yoga (30 min dynamic + 30 min static stretching) with HA_{DEH} in 30°C, 48%RH	6 sessions (daily)	23°C, 27%RH	↑ VT1 (3.9%), VT2 (4.5%). No change in $\dot{V}O_{2max}$, TTE
Pethick et al., 2018	18F	90 min soccer-specific circuit training in 35°C, 37%RH	5 sessions (daily)	20°C	↑ 30-15 intermittent running test (1.5%)
Sotiridis et al., 2019	12M	90 min isothermic (38.5°C T_{rec}) cycling in 35°C, 56%RH	10 sessions (daily)	23°C, 51%RH	↑ PPO (+8.2%). No change in $\dot{V}O_{2peak}$
Tebeck et al., 2019	HA_{DRY}: 11M HA_{WET}: 11M*	90 min isothermic (38.5°C GI temp) cycling in 43°C, 20%RH (HA_{DRY}) or 32°C, 80%RH (HA_{WET})	5 sessions (daily)	21°C, 53%RH	↑ LT in HA_{WET} (+4.0%), ↓ HR at LT (both groups). No change in $\dot{V}O_{2max}$
Barley et al. 2019	20M	90 min passive heating (infrared sauna) in 45°C 38%RH, wearing plastic sweat suit	12 sessions separated by 1-3 days	22-24°C	↑ repeated sled-push performance (total test time: +2.3%; mean sled-push effort +9.8%)
Mang et al., 2020	7M, 6F	2x45 min (10 min rest) walking at 30-40% $\dot{V}O_{2max}$ in ~43°C, 30-50%RH	10 sessions (across 14 days)	"temperate"	↑ $\dot{V}O_{2max}$ (+3.6%). No change in VT1, VT2

Travers et al., 2020	HA_{EUH}: 8M HA_{DEH}: 8M*	90 min cycling at HR equivalent to 65% $\dot{V}O_{2max}$ in 40°C, 40%RH (with HA_{DEH} or HA_{EUH})	10 sessions (daily)	19°C, 63%RH	↑ $\dot{V}O_{2max}$ when groups combined (+3.8%). No change in PPO

Note: %change refers to mean value, only indicated when results were significant
*Participants cross-over groups.
CON, control group; DBT, dry bulb temperature; F, female participants; GI temp, gastro-intestinal temperature; HA, heat acclimation group; HA_{EUH}, euhydrated heat acclimation group; HA_{DEH}, dehydrated heat acclimation group; HA_{DRY}, hot-dry conditions heat acclimation group; HA_{WET}, hot-wet conditions heat acclimation group; HIIT, high-intensity interval training; HR, heart rate; HWI, hot-water immersion; LT, lactate threshold; M, male participants; PPO, peak power output attained during a graded exercise test; RH, relative humidity; RPE, ratings of perceived exertion; T_{rec}, rectal temperature; TT, time trial; $\dot{V}O_{2max}$, maximal aerobic capacity; $\dot{V}O_{2peak}$, peak aerobic capacity; VT, ventilatory threshold; WBGT, wet bulb globe temperature

Both thermal and non-thermal mechanisms have been suggested to contribute to improved exercise performance (Corbett et al., 2014). The thermal adaptations discussed in **Sections 2.3.1**, **2.3.3** and **2.3.4** (i.e. reduced resting temperatures and thermoeffector onset thresholds, and preferential peripheral thermoeffector adaptations to augment heat loss) allow for a greater heat storage capacity and a slower rate of rise in body temperatures. Whilst high core body temperatures are most deleterious to performance when accompanied by the high skin temperatures experienced in the heat (Sawka et al., 2012), these adaptations may also be relevant for performance in cooler conditions. Increased skin blood flow and sweating during exercise in cooler (14-22°C) conditions indicate that thermal strain is still a factor worth considering (Maw et al., 1998; Johnson, 2010). Indeed, Beis et al. (2012) reported that elite male marathon runners lose ~8% of their body mass during races in cool (~15°C) conditions and Ely et al. (2009) observed that trained distance runners (7M, 6F) reached rectal temperatures of ~39.8°C during an 8-km track TT (lasting only 25-30 min) in cool conditions (~17°C, 45%RH). Together, these data indicate that endurance exercise performance in cool conditions represents a notable thermal burden, which further impacts exercise performance with every degree differences from “optimal” temperatures (i.e. ~10°C; Galloway & Maughan, 1997; Ely et al., 2007). Thus, alleviating thermal strain via heat acclimation-mediated thermoregulatory adaptations may independently contribute to improved exercise performance in cool or temperate conditions.

Regarding non-thermal effects, Lorenzo et al. (2010) suggest that the cardiovascular adaptations associated with heat acclimation (i.e. plasma volume expansion and increased maximal cardiac output; **Section 2.3.2**) improve $\dot{V}O_{2max}$ in cooler conditions. In general, STHA protocols have not been effective in increasing $\dot{V}O_{2max}$ (Chen et al., 2013; Neal et al., 2016a; Perrotta et al., 2018; Tebeck et al., 2019), unless adaptations were given more time to manifest post-intervention (i.e. ~4 days; Waldron et al., 2020). However, a number of studies have reported mean increases in $\dot{V}O_{2max}$ ranging from 2.5-4.9% in recreationally to highly trained individuals following a medium-term heat acclimation protocol (Sawka et al., 1985; Lorenzo et al., 2010; Pascoe et al., 2013; Willmott et al., 2018; Waldron et al., 2019; Mang et al., 2020; Travers et al., 2020b). Greater increases have been observed using long-term protocols (6-13%; Pivarnik et al., 1987; Bailey et al., 2016; Glazachev et al., 2020) and in sedentary or untrained cohorts (6-23%; Nadel et al., 1974; Shvartz et al., 1977). In untrained cohorts, a large portion of aerobic capacity improvements are probably exercise training-mediated and not specific to heat acclimation *per se* (Sotiridis et al., 2020b). However, trained athletes have historically been reported to respond better to heat acclimation than untrained cohorts (Pandolf et al., 1977). As such, Mang et al. (2020) found that improvements in $\dot{V}O_{2max}$ in temperate conditions (specific temperature not reported) following heat acclimation were positively correlated with baseline $\dot{V}O_{2max}$, whereby those

with higher baseline fitness tended to improve more following their low-intensity (walking at 30-40% $\dot{V}O_{2max}$) heat acclimation protocol. However, given the difference in fitness between the 6 female and 7 male participants (mean $\dot{V}O_{2max}$ 42.3 mL·kg^{-1}·min^{-1} and 56.4 mL·kg^{-1}·min^{-1}, respectively), it is unknown whether this was a fitness effect or a sex effect, *per se*.

A number of studies report that improvements in $\dot{V}O_{2max}$ in heat acclimation intervention groups are not reflected by control groups exercising in cool or temperate conditions (Lorenzo et al., 2010; Willmott et al., 2018; Waldron et al., 2019; Glazachev et al., 2020; Waldron et al., 2020). However, both Takeno et al. (2001) and Ronnestad et al. (2020) reported that increases in $\dot{V}O_{2max}$ in trained males following heat acclimation (6.5% and 4.6%, respectively) were similar to those exhibited by a control group exercising at matched $\%HR_{max}$ and RPE, respectively. Yet, it remains that these outcomes still support the notion that heat acclimation is an ergogenic aid, by broad definition. If similar improvements in exercise performance can be attained as those of a control group, but via a much lower mechanical load (~30%; Pethick et al., 2019), this can arguably still be classified as being ergogenic.

Conversely, there are also a number of studies that report that medium- or long-term active heat acclimation (10-28 heat exposures) do not improve $\dot{V}O_{2max}$ (Fortney & Senay, 1979; Karlsen et al., 2015; Keiser et al., 2015; Neal et al., 2016b; Mikkelsen et al., 2019; Sotiridis et al., 2019). Various methodological factors may have prevented these previous studies from observing anticipated improvements in $\dot{V}O_{2max}$. Firstly, Fortney and Senay (1979) may not have employed an exercise stimulus of sufficient intensity (i.e. cycling at ~40% $\dot{V}O_{2max}$ with no training outside the intervention). Next, all training by Karlsen et al. (2015) was in hot conditions and thus the quality of participants' high-intensity training may have been compromised (Minson & Cotter, 2016). Somewhat analogous to the "live-high train-low" altitude training model, Corbett et al. (2014) suggest that preserving the quality of high-intensity training by keeping it independent of exercise-heat stress is a key factor in facilitating performance gains when undertaking active heat acclimation. Also, in the study by Keiser and colleagues (2015), pre-heating before the performance tests may have interfered with the aforementioned thermoregulatory advantages conferred by heat acclimation (i.e. lower resting core body temperature). Additionally, the statistical analysis of the data by Keiser et al. (2015) may have been underpowered (heat acclimation group n=7), especially given that they used a two-way repeated-measures analysis of variance (group × time), whereas Lorenzo et al. (2010) compared the effect of the intervention independently within-groups using *t*-tests. In the study by Keiser et al. (2015), the heat acclimation group's mean improvement in absolute $\dot{V}O_{2max}$ of ~200 mL (4.4%) was not statistically significant (pooled heat acclimation and control groups effect of *time*: p=0.08), however, this

improvement was very similar to that found by Lorenzo et al. (2010) in their 12-participant cohort (~210 mL [4.9%] increase).

Inconsistent outcomes are undoubtedly affected by a multitude of other methodological factors, including participant age (Pascoe et al., 2013), modality of heat administration (i.e. passive or active; Scoon et al., 2007) and how the heat acclimation protocol is integrated into the participants' training program (Corbett et at., 2014). Timing of post-intervention testing has also been highlighted as a potential confounding factor. Waldron et al. (2019) examined the time course of changes in $\dot{V}O_{2max}$ during and in the days following a 10-day heat acclimation protocol. Maximal aerobic capacity was not only suppressed during the heat acclimation protocol, but at one- (-6.7%) and two-days (-6.0%) post-intervention. By 4 days post-intervention, a rapid rebound effect was observed, and participants' $\dot{V}O_{2max}$ was increased 4.6% from pre-heat acclimation values. A similar acute reduction and rebound pattern of exercise performance has been observed when athletes are "over-reached" by a novel or more-intense stressor (Jeukendrup et al., 1992; Aubry et al., 2015). This could explain some of the discrepancies in the heat acclimation literature with regards to use as an ergogenic aid.

Mechanistically, any improvements in $\dot{V}O_{2max}$ in cool conditions following heat acclimation must be related to the two main components of the Fick equation (either independently or in combination; **Figure 2.22**). Namely, these components are maximal cardiac output ('central' adaptations) and arterio-venous oxygen difference ("peripheral" adaptations)(Ekblom et al., 1968; Saltin et al., 1968). Firstly, with regards to "central" adaptations, plasma volume expansion (Bass et al., 1955; Senay et al., 1976; Nielsen et al., 1993) and to a lesser extent, increased red cell volume (Scoon et al., 2007; Ronnestad et al., 2020), additively augment total blood volume following heat acclimation. An increase in total blood volume would independently increase end-diastolic volume and stroke volume via the Frank-Starling mechanism (Kanstrup & Ekblom, 1982; Convertino et al., 1991). Indeed, Lorenzo et al. (2010) suggested that an increased plasma and blood volume supported the greater maximal cardiac output (and thus $\dot{V}O_{2max}$) that they observed following heat acclimation. Likewise, Minson & Cotter (2016) proposed that plasma volume expansion is the fundamental mechanism driving ergogenic effects of heat acclimation, given that Scoon et al. (2007) observed strong correlations between improved running time-to-exhaustion performance and plasma volume expansion post-acclimation (~10 intermittent post-exercise sauna sessions over 3 weeks).

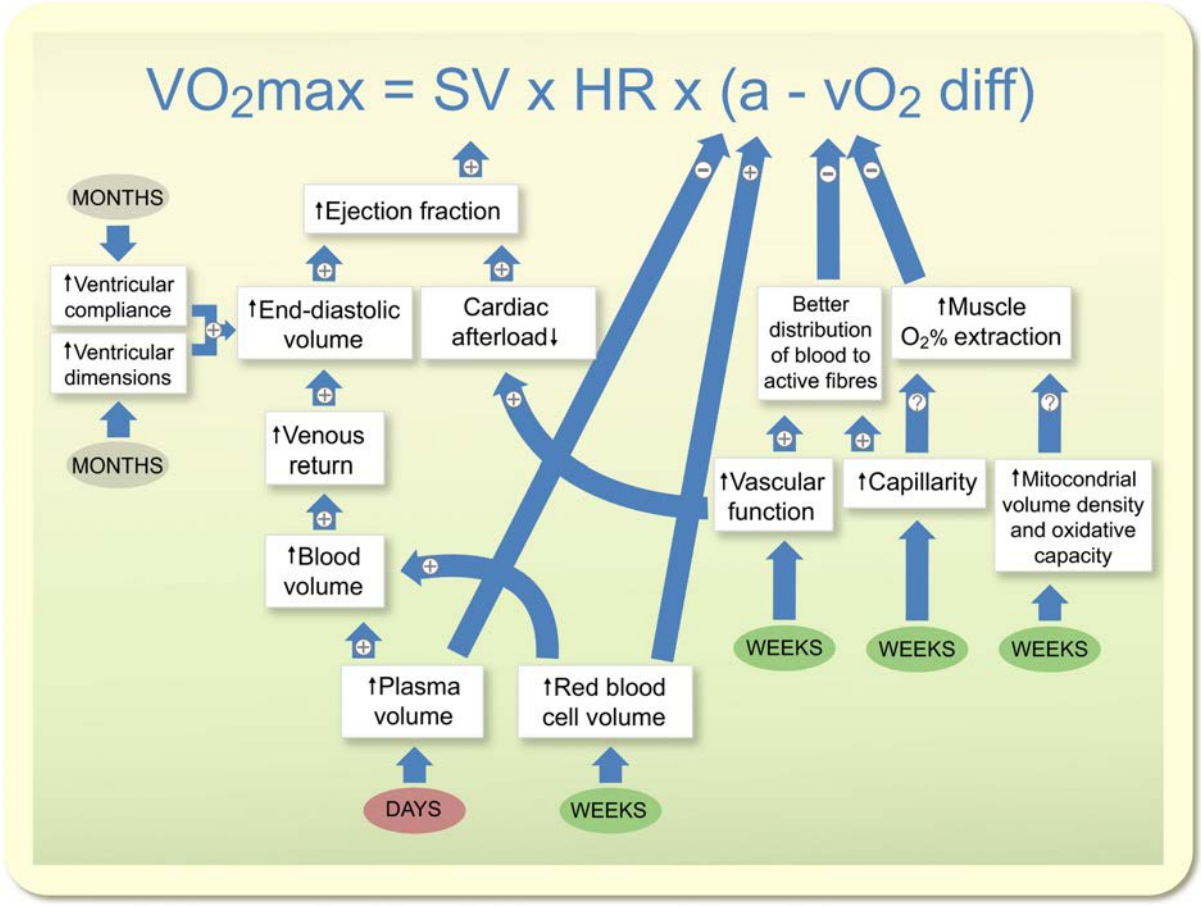


Figure 2.22 Physiological adaptations underlying improvements in maximal aerobic capacity ($\dot{V}O_{2max}$) with exercise training are similar to those proposed to underlie improvements with heat acclimation. Adaptations can contribute to "central" (cardiac output: stroke volume [SV] x heart rate [HR]) and/or "peripheral" (arterio-venous oxygen difference [a−vO_2 diff]) elements of $\dot{V}O_{2max}$. Taken from Lundby et al. (2017).

The increases in red cell mass observed following chronic heat exposure (Scoon et al., 2007; Ronnestad et al., 2020) would be expected to offset the haemodilution that accompanies plasma volume expansion, improve oxygen delivery, and thus improve $\dot{V}O_{2max}$ following heat acclimation. Infusion of red blood cells has previously been observed to independently increase $\dot{V}O_{2max}$ and time trial performance, even in trained athletes (Ekblom et al., 1972; Bejder et al., 2019). An augmented red cell mass did not, however, appear to independently increase $\dot{V}O_{2max}$ following LTHA, as Ronnestad et al. (2020) reported that whilst 5 weeks of heat acclimation increased haemoglobin mass more so than RPE- and duration-matched exercise in cool conditions, $\dot{V}O_{2max}$ improved equally in both groups. Moreover, Scoon et al. (2007) observed that increases in red cell mass were not correlated with performance improvements following heat acclimation (though plasma volume expansion was). Thus, increased blood volume, primarily via plasma volume expansion, is likely the most important "central" adaptation for improving $\dot{V}O_{2max}$ in cool conditions.

Plasma volume expansion may only be ergogenic for team sport and sub-elite endurance athletes, not elite endurance athletes (Minson & Cotter, 2016; Nybo & Lundby, 2016). Increases in red cell mass probably take at least three weeks (Girard et al., 2013), and thus plasma volume expansion during active heat acclimation protocols lasting 1-2 weeks likely cause some degree of haemodilution. This “trade-off” of slight haemodilution for increased cardiac output is still a net benefit for team sport and sub-elite endurance athletes with sub-optimised haematological, myocardial and vascular function (Hopper et al., 1988; Minson & Cotter, 2016). However, elite endurance athletes may already be approaching the limit of their diastolic reserve capacity (Warburton et al., 1999), whereby further plasma volume expansion probably does not further increase stroke volume and cardiac output (Hopper et al., 1988). Therefore, elite athletes may be at an optimal, hypervolemic blood volume at which any further plasma volume expansion and ensuing haemodilution causes a reduced oxygen carrying capacity. As a consequence, this “trade-off” has nullifying effects or even a net detriment (Keiser et al., 2015; Neal et al., 2016b). The optimal balance of cardiovascular benefits and haemodilution varies on an individual basis (Racinais et al., 2012), which indicates that some individuals could benefit from plasma volume expansion, whilst others may not. Overall, enhanced haemodynamic outcomes due to plasma volume expansion perhaps only consistently augment $\dot{V}O_{2max}$ in cool conditions in sub-elite athletes.

Waldron et al. (2019) refute the popular emphasis on central cardiovascular factors for facilitating the increase in $\dot{V}O_{2max}$ following heat acclimation (or at least following short- and medium-term heat acclimation). These authors demonstrated a marked suppression of $\dot{V}O_{2max}$ at 5- and 10-days heat acclimation as compared to baseline, amid the ~9% plasma volume expansion and reduction in exercising heart rate present at both 5 and 10 days heat acclimation (**Figure 2.23**). Thus, the authors contend that improved $\dot{V}O_{2max}$ following heat acclimation cannot be independently due to plasma volume expansion. Instead, they proposed that peripheral factors, namely improved perfusion and oxygen extraction at the working muscles (i.e. increasing arterio-venous oxygen difference), increase $\dot{V}O_{2max}$ following heat acclimation. There are three plausible pathways to increase oxygen extraction at $\dot{V}O_{2max}$. The first is through improved vascular function for better skeletal muscle perfusion and increased absolute muscle $\dot{V}O_2$. Improved vascular function could influence a main limiting factor of $\dot{V}O_{2max}$, since leg blood flow exhibits a severe drop-off when approaching exhaustion during $\dot{V}O_{2max}$ tests in both hot and cool conditions (Gonzalez-Alonso & Calbet, 2003). Evidence of this adaptive pathway is supported by data from Lorenzo and Minson (2010), who observed improved *cutaneous* microvasculature function (indexed by cutaneous vascular conductance in response to acetylcholine microdialysis at rest) following 10 days of active heat acclimation. Although Lorenzo and Minson (2010) observed these functional improvements at the cutaneous microvasculature, data from rats (Laughlin et al., 2004;

McAllister et al., 2005) indicate that the greatest increases in vascular function (at least with exercise training) are specific to the most active muscle fibres, which are presumably experiencing the greatest sheer stress (Padilla et al., 2011). Considering the high metabolic (and thus skeletal muscle blood flow) demands of exercise-heat stress (Febbraio et al., 1994a), it is possible that heat acclimation causes similar microvasculature functional adaptations at the active muscles as at the skin. The second plausible pathway for increased oxygen extraction is via increased capillary density (Hesketh et al., 2019; Kim et al., 2020). Increased capillary density around the active muscle fibres allows for a longer mean transit time and greater surface area for O_2 extraction (Saltin et al., 1986) and thus a great *relative* O_2 extraction from the blood. This is a particularly viable peripheral pathway for improved $\dot{V}O_{2max}$ following short- and medium-term heat acclimation, since Zhou et al. (1998) reported capillary growth within just 7-14 days of drug-induced sheer stress in rats. Data from Waldron et al. (2020) also support these two vascular adaptive pathways, since they observed increased leg blood flow during reactive hyperaemia at rest following short-term active heat acclimation (5-days of 60 min at 55%$\dot{V}O_{2max}$ in 36°C, 40%RH).

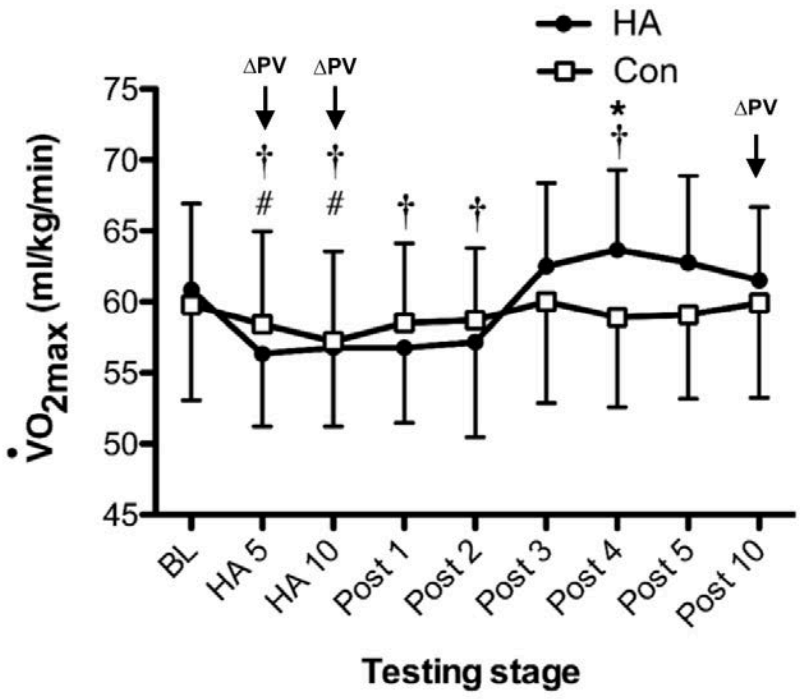


Figure 2.23 Changes in $\dot{V}O_{2max}$ in 20°C across 5 and 10 days of cycling at 50% $\dot{V}O_{2max}$ in 38°C (heat acclimation, HA; *n*=12) or 20°C (control, Con; *n*=10) plus 10 days of normal training post-intervention (Post 1, 2, 3, 4, 5 and 10). *Significant difference between groups at time-point (p<0.05); †Significant difference from baseline (BL) in HA group (p<0.05); #Significant difference from BL in Con group (p<0.05). Taken from Waldron et al. (2019). Arrows added to denote time-points when plasma volume was measured and was significantly elevated (+8-10%) from baseline in the HA group (p<0.05).

Lastly, increased oxygen extraction could be related to mitochondrial adaptations. Heat-mediated increases in signalling for (and eventual synthesis of) mitochondrial protein

content and for improved mitochondrial function have been previously observed (Tamura et al., 2014; Hafen et al., 2018; Ihsan et al., 2020). However, improved aerobic capacity following 10 days of heat acclimation has been observed without any changes in proteins for mitochondrial biogenesis or content in the skeletal muscle (Mang et al., 2020), which indicates that adaptations at the mitochondria are not a key mechanism responsible for rapid improvements in $\dot{V}O_{2max}$ following heat acclimation. Indeed, mitochondrial oxidative capacity typically exceeds O_2 delivery at maximal intensities during graded exercise tests (Boushel et al., 2011), and is therefore unlikely to be a limiting factor in $\dot{V}O_{2max}$, *per se*. Taken together, peripheral factors facilitating improved $\dot{V}O_{2max}$ following heat acclimation are probably related to vascular adaptations that improve muscle perfusion and not mitochondria function and/or content.

Alongside improvements in maximal aerobic capacity, Lorenzo et al. (2010) perhaps demonstrated the most holistic collection of improvements to performance parameters following heat acclimation to date (**Figure 2.24**). Following 10 days of heat acclimation (2 x 45 min bouts of cycling at 50%$\dot{V}O_{2max}$ in 40°C, 30%RH), participants improved $\dot{V}O_{2max}$ (+4.9%), power at 4 mmol·L^{-1} [La^-] (+5.1%) and work done during a 60-min time trial (+6.2%) in cool conditions (13°C, 30%RH). None of the improvements listed were observed in a control group (10 days cycling in 13°C, 30%RH) of cyclists from the same club.

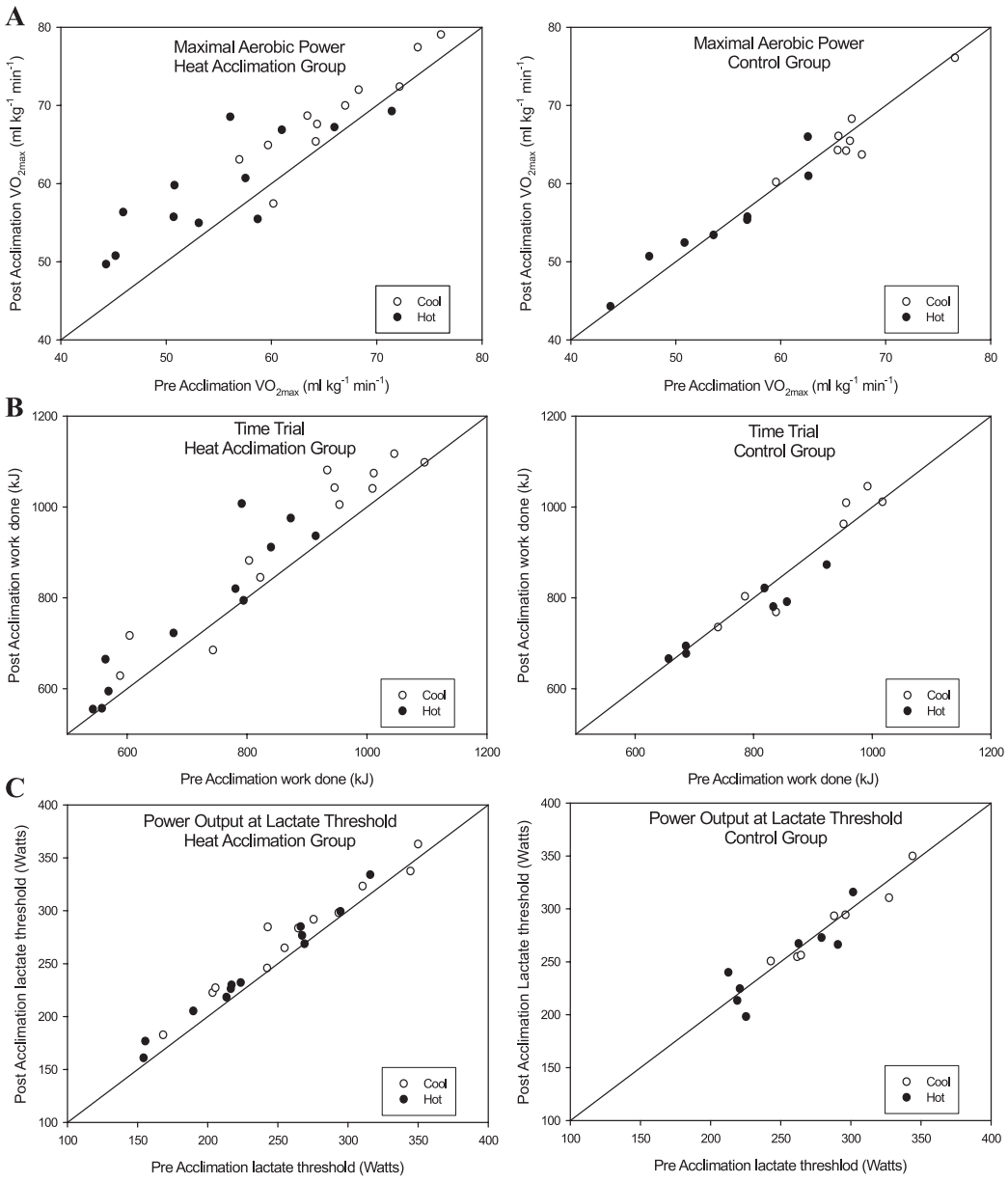


Figure 2.24 Individual data for the relationship between pre- and post-heat acclimation (10 days of 90 min cycling at 50%$\dot{V}O_{2max}$ in 40°C, 30%RH) in performance variables of heat acclimation (*left*) and control (*right*) groups under hot (38°C, 30%RH) and cool (13°C, 30%RH) conditions. **A**: maximal aerobic power ($\dot{V}O_{2max}$); **B**: 60-min time trial performance; **C**: lactate threshold (power at 4 mmol·L^{-1} blood lactate concentration). Straight line represents line of equality. Taken from Lorenzo et al. (2010).

Time trial performance is modified and limited by numerous factors, with aerobic capacity being only one of many (Jacobs et al., 2011). Thus, improvements in cool or temperate time trial performance following heat acclimation have been observed alongside (Lorenzo et al., 2010; Ronnestad et al., 2020) as well as in the absence of improvements to $\dot{V}O_{2max}$ (Neal et al., 2016a; Mikkelsen et al., 2019). Barley and colleagues (2019) investigated the effects of 12 sessions of passive heating via infrared sauna on repeated sled-push performance, whereby participants were instructed to push a weighted sled 10 m

back and forth for 30 repetitions as quickly as possible, giving maximal effort during each push. Following heat acclimation, the total test time was 2.3% faster and mean sled-push effort was 9.8% faster, however, there was no control group in this study. Similarly, Hue et al. (2007) reported a 10% increase in 400-m swim time and McCleave et al. (2017) reported a 3.3% increase in 3-km run time. Although both studies observed these improvements at 3-4 weeks post-acclimation, there were no such improvements in their respective control groups. Conversely, others have shown trivial (i.e. <2.0%; Morrison et al., 2002; Creasy et al., 2003; Neal et al., 2016b; McCleave et al., 2017), similar (Mikkelsen et al., 2019; Ronnestad et al., 2020) or no improvements (Bradford et al., 2015; Karlsen et al., 2015; Keiser et al., 2015; Zurawlew et al., 2016) in time trial performance following heat acclimation, as compared to their respective control groups.

Lactate (or ventilatory) thresholds have been observed to be well-correlated with time trial performance (Fay et al., 1989; Bassett & Howley, 2000; Lucia et al., 2004; Lorenzo et al., 2011). Importantly, Lorenzo et al. (2011) showed strong correlations and a similar mean bias between lactate threshold and time trial performance before *and* after heat acclimation (Lorenzo et al., 2011). This indicates that an improvement in lactate threshold in cool conditions following heat acclimation is a suitable proxy for predicting changes in time trial performance. Moreover, lactate (or ventilatory) threshold tests could be a more accurate indicator of physiological changes underpinning exercise performance improvements, as such tests are free of psychological or motivational factors that can sometimes confound laboratory-based time trials (Corbett et al., 2018b). Interestingly, improved power/speed at lactate thresholds (of various definition; further discussion on determination of “lactate threshold” in **Section 3.7.5**) and ventilatory thresholds have been observed following only 5-6 heat exposures (Neal et al., 2016a; Perrotta et al., 2018; Tebeck et al., 2019; Waldron et al., 2020). Likewise, following 10 days of heat acclimation, Lorenzo et al. (2010) and Willmott et al., (2018) reported 4-5% improvements in power at lactate threshold (defined as 4 $mmol \cdot L^{-1}$ [La^-] and 1 $mmol \cdot L^{-1}$ [La^-] increase from rest, respectively).

The specific mechanism(s) that underpin an improved lactate threshold following heat acclimation have not been delineated, but it is likely that a myriad of adaptations contribute. Firstly, as these exercise thresholds are largely determined by skeletal muscle oxidative capacity (Jacobs et al., 2011), recent evidence of heat acclimation-related increases/improvements in mitochondrial biogenesis and function could largely explain reduced blood lactate levels (Hafen et al., 2018; Hafen et al., 2019; Ihsan et al., 2020). Indeed, as little as 6 days of heat acclimation (100 min walking in 49°C, 20%RH) is sufficient to improve exercise efficiency (i.e. lowered metabolic rate) in both males and females by ~5% during temperate exercise (20°C, 40%RH; Sawka et al., 1983a). This timeline corresponds to the increases in PGC-1α and mitochondrial electron transport protein

complexes after just 6 days of passive heating, as observed by Hafen et al. (2018); as well as increased metabolic efficiency (indexed by muscle $\dot{V}O_2$ during reactive hyperaemia at rest) following 5 days of active heat acclimation, as observed by Waldron et al. (2020). However, mitochondrial oxidative capacity is likely one of a number of factors. It is also possible that the improved efficiency during exercise is due to a shift in motor unit recruitment patterns towards more efficient, slow-twitch fibres (Sawka et al., 1983a). Moreover, if heat acclimation does increase maximal aerobic capacity and maximal muscle strength (Goto et al., 2011; Racinais et al., 2017b), post-acclimation submaximal workloads will be performed at a lower *relative* exercise intensity if the absolute workload is matched. Equally, an augmented cardiac output would support splanchnic circulation and consequently enhance lactate removal (Lorenzo et al., 2010). Thus, it seems most probable that these factors collectively and additively enhance speed/power at submaximal exercise thresholds, such as lactate or ventilatory thresholds.

Other authors have reported no change in lactate or ventilatory thresholds with short- or medium-term heat acclimation (Neal et al., 2016b; Mang et al., 2020) or trivial changes that were not superior to those exhibited by a control group (Chalmers et al., 2016; Gollan et al., 2017). Interestingly, Mang et al. (2020) reported increased $\dot{V}O_{2max}$ following heat acclimation without changes in either the first or second ventilatory threshold or increases in markers of mitochondrial biogenesis or function. Thus, perhaps the increase in the performance "ceiling" (i.e. $\dot{V}O_{2max}$) has a lesser role in improving submaximal exercise thresholds following heat acclimation than does improved peripheral factors related to oxidative capacity. This is a reasonable suggestion, as peripheral factors primarily moderate improvements in submaximal exercise performance markers (i.e. lactate/ventilatory thresholds) with endurance training (Holloszy & Coyle, 1984). Still, the possibility remains that reduced blood lactate concentration for a given submaximal intensity is merely an effect of haemodilution due to plasma volume expansion and not improvements in mechanisms responsible for lactate production or clearance, *per se*.

Recently, Tebeck et al. (2019) highlighted that different environmental conditions (i.e. hot-wet *vs* hot-dry) could elicit different performance outcomes in temperate conditions (21°C, 53%RH). Tebeck et al. (2019) suggested that heat acclimation using hot-wet (32°C, 80%RH) conditions was better for driving improvements to lactate threshold as compared to in hot-dry (43°C, 20%RH) conditions (+4% *vs* 0% improvement in power output at 4 $mmol \cdot L^{-1}$, respectively). However, none of the studies discussed above (either supporting or refuting improvements in lactate or ventilatory thresholds) have utilised particularly humid conditions for heat acclimation (all ≤50%RH). Thus, the conflicting outcomes in the literature cannot be explained by environmental conditions.

Lastly, time-to-exhaustion trials also represent a measure of exercise performance, despite their relative variability when compared to other performance measures (Hopkins et al., 2001; Laursen et al., 2007). Time-to-exhaustion protocols may include constant workloads, intermittent work-rest or peak power attained in a graded exercise test. Time-to-exhaustion protocols can also be modified to be more team sport-specific (e.g., Yo-yo or 30-15 Intermittent Running Tests). As with other measures of exercise performance in cooler conditions following heat acclimation, there are conflicting findings in the literature. A number of studies report improvements in the range of 2-8% (Sawka et al., 1985; Neal et al., 2016a; Neal et al., 2016b; Sotiridis et al., 2019; Waldron et al., 2020), with some reporting a larger magnitude of improvement (i.e. >15%; Scoon et al., 2007; Buchheit et al. 2012; Glazachev et al., 2020). For example, Scoon et al. (2007) observed a 33% increase in running time-to-exhaustion at participants' approximate best 5-km race-pace (although this was calculated to translate to ~2% increase in time trial performance) following 3 weeks of post-exercise sauna bathing. Buchheit et al. (2013) observed a 25% and 44% increase in the Yo-Yo Intermittent Run Test following 5 and 10 football-specific heat acclimatisation sessions, respectively. Although Pethick et al. (2018) reported only a 1.5% improvement in the 30-15 intermittent running test in female team sport athletes following 5 days of circuit training in the heat (35°C, 37%RH), the authors maintain that this was a meaningful improvement. These results represented the team's best performance to date in a highly familiar test, which required a "smallest worthwhile change" of just 0.6%. Philp et al. (2017) also tested games-players using the 30-15 Intermittent Running Test following 5 days of heat acclimation, although heat acclimation consisted of 50-min constant-load cycling at 70%HR reserve in 35°C, 56%RH. Contrary to findings by Pethick et al. (2018), Philp et al. (2017) observed only a 0.5% improvement, which was similar to the control group after training in 15°C. Mackay et al. (2018) also found similar changes in time-to-exhaustion following short-term heat acclimation in their heat acclimation and control groups. Whilst Willmott et al. (2018) did observe increased $\dot{V}O_{2max}$ and lactate threshold as compared to a temperate control group, they found no significant improvements in time-to-exhaustion following 10 heat acclimation sessions (60 min isothermic cycling in 45°C, 20% RH) completed either on every weekday or twice daily on every other weekday. Willmott et al. (2018) and others (Chen et al., 2013; Karlsen et al., 2015; Perrotta et al., 2018; Mikkelsen et al., 2019; Travers et al., 2020b) also did not observe any effects of heat acclimation on peak power or time-to-exhaustion during a graded exercise test.

Nine of the 42 studies investigating performance outcomes summarised in **Table 2.3** included female participants. Four studies (Lorenzo et al., 2010; Gollan et al., 2017; McCleave et al., 2017) included less than 3 female participants in the heat acclimation intervention group. Mang et al. (2020) included 6 female participants (though their results

were pooled with 7 males). Four studies (Fortney & Senay, 1979; Bailey et al., 2016; Perrotta et al., 2018; Pethick et al., 2018) included separate female cohorts. However, only the female cohorts in Pethick et al. (2018) and Perrotta et al. (2018) were trained, limiting the amount of information on heat acclimation's ergogenic potential for female athletes as compared to males. There are reasons to believe that the outcomes related to temperate exercise performance following heat acclimation may be different in females. Firstly, if improvements in aerobic capacity are to be credited in part to increased haemoglobin mass (Jacobs et al., 2011), female athletes may need to consider using iron supplementation. Adequate iron levels are required for altitude-induced increases in haemoglobin mass (Govus et al., 2015), however female athletes are prone to iron deficiency (with or without anaemia; McClung et al., 2014). Assuming heat and altitude-mediated increases in haemoglobin mass share a similar induction pathway (i.e. stabilisation of HIF-1α for downstream elevations of EPO and eventually red cell mass, as discussed in **Section 2.4.3**), iron supplementation is likely to be especially beneficial for haematological adaptations in females undertaking heat acclimation.

Trained females already exhibit a higher arterio-venous difference at the attainment of $\dot{V}O_{2max}$ as compared to trained males (Wiebe et al., 1998), and thus there may be less reserve for peripheral improvements, especially following STHA. Alternatively, females, who typically have lower blood volumes than males (Wiebe et al., 1998), may have a greater capacity for central cardiovascular adaptations to improve $\dot{V}O_{2max}$. With regards to time trial performance and submaximal exercise thresholds, trained females already exhibit greater mitochondrial volume density than trained males (Montero et al., 2018), a main physiological determinant of speed at 4 $mmol \cdot L^{-1}$ $[La^-]$ (Holloszy & Coyle, 1984). Overall, there is a dearth of information regarding females' exercise performance responses to heat acclimation, and very little literature to date provides evidence of heat acclimation's ergogenic potential in females.

2.8 Summary

This review of the literature has described the acute and chronic physiological responses to heat stress, including active and passive heating. Throughout, this review has considered how sex differences may affect these acute physiological responses, and subsequently, adaptation and performance responses to exercise-heat stress. Additionally, this review compared and contrasted the evidence for the time course of heat acclimation adaptations and select emerging methods and aspects of heat acclimation. This included short- and long-term heat acclimation, as well as decay of heat acclimation adaptations. It examined pragmatic methods of inducing heat acclimation such as post-exercise sauna

bathing. Is also examined the evidence for the ergogenic benefits of heat acclimation for exercise performance in cooler conditions.

2.9 Research questions arising from the review of the literature

This review of the literature has identified a number of knowledge gaps and avenues of investigation within the broad topics of heat acclimation and female athlete performance. Self-paced exercise performance responses to active heat acclimation have not previously been investigated in females. Investigation of this response is warranted, because there is a myriad of physiological sex differences that may modify the adaptive response to active heat acclimation, and specifically short-term active heat acclimation, in females. Furthermore, understanding of emerging, pragmatic heat acclimation methods such as post-exercise sauna bathing is incomplete and has not previously been investigated in a female cohort. There is considerable debate surrounding the potential for heat acclimation to be an ergogenic aid for performance in cool or temperate conditions. However, studies including female participants are limited, and thus female athlete responses have been largely omitted from this debate. Therefore, the overall aim of the series of studies in the current thesis was to contribute to the literature surrounding emerging methods of heat acclimation that are more practical than traditional, active heat acclimation (i.e. short-term heat acclimation and post-exercise sauna bathing) and the potential for heat acclimation as an ergogenic aid, with a focus on the female athlete. This information will be useful to both female and male athletes, practitioners and military and occupational personnel, as well as all those proposing relevant policy and recommendations for these populations. Specific study aims and hypotheses for **Chapters 4-8** are as follows:

Chapter 4: Nine-, but not four-days heat acclimation improves self-paced endurance performance in females

Aim: To investigate self-paced endurance performance in hot conditions following 4 days (STHA) and 9 days of a high-intensity exercise, isothermic heat acclimation protocol with permissive dehydration in a female cohort.

Hypotheses: Females will not exhibit performance improvements in self-paced exercise following STHA, as previous studies indicate that 4 days of 90-min heat acclimation is unlikely to be a sufficient stimulus for the thermoregulatory and cardiovascular adaptations necessary for performance improvements in the heat. Therefore, it is further hypothesised

that performance improvements in power output and time trial distance will occur following 9 days of heat acclimation.

Chapter 5: Is active heat acclimation ergogenic for performance in cool conditions in females?

Aim: To investigate self-paced endurance performance in cool conditions following 5 and 10 days of a high-intensity exercise, isothermic heat acclimation protocol with permissive dehydration in a female cohort.

Hypotheses: Females will exhibit performance improvements in self-paced exercise following 10, but not 5 days of heat acclimation, as previous literature (Mee et al., 2015b) indicates that 5 days of 90-min heat acclimation is unlikely to be a sufficient stimulus for the thermoregulatory and cardiovascular adaptations that could underpin performance improvements in cool conditions.

Chapter 6: Intermittent post-exercise sauna bathing improves markers of exercise capacity in hot and temperate conditions in trained middle-distance runners

Aim: To determine the efficacy of adding intermittent post-exercise sauna bathing to normal endurance training in order to induce heat acclimation adaptations in trained athletes, and to determine the impact of these adaptations on maximal and submaximal exercise performance markers in temperate conditions. The subsidiary aim was to determine the efficacy of a long-term intermittent post-exercise sauna bathing protocol to further improve heat acclimation adaptations.

Hypotheses: Intermittent post-exercise sauna bathing will: i) elicit hallmark heat acclimation adaptations and improve thermoregulation during exercise-heat stress more than endurance training alone, and ii) improve physiological markers of exercise performance in temperate conditions to a greater extent than endurance training alone.

Chapter 7: Sex differences in adaptation to intermittent post-exercise sauna bathing in trained middle-distance runners

Aim: To investigate the effect of sex on the efficacy of intermittent post-exercise sauna bathing to induce heat acclimation and improve markers of maximal and submaximal exercise performance in temperate conditions in trained athletes.

Hypotheses: An acute bout of post-exercise sauna bathing will be more physiologically challenging in females. However, despite this greater thermoregulatory strain, 3 weeks of post-exercise sauna bathing will be more effective in males for inducing desirable adaptations during both exercise-heat stress and temperate exercise.

Chapter 8: Heat acclimation is partially retained for two weeks following three weeks of intermittent post-exercise sauna bathing

Aim: To determine whether a 3-week post-exercise sauna bathing intervention would induce heat acclimation adaptations that would be retained for 2 weeks without exogenous heat exposure.

Hypotheses: Three weeks of post-exercise sauna bathing will induce heat acclimation adaptations that will be partially lost (i.e. decay at a rate of ~2.5% per day) after 2 weeks without post-exercise sauna bathing, equating to ~35% decay, in line with current literature on heat acclimation decay.

3. GENERAL METHODS

3.1 Ethical approval

Each study received ethical approval by the University of Birmingham's Science, Technology, Engineering and Mathematics Ethical Review Committee (**Chapters 4 and 5**: ERN_17-0019; **Chapters 6-8**: ERN_18-0958) and conformed to the standards set by the Declaration of Helsinki (2013). All participants were informed of the experimental procedures and possible risks involved in the study before providing verbal and written consent.

Experimental sessions were stopped if any of the following criteria were met:

i) The participant asked to stop the test or did not wish to proceed with the test. Participants were not required to provide a reason for this
ii) A member of the research team felt it appropriate to stop the test (technical or equipment problems or participant appeared ill or overly uncomfortable)
iii) A participant's rectal temperature reached, or would clearly soon reach the 39.5°C safety limit set by the Ethical Review Committee

3.2 Participants

Healthy, moderately to well-trained female and male participants were recruited between March 2017 and December 2019. A total of 89 participants were recruited for the studies detailed in the experimental chapters; 9 for the studies involving active heat acclimation (**Chapters 4 and 5**), and 80 for the studies involving post-exercise sauna bathing (**Chapters 6-8**). However, of those recruited, 8 and 41 participants were included in the respective series of studies, after participant dropout due to various reasons (injury, scheduling, etc.). An overview of the progression of participants through each study is presented in **Figure 3.1**. More details of the participants' progression, as well as relevant participant characteristics are detailed in each of the experimental chapters.

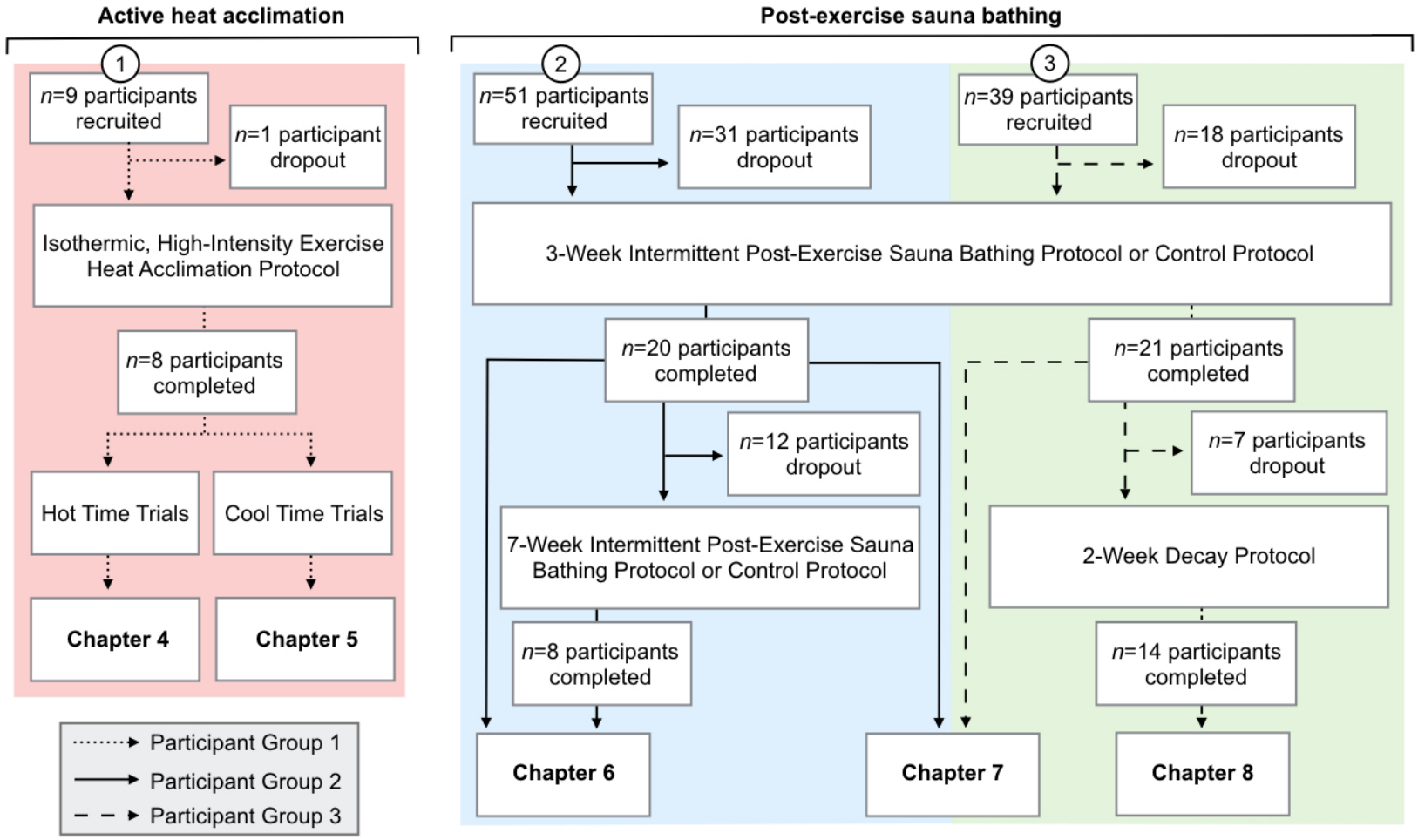


Figure 3.1 Overview of the progression of participants through the five data chapters of the current thesis (**Chapters 4-8**). Participants were recruited in three distinct groups. The first participant group completed the isothermic, high-intensity exercise heat acclimation protocol with hot and cool time trials. Their data were included in the studies in **Chapters 4** & **5**. The second participant group completed the 3-week intermittent post-exercise sauna bathing protocol or control, followed by an additional 4 weeks of sauna bathing or control, to complete the 7-week intermittent sauna bathing protocol. Their data were included in the studies in **Chapter 6**. The third participant group completed the 3-week intermittent post-exercise sauna bathing protocol or control, followed by the 2-week decay protocol. Their data were included in the studies in **Chapter 8**. **Chapter 7** pooled data from participants in the second and third participant groups to investigate sex differences following 3 weeks of intermittent post-exercise sauna bathing or control.

3.3 General participant instructions

Participants completed a general health questionnaire at the commencement of each study. Participants were not exposed to hot ambient conditions either through natural acclimatisation or through means of a heat acclimation protocol for at least one month prior to beginning any of the studies detailed herein. Before experimental testing sessions, participants were advised to arrive well-hydrated and avoid caffeine ≥4 h before arrival, as well as overly exhaustive exercise and alcohol consumption ≥24 h before arrival. Participants were asked to recall the food they had eaten prior to experimental tests, and to repeat the same diet when re-tested. Participants were to consider heat acclimation in either a complimentary fashion to their normal training (**Chapters 4** and **5**) or in addition to maintaining their normal training (**Chapters 6-8**), in agreement with current recommendations (Corbett et al., 2014).

3.3.1 Iron supplementation

Participants took daily iron tablets (65mg ferrous sulfate; Nature Made, West Hills, CA, United States) from two weeks prior to the first experimental trial until the completion of the protocols in **Chapters 6-8**. Participants were instructed to take iron supplements first thing in the morning on an empty stomach. Compliance was monitored by asking participants how many iron tablets they had left. Since iron supplements are equally effective when taken each day or every other day (Düzen Oflas et al., 2020), participants that reported missing a few days' supplementation were not cause for concern for the efficacy of the supplement.

Participants took iron supplements for two main reasons. Firstly, high physical activity levels are known to reduce serum ferritin, especially in female athletes (Malczewska et al., 2001; Fallon, 2008; Parks et al., 2017). This could inhibit performance improvements from endurance training (Garvican et al., 2011). Second, iron supplementation is known to be effective for facilitating haematological adaptations when training at altitude (Govus et al., 2015). Haematological adaptations to long-term heat acclimation interventions may overlap with that of altitude training (see **Section 2.4.3**), and thus may be aided by iron supplementation. Participants also took iron supplements two weeks prior to the first experimental trial in accordance with current literature on altitude training (Garvican-Lewis et al., 2018).

3.4 Environmental conditions

3.4.1 Environmental chamber

Ambient temperature and humidity were controlled using an environmental chamber (TIS Services, Hampshire, United Kingdom) at the University of Birmingham during active heat acclimation (**Section 3.7.2.1**), time trials (**Section 3.7.2.2**) and running heat tolerance tests (**Section 3.7.2.4**). Conditions were maintained between 15-40°C and 30-40%RH, and front-facing, fan-generated airflow was ~3-4 $m \cdot s^{-1}$, depending on the experimental test.

3.4.2 Human Performance Laboratory

Temperature was controlled using a commercial air conditioning system at ~18°C, and front-facing, fan-generated airflow was ~3 $m \cdot s^{-1}$ for tests conducted in the Human Performance Laboratory at the University of Birmingham, including tests of maximal oxygen consumption (**Section 3.7.1.1**), time to exhaustion (**Section 3.7.1.3**) and lactate profile (**Section 3.7.1.4**).

3.4.3 Sauna

Saunas used for post-exercise sauna bathing (**Section 3.7.2.3**) were housed in the University of Birmingham Sport and Fitness Centre. The air in the sauna was heated using electric heating elements, and temperature at 1.8 m was recorded at each post-exercise sauna bathing session using a commercially available thermometer mounted to the wall (101-108°C). Additionally, temperature and humidity were recorded at chest (~86°C at ~1 m) and seat (~60°C at ~0.6 m) height using an iButton Hygrochron Logger (Maxim Integrated, California, USA).

3.5 Anthropometric measures

Height was measured using a Stadiometer (Seca 217, Seca, Hamburg, Germany). Nude body mass (NBM) was measured to 0.1 kg precision using digital scales (Seca 877, Seca, Hamburg, Germany). Body mass on maximal oxygen consumption and lactate profile test days (**Sections 3.7.6** and **3.7.5**, respectively) was measured to 0.1 kg precision using digital scales, whilst participants stood in socks and shorts (males) and a sports bra (females). Body surface area (BSA) was calculated using the formula derived by Du Bois & Du Bois (1916), which is as follows:

$$BSA = 0.007184 \cdot Height^{0.725} \cdot Weight^{0.425}$$

Forearm volume for the purpose of normalisation of forearm blood flow (**Section 3.9.5**) was measured using the water displacement method, whereby participants first inserted their hand (up to the wrist) into a fully filled container of water. Water overflow was subsequently discarded. Participants then continued to insert their forearm (up to the crease of the elbow) into the container, and the quantum of water that overflowed through the spill tube (measured to 1 g) was deemed the equivalent of forearm volume. The water displacement method is generally preferred for both feasibility and accuracy in reporting limb volumes, when compared to methods based on circumferential measures such as Computed Tomography or Magnetic Resonance Imaging (Chromy et al., 2015).

3.6 Menstrual cycle questionnaire and self-reported log

Before commencing experimental sessions, female participants were asked to complete a menstrual cycle questionnaire, detailing last known cycle start dates, cycle regularity, adverse menstrual or pre-menstrual symptoms and hormonal contraceptive use (**Appendix 1**). Female participants reported that they did not experience any negative menstrual or pre-menstrual symptoms that may have affected performance, or otherwise were not scheduled for experimental tests near menstruation (Giacomoni et al., 2000). Additionally, female participants were asked to self-report menstruation throughout the duration of the protocols.

3.7 Experimental procedures

3.7.1 Active heat acclimation

Participants performed all active heat acclimation and experimental testing sessions detailed in **Chapters 4 & 5** at the same time of day (±2 h), and at similar times to their normal training sessions so as not to disrupt their normal circadian rhythms (Reilly & Brooks, 1986). This included mornings, afternoons or evenings (start times ranging from 7:00-19:00). A combination of high-intensity intervals, permissive dehydration and isothermic heat acclimation (Sunderland et al., 2008; Garrett et al., 2014) was used to construct a “high-intensity” active heat acclimation protocol. Conditions in the environmental chamber during active heat acclimation were 40°C, 30%RH with a fan-generated airflow of ~3 $m \cdot s^{-1}$ facing participants (MIW Office Solutions, County Durham, UK; **Figure 3.2**). Sessions were completed on the Velotron cycle ergometer (**Section 3.8.1**). Following a 5-min, self-selected

intensity warm-up, participants completed 15 min of high-intensity intervals, where participants were asked to give maximum effort for 15 s, with 45-s active recovery. The aim of the high-intensity intervals was to rapidly increase T_{rec}. This was followed by an additional 75-min continuous cycling at an intensity manipulated with the aim to further increase T_{rec} and maintain it at ~38.5°C (Patterson et al., 2004b; Garrett et al., 2012), totalling 90-min heat acclimation plus 5-min warm-up. On days that time trials (**Section 3.7.2**) preceded heat acclimation sessions, the time trial was used in place of the high-intensity intervals. On these test days, the temperature of the environmental chamber was immediately increased to 40°C, 30%RH following the time trial. Participants were instructed to refrain from fluid consumption as much as could be tolerated during active heat acclimation sessions to induce the added stressor of dehydration (i.e. permissive dehydration; Garrett et al., 2014).

3.7.2 Time trials (Chapters 4 & 5)

Time trials were performed in the environmental chamber in hot (35°C, 30%RH; TT_{HOT}) or cool (15°C, 30%RH; TT_{COOL}) conditions with a fan-generated airflow of ~3 $m \cdot s^{-1}$ facing participants. Time trials were completed on the Velotron cycle ergometer (**Section 3.8.1**). Participants commenced a 5-min warm up at a self-selected pace before completing a 15-min, self-paced cycling time trial. Participants were aware of the time elapsed, as displayed by a stop-clock mounted to the handles of the cycle ergometer, however, they were blinded to any other physiological or performance feedback (i.e., HR, power output, distance cycled, etc.). Participants were given equal verbal encouragement by the same researchers at similar time points during the TTs. Drinking was permitted ad libitum during TTs. Time trials were selected as the performance measure for their high reproducibility (e.g. 0.8-1.6% CV for familiarised 10-km TT in recreational athletes; Borg et al., 2018). A 15-min TT was also long enough to observe a detrimental effect of the heat in the unacclimated state (~17% performance reduction in 40°C *vs* 21°C observed by Ely et al., 2010), but of a length that was not overly fatiguing, considering it would be completed six times throughout the protocol, plus two familiarisations.



Figure 3.2 Active heat acclimation was completed in hot (40°C, 30%RH) conditions on a cycle ergometer

3.7.3 Post-exercise sauna bathing (Chapters 6-8)

Participants ended their outdoor continuous, low-intensity runs (i.e. “easy runs” or “long runs”) at the sport and recreation centre, and entered the sauna as soon as possible. Participants were asked to remain in the sauna for 30 min or until they could no longer tolerate the discomfort, as per previous investigations (Scoon et al., 2007; Stanley et al., 2015). In the sauna, participants sat upright (**Figure 3.3**) and were allowed to drink water *ad libitum*. Passive heating is recommended post-exercise (Racinais et al., 2019a), as athletes begin passive heating bouts at body temperatures which have already been elevated by exercise (Ridge & Pyke, 1986; Heathcote et al., 2019), a key driver of adaptation.



Figure 3.3 Participants undergoing the post-exercise sauna bathing intervention.

3.7.4 Running heat tolerance test (Chapters 6-8)

The running heat tolerance test (HTT) was performed in the environmental chamber in hot (40°C, 40%RH) conditions with a fan-generated airflow of ~4 $m \cdot s^{-1}$ facing participants (**Figure 3.4**). Participants wore socks, shoes, and either shorts (males) or shorts and a sports bra (females). Participants could opt to wear a t-shirt (which was also worn at subsequent tests if chosen). Participants ran on a treadmill at 9 $km \cdot h^{-1}$ and 2% gradient for 30 min, as described by Mee et al. (2015a). Drinking was not permitted during the test. Participants performed the HTT at the same time of day (±2 h) pre- and post-intervention (start times ranging from 8:00-17:00).

This test was selected as it has been validated as a repeatable protocol, which provokes physiological strain within ethical safety limits in trained female and male cohorts. Mee et al. (2015a) report low coefficients of variation (CV) for main outcome measures when using similar equipment and methods as contained in the current thesis, including T_{rec} (0.3%), T_{sk} (0.4%), HR (1%) and sweat rate (9%).



Figure 3.4 Flir™ thermal camera images of a male (left) and female (centre, right) participant during the running heat tolerance test (40°C, 40%RH). The image of the male participant was taken at the 28th minute of the test, and the images of the female participant were taken at 10th (centre) and 28th minute of the test (right). Relative temperatures are indicated by the thermal palette.

3.7.5 Lactate profile (Chapters 6-8)

The lactate profile test was performed in the Human Performance Laboratory in temperate conditions (~18°C) with a fan-generated airflow of ~3 $m \cdot s^{-1}$. A schematic detailing the lactate profile test is presented in **Figure 3.5**. Participants completed a light 10-min warm-up before commencing the step-style incremental test on the treadmill (**Section 3.8.2**). Participants completed 3-min stages with 30-s stops between stages when concentration of blood lactate ($[La^-]$) was measured. The test was completed at 1% gradient (Jones & Doust, 1996) and began at a speed 4 $km \cdot h^{-1}$ slower than a recent 5-km race-pace, increasing by 1 $km \cdot h^{-1}$ for each stage thereafter. The test was terminated when $[La^-]$ exceeded 4 $mmol \cdot L^{-1}$, which occurred following 4-7 stages. The facemask was removed and the fan was switched off immediately. Participants were then seated quietly on a chair placed on the treadmill at 30 s from the cessation of exercise until 3-min post-exercise, for measurement of HR recovery (**Section 3.9.4.1**). Participants performed the temperate exercise tests (i.e. lactate profile and maximal aerobic capacity) at the same time of day (±2 h) pre- and post-intervention (start times ranging from 8:00-17:00).

The test speeds were individualised with the aim for participants to complete at least four stages, providing sufficient [La^-] data to fit a third-order polynomial curve to each individual dataset and interpolate the running speed at 4 mmol · L^{-1} [La^-]. Running speed at 4 mmol·L^{-1} [La^-] was determined to 0.01 km·h^{-1} using a custom Matlab script (Mathworks Inc, Natick, USA).

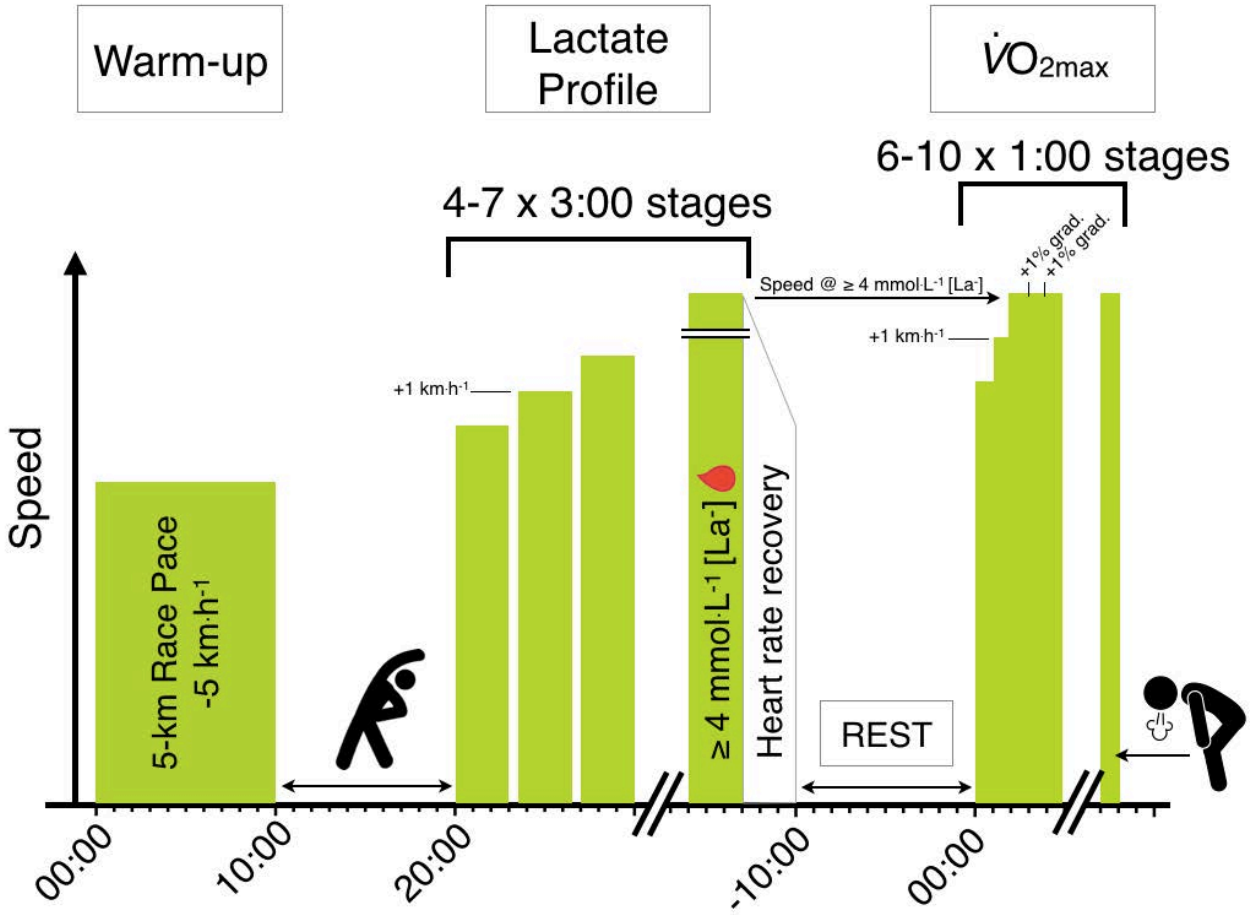


Figure 3.5 Schematic of the temperate exercise (18°C) laboratory visit. Participants completed a 10-min warm up at 5 km·h^{-1} slower than a recent 5-km race pace, stretched for 10-min and began the step-style incremental lactate profile test. The test was terminated following the attainment of blood lactate concentration ([La^-]) of ≥4 mmol·L^{-1}. After assessment of heart rate recovery and 10-min rest, participants began the test of maximal oxygen consumption ($\dot{V}O_{2max}$), which was terminated at volitional exhaustion.

Part of the rationale for the use of a 4 mmol · L^{-1} [La^-] threshold protocol was that coaching staff used this test to assess high performers in this participant cohort. Thus, it was a familiar and meaningful test to this cohort. The 4 mmol · L^{-1} [La^-] threshold was initially proposed by Mader (1976) and is sometimes referred to as the onset of blood lactate accumulation (OBLA; Svedahl & MacIntosh, 2003). However, the use of this protocol to determine a true 'lactate threshold', and therefore its usefulness, is controversial (Faude et

al., 2009). Alternative assessments of lactate threshold have been proposed, one category of which being D-max or modified D-max methods (maximal distance from [La^-] curve to the line formed by its endpoints [Cheng et al., 1992] or by the point before the first rise in [La^-] and the value at cessation of exercise [Bishop et al., 1998], respectively). However, the modified D-max method significantly underestimates 5-km race times in runners (Chalmers et al., 2015). Training aims of the running club sampled in the current thesis were largely centred around cross-country races (typically ranging between 4-6 km), and thus, D-max methods were less meaningful for the cohort than running speed at 4 mmol $\cdot$ L^{-1} [La^-]. Indeed, Fay et al. (1989) observed that running speed at 4 mmol $\cdot$ L^{-1} [La^-] in female endurance athletes was almost exactly the same as 5-km race-pace (15.47±1.72 km$\cdot h^{-1}$ *vs* 15.51±1.16 km$\cdot h^{-1}$, respectively), though the protocol used 10-min stages.

Maximal lactate steady-state (MLSS), the highest intensity at which lactate production and elimination are in equilibrium, has also been suggested as an appropriate measure of 'lactate threshold' (Urhausen et al., 1993). However, this method requires long or even multiple testing sessions, and only yields a single performance measure. Thus, this test was not suitable from a feasibility standpoint and could not be used to generate the training zones and extensive feedback for athletes that were crucial for recruitment and retention.

Jamnick et al. (2018) observed that MLSS occurs when cycling at a considerably lower power output (~41 W lower; effect size: d=1.05) than 4 mmol $\cdot$ L^{-1} [La^-] in a 3-min stage protocol. However, Figueira et al. (2008) observed a difference between MLSS and power/speed at 3.5 mmol $\cdot$ L^{-1} [La^-] (3-min stage protocol) whilst cycling, but not whilst treadmill running. As Figueira et al. (2008) showed that MLSS and 3.5 mmol $\cdot$ L^{-1} [La^-] values were strongly correlated in treadmill running (r = 0.94, p<0.001), the current 4 mmol $\cdot$ L^{-1} [La^-], 3-min stage treadmill running protocol likely produces a similar result and an acceptable index of this response whilst using a more pragmatic method than MLSS or D-max methods.

3.7.6 Maximal oxygen consumption

Two different, exercise modality specific (Beltz et al., 2016) styles of maximal oxygen consumption tests were used in this thesis. The first was to determine $\dot{V}O_{2peak}$, and was included as a preliminary measure in **Chapters 4** and **5.** This was a ramp-style graded exercise test performed in the Human Performance Laboratory using the Lode Excalibur Sport cycle ergometer (**Section 3.8.1**). Participants completed a 3-5 min warm-up, whereby power output was adjusted to elicit a "light" exercise intensity (i.e. "11", as classified by $RPE_{6\text{-}20}$ [**Section 3.10**]; Borg, 1962). Power increased gradually by 20 W$\cdot$$min^{-1}$, until participants reached volitional exhaustion and/or cadence could no longer be maintained at

80±5 rpm despite strong verbal encouragement. Respiratory gas exchange, as measured using open-circuit spirometry (**Section 3.9.2.1**), and HR (**Section 3.9.4**) were measured continuously. $\dot{V}O_{2peak}$ was determined as the highest rolling 30-s average of 5-s binned $\dot{V}O_2$ attained during the test.

A test of $\dot{V}O_{2peak}$, representing the highest value attained during exercise and not necessarily the maximal physiological value attainable (Day et al., 2003), was deemed most appropriate to quantify participants' fitness in **Chapters 4** and **5.** The "plateau" in $\dot{V}O_2$, required to ascertain $\dot{V}O_{2max}$, is more elusive on a cycle ergometer, due to the concentric nature of cycling as compared to running (Gordon et al., 2012).

The maximal oxygen consumption test used to determine $\dot{V}O_{2max}$, a main outcome measure in **Chapters 6-8**, was also completed in the Human Performance Laboratory (**Figure 3.6**). Participants performed a step-style graded exercise test ~10 min following the Lactate Profile test (**Figure 3.5**) on the H/P/Cosmos Quasar 4.0 treadmill (**Section 3.8.2**). The test began at 1% gradient and at the speed 2 $km \cdot h^{-1}$ slower than the speed at which the participant had previously surpassed a 4 $mmol \cdot L^{-1}$ [La^-] in the lactate profile test. Speed increased 1 $km \cdot h^{-1}$ each minute for the next two minutes (until the treadmill was the speed at which 4 $mmol \cdot L^{-1}$ [La^-] had been reached). At this point the gradient was increased by 1% each minute until volitional exhaustion. Participants were given consistent and loud verbal encouragement during the test. When participants were re-tested following the intervention or control, the starting speed and progression of the protocol was repeated to allow for measurement of time-to-exhaustion (TTE). HR and respiratory gas exchange were continuously measured. Concentration of blood lactate was measured 5 min from the time of exhaustion.

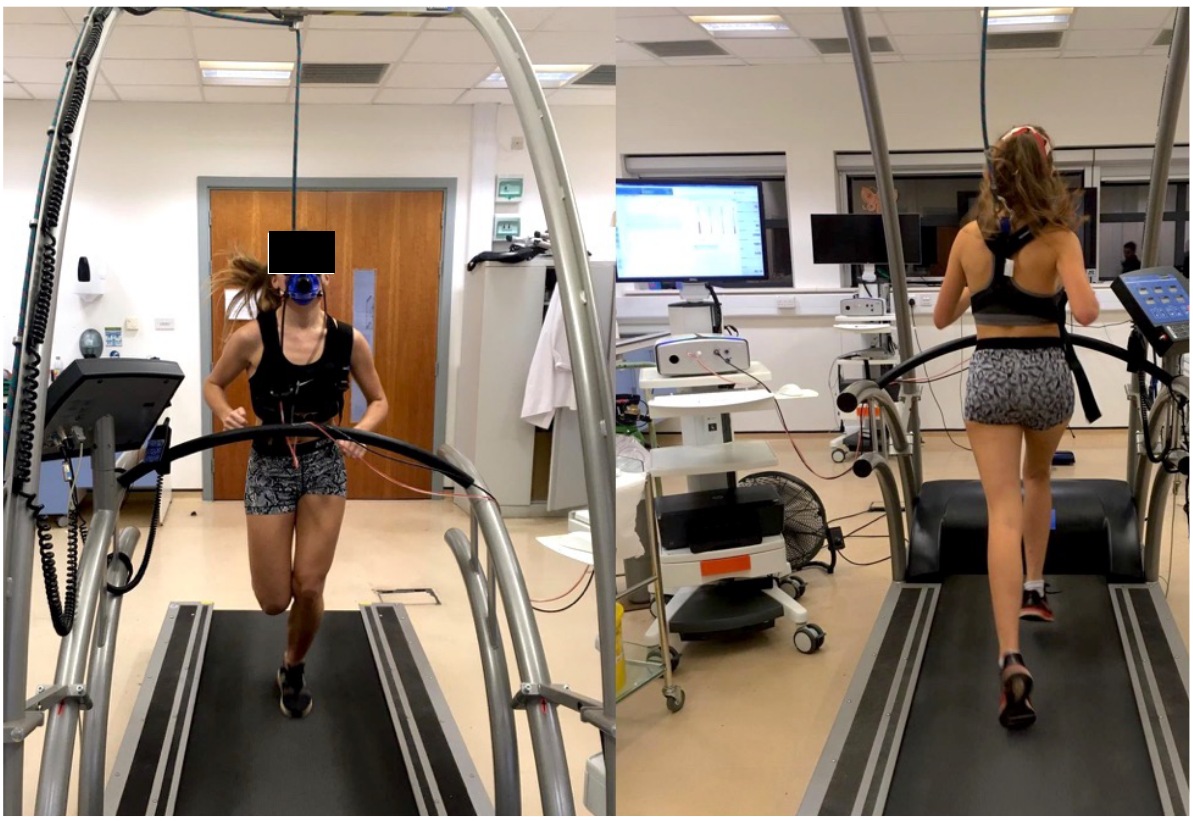


Figure 3.6 The ramp-style maximal oxygen consumption ($\dot{V}O_{2max}$) test was completed on a treadmill in the Human Performance Laboratory. Participants ran at an increasing speed, and subsequently gradient, until volitional exhaustion. Respiratory gas exchange was measured continuously via open-circuit spirometry.

$\dot{V}O_2$ was averaged into 5-s bins, and $\dot{V}O_{2max}$ was calculated as the highest rolling 30-s average attained during the test. Successful attainment of $\dot{V}O_{2max}$ required meeting 2 of the following 3 criteria: 1) $[La^-] \geq 8$ mmol $\cdot$ L^{-1} (Howley et al., 1995), 2) RER ≥1.10 (Edvardsen et al., 2014), 3) maximal HR ≥90% of age-predicted maximal HR (220−age). Additionally, a plateau was confirmed both visually and systematically by a 5-s average value ≥2 standard deviations lower than the extrapolated linear increase of $\dot{V}O_2$ from 2:00-4:00 of the test.

The average $\dot{V}O_{2max}$ test in studies in **Chapters 6-8** lasted 7:04 ± 1:08 min. This falls below the recommended protocol duration of 8-12 min (Buchfuhrer et al., 1983). However, the 8-minute threshold was set to be more inclusive of untrained cohorts, who may exhibit premature muscular fatigue and central cardiovascular limitations in response to the steeper $\Delta \dot{V}O_2/\Delta$intensity slope experienced in shorter protocols (Yoon et al., 2007). Thus, the ~7-min test was appropriate for the trained cohorts in the current thesis.

3.8 Exercise equipment

3.8.1 Cycle ergometers

All active heat acclimation sessions and TTs (**Chapters 4 and 5**) were completed using a Velotron cycle ergometer (Velotron, RacerMate Inc., Seattle, WA, United States). Pilot testing was conducted in both cool (15°C, 30%RH) and hot (35°C, 30%RH) ambient temperatures to determine the calibration settings specific to each environment. Before each TT and active heat acclimation session, the Velotron cycle ergometer was calibrated to <1% deviation from the calibration settings (specific to the chosen temperature) using the Accuwatt^{TM} verification program. Power output, distance cycled and speed were recorded continuously via the Velotron Coaching Software (RacerMate Inc., Seattle, WA, United States).

During $\dot{V}O_{2peak}$ tests reported in **Chapters 4 and 5**, participants cycled on the Lode Excalibur cycle ergometer (Sport Excalibur, Lode, Groningen, Netherlands). The researcher manually adjusted the controls so that resistance was applied to the flywheel to gradually increase intensity.

3.8.2 Treadmill running

During exercise tests reported in **Chapters 6-8**, participants ran on an electrically motorised treadmill (H/P/Cosmos Quasar 4.0, H/P/Cosmos, Germany). Speed (to 0.1 km·h^{-1} precision) and gradient (to 0.1% precision) controls were adjusted manually by the researcher.

3.9 Physiological measures

3.9.1 Urine osmolality

Participants were instructed to maintain normal hydration before each experimental testing session, which was verified with a urine osmolality value of ≤700 mOsm·kg^{-1} (Sawka et al., 2007) using a hand-held digital refractometer (Osmocheck, Vitech Scientific Ltd., West Sussex, United Kingdom) to a precision of 10 mOsm·kg^{-1}. The refractometer was cleaned and zeroed with tap water before each use.

3.9.2 Respiratory gas exchange

During the lactate profile, $\dot{V}O_{2peak}$ and $\dot{V}O_{2max}$ tests, respiratory gas exchange was measured via open-circuit spirometry. Participants exercised wearing a leak-free facemask connected to the Vyntus CPX system (Jaeger, Wuerzberg, Germany). Breath-by-breath analysis of CO_2 and O_2 fractions of expired air were digitally analysed in the Vyntus CPX system via an electrochemical sensor (commercially specified accuracy of 0.2%) and an infrared sensor (commercially specified accuracy of 1%), respectively. Inspired and expired air volume was calculated via a digital volume transducer and automatically adjusted according to ambient temperature and air pressure as recorded by sensors during calibration. Gas and volume calibration were completed according to manufacturer instruction immediately prior to each test.

$\dot{V}O_2$ and $\dot{V}CO_2$ were used to calculate $\dot{V}O_{2peak}$, $\dot{V}O_{2max}$, RER and running economy. Running economy ($mL \cdot kg^{-1} \cdot km^{-1}$) was calculated as the quotient of oxygen consumption ($mL \cdot kg^{-1} \cdot min^{-1}$) divided by treadmill speed ($km \cdot h^{-1}$) (Barnes & Kilding, 2015).

3.9.3 Body temperatures

3.9.3.1 Rectal temperature

Rectal temperature (T_{rec}) was measured using a rectal thermistor self-inserted 10 cm past the anal sphincter (Mon-a-Therm, Covidien, Mansfield, MA, United States) and continuously logged at 30-s intervals (Squirrel 2020 series, Eltek, Ltd., United Kingdom). Rectal temperature was used as an index of deep body temperature. The tissues of the rectum that influence T_{rec} are supplied by the middle and superior haemorrhoidal arteries. However, the large tissue mass relative to blood perfusion creates a thermal inertia that makes the rectum a stable measurement site, accurate to 0.1°C (Durotoye & Grayson, 1971; Ganio et al., 2009). Although T_{rec} is not as dynamic or quick to respond to rapid, exercise intensity-mediated changes in deep body temperature as telemetric pills or oesophageal temperature (Kolka et al., 1993), T_{rec} is a stable index of core temperature when exercise intensity does not fluctuate considerably (Eichna et al., 1951). Additionally, rectal thermistors may be better tolerated (especially whilst exercising) than oesophageal thermistors because of the gag reflex response to the latter. Structural nasal abnormalities can also make oesophageal thermistors difficult or even impossible to insert. With regards to feasibility, rectal probes are less expensive than telemetric pills. Lastly, T_{rec} was selected over other less invasive measurement sites, including forehead, sublingual, temporal, aural and axillary measurements, as these sites are less reliable and have less validity in comparison (i.e.

deviate >0.2°C; Ganio et al., 2009). Thus, T_{rec} was selected because it is a valid, stable and reliable measure when conducting exercise interventions with a repeated-measures design (Mee et al., 2015a).

Peak T_{rec} was calculated as the average T_{rec} in the final minute of each test. $T_{recRISE}$ was calculated as the change in T_{rec} from the first until the final measurement during each test. T_{rec75} represents mean T_{rec} for the final 75 min of active heat acclimation sessions, which followed the 15-min high-intensity intervals.

3.9.3.2 Skin temperatures

Skin temperatures were recorded using skin thermistors (Squirrel Thermal Couples, Grant Instruments, Cambridge, UK) attached to four sites on the left side of the body: pectoralis major (T_{chest}), biceps brachii (T_{arm}), rectus femoris (T_{thigh}), and gastrocnemius lateral head ($T_{lower\ leg}$). Skin temperatures were continuously logged at 30-s intervals (Squirrel 2020 series, Eltek, Ltd., United Kingdom). Skin temperature (T_{sk}) was calculated as a weighted average according to Ramanathan (1964):

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

This four-site method is favourable during exercise over a 12- or 15-site method, because it allows greater freedom of movement, whilst still providing a valid index of skin temperature (Mitchell & Wyndham, 1969).

3.9.4 Heart rate

Heart rate (HR) was recorded by short-range telemetry (Polar H7 & H10, Polar Electro, Kempele, Finland) at a sampling rate of 1 Hz. Data were recorded on the Velotron Coaching Software (Velotron CS 2008, RacerMate Inc., Seattle, WA, United States) in **Chapters 4** and **5**, and using the Polar Beat application (Polar, Kempele, Finland) in **Chapters 6-8.**

Peak HR was calculated as the average HR in the final minute of each test. Minute average data were used for all other analyses of HR. Percentage of age-estimated maximal heart rate ($\%HR_{max}$) was calculated as follows:

$$\%\mathrm{HR}_{\max} = \frac{\mathrm{HR}}{220 - \mathrm{age}} \cdot 100\%$$

3.9.4.1 Heart rate recovery

Immediately following the lactate profile test, HR recovery was recorded and calculated at four intervals, using the 10-s average HR proceeding the immediate cessation of exercise, and the 10 s preceding 1-, 2- and 3-minutes post-exercise.

HR recovery has previously been employed to indicate autonomic nervous system activity in an athletic population (Aubry et al., 2015). HR recovery immediately post-exercise is characterised by an initial “fast phase”, predominantly promoted by vagal reactivation, and the subsequent “slow phase”, whereby sympathetic withdrawal also influences HR (Peçanha et al., 2014; **Figure 3.7**). Thus, HR recovery was measured in **Chapter 7** to detect changes in autonomic excitability that accompany the initial transient phase of heat acclimation (Horowitz & Meiri, 1993). HR recovery was measured following submaximal exercise (i.e. the lactate profile test) because a change is more likely to be detected following submaximal as opposed to maximal exercise (Le Meur et al., 2017).

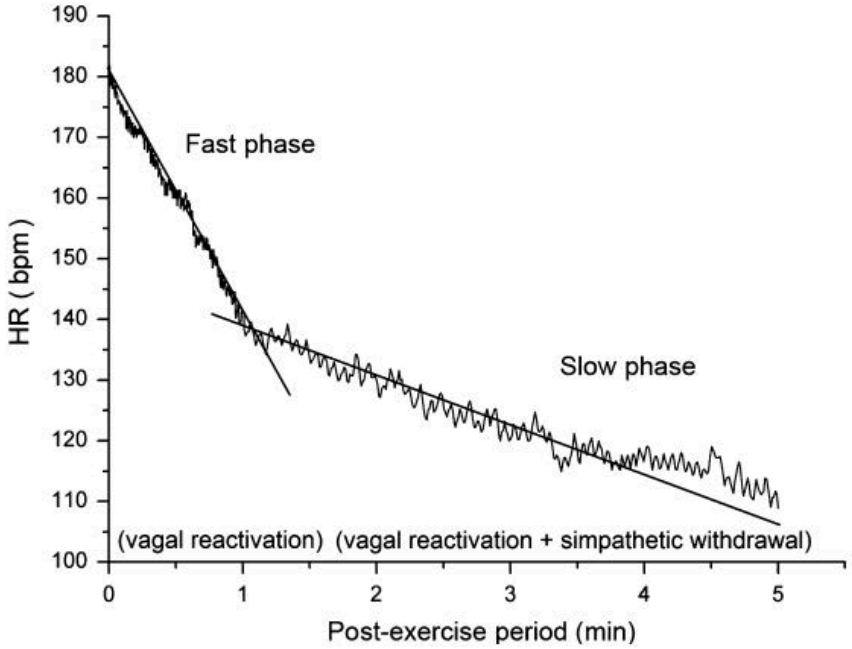


Figure 3.7 Heart rate recovery from exercise is biphasic, and influenced by the autonomic nervous system. Figure taken from (Peçanha et al., 2014).

3.9.5 Forearm blood flow

Forearm blood flow (FBF) was quantified using venous occlusion plethysmography (Whitney, 1953) immediately post-exercise with the participant seated and the forearm supported at heart level. A strain-gauge was secured (though not in a restricting manner) onto the widest part of the forearm (position measured from the elbow to replicate strain

gauge position across trials). Blood flow to the hand was occluded using a wrist cuff at a pressure >220 mmHg to prevent inclusion of hand circulation in the calculations of FBF. An upper arm cuff was occluded at a pressure of 45 mmHg for 10 s, and voltage output was recorded (PowerLab, ADInstruments, Dunedin, New Zealand). Two-point calibration (0.1% and 1% change in strain) was conducted immediately prior to each measurement. Outputs of 4-8 s post-upper arm occlusion were analysed offline (Labchart; ADInstruments) for maximal flow response, as detailed by Wythe and colleagues (2015) and depicted in **Figure 3.8**. The maximal flow response was selected because other methods (which use multiple samples) incur greater variation, as they are affected by: i) the increasing time post-exercise, and ii) considerable individual variation in the rate at which FBF declines post-exercise (Wythe et al., 2015).

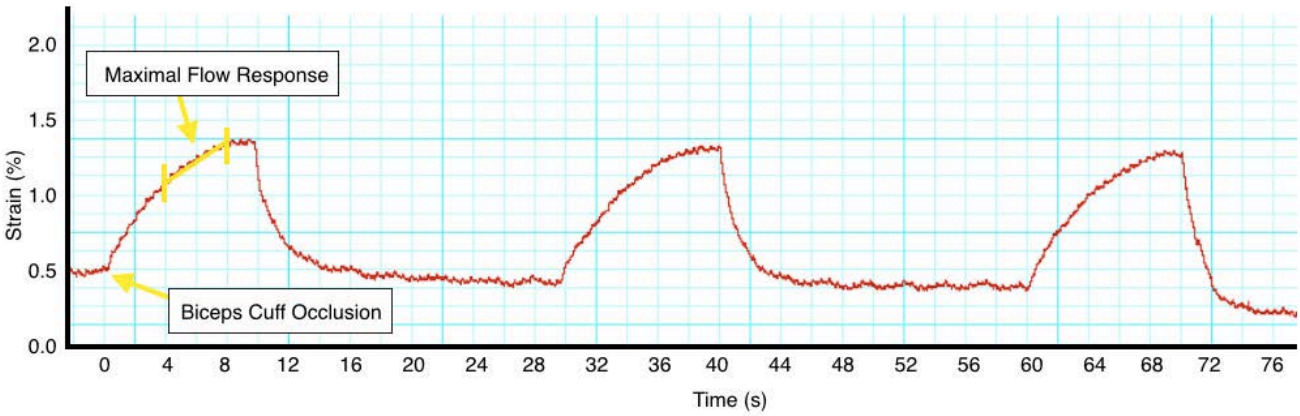


Figure 3.8 Individual participant trace of forearm blood flow (FBF) as measured using venous occlusion plethysmography. Maximal flow response of FBF was measured from the first sample post-exercise as the average slope from 4-8 s post-biceps cuff occlusion.

Forearm blood flow was recorded as $\%\cdot s^{-1}$ change (Δ), normalised to forearm volume (**Section 3.5**) and expressed in $mL \cdot dL\ tissue^{-1} \cdot min^{-1}$:

$$FBF\ (mL \cdot dL\ tissue^{-1} \cdot min^{-1}) = \frac{slope\ (\Delta\%\cdot s^{-1}) \cdot Forearm\ Volume\ (mL) \cdot 60\ s}{100\ mL\ Forearm\ Volume}$$

Although venous-occlusion plethysmography detects blood flow changes within all vascular beds, these measurements primarily reflect cutaneous blood flow at rest (Edholm et al., 1956; Detry et al., 1972) or during exercise (Johnson & Rowell, 1975), provided the arm remains inactive. Thus, FBF was assumed as an index of skin blood flow in **Chapter 7**.

Laser Doppler flowmetry is an alternative method of measuring skin blood flow, whereby skin blood flow is quantified according to the Doppler shift of incident laser light reflected off moving red blood cells (Oberg, 1990). Laser Doppler flowmetry is advantageous

for measuring skin blood flow continuously, whereas venous-occlusion plethysmography takes discontinuous measurements every ~30 s (**Figure 3.8**). However, venous-occlusion plethysmography provides absolute rates of forearm blood flow, whereas Laser Doppler flowmetry only provides relative flux, typically normalised as a percentage of maximal skin blood flow.

3.9.6 Sweating responses

Estimated sweat loss was calculated as the difference between pre- and post-test nude body mass, and was expressed as an absolute value (**Chapter 6**), as a percentage of body mass (**Chapters 4, 5, 7 and 8**) and/or as a rate normalised by body surface area (BSA; **Chapters 4 and 5**):

$$SR_{BSA}\ (g \cdot h^{-1} \cdot m^{-2}) = (\Delta NBM\ [g] - \text{fluid consumption})\ [g]) \cdot (\text{exercise time}^{-1}\ [h])^{-1} \cdot (BSA\ [m^2])^{-1}$$

$$\text{Sweat loss}\ (\%BM) = 100\% \cdot \frac{(\Delta NBM\ [kg] - \text{fluid consumption}\ [kg])}{(BM\ [kg])}$$

Active sweat glands were quantified using a modified-iodine paper technique with blinded computer aided analysis (Gagnon et al., 2012). Briefly, 100% cotton paper was impregnated with iodine (≥99.8%; Sigma-Aldrich, Dorset, UK) for ~48 h and cut into equal 28 mm diameter discs. The skin was wiped of sweat and the disc was immediately pressed firmly and evenly onto the skin using a flat plastic surface for 2 (males) or 3 (females) seconds. Samples were collected in duplicate by the same researcher from the dorsal side of the thickest segment of the forearm (**Chapters 4, 6, 7** and **8**) and mid-scapula on the upper back (**Chapters 6-8**). Discs were scanned at 600 dpi and analysed using ImageJ software. Gagnon et al. (2012) reported this method to have a coefficient of variation of 11%.

3.9.7 Phlebotomy and biochemistry

3.9.7.1 Venous sampling

Following a minimum of 10-min supine rest, venous blood was drawn from the antecubital vein into a K2EDTA-coated vacutainer for measurement of plasma vascular endothelial growth factor (VEGF) and an uncoated vacutainer for measurement of serum erythropoietin (EPO; **Section 3.9.7.5**). Vacutainers were kept on ice (plasma) or at room temperature (serum) for 45 min before centrifugation (11,000 x *g* for 10 min at 4°C; Heraeus

Multifuge X1R, Thermo Scientific, Loughborough, UK) and were frozen at -80°C until analysis.

3.9.7.2 Fingertip capillary sampling

Fingertip capillary blood samples were taken with a pressure-activated safety lancet (StariLance Press 2, Vitrex Medical, Denmark) for measures of resting and exercising blood lactate concentration (**Section 3.9.7.4**) or following a minimum 10-min supine rest and hand warming to arterialise samples for determination of relative plasma volume (**Section 3.9.7.3).** The fingertip was cleaned with alcohol before being punctured, and the first drop of blood was wiped away and safely discarded.

3.9.7.3 Determination of plasma volume

Haemoglobin concentration ([Hb]) and haematocrit (Hct) were measured to estimate plasma volume as a percentage of total blood volume. A 60-100 µL arterialised fingertip capillary blood sample (**Section 3.9.7.2**) was collected into a sodium-heparinised capillary tube (Abbot Point of Care Inc., Princeton, NJ, USA), immediately deposited into a single-use test cartridge (E3+ or EG6+, Abbot Point of Care Inc., Princeton, NJ, USA) and analysed using an iSTAT portable blood analyser (iSTAT1, Abbot Point of Care Inc., Princeton, NJ, USA). The iSTAT analyser was electronically calibrated once per month and quality control checked (iSTAT TriControls Verification Set, Abbot Point of Care Inc., Princeton, NJ, USA) once per week during the interventions. Previous investigations have observed iSTAT [Hb] values to be reliable (CV 2.8%) within paired-samples (Ng et al., 2014). The iSTAT system uses a conductometric biosensor to measure the conductivity of the sample, which is inversely proportional to the number of cells in the sample. Plasma conducts electrical current, whilst cells are electrical insulators. The conductivity of the sample is compared to that of the calibration solution, adjusted for electrolyte concentration and expressed as the percent packed cell volume of the sample. Haemoglobin concentration is then derived as the product of measured Hct and a constant:

$$\text{Hb } (g \cdot dL^{-1}) = \text{Hct (\% packed cell volume)} \times 0.34$$

The iSTAT system has been reported to produce similar results to the widely used HemoCue system (0.23% mean bias for [Hb] measurements; Kolotiniuk et al., 2018), which uses an optical-based method to assess the absorbance of the sample following a chemical

reaction with the Hb in the sample. When [Hb] and Hct are known, the change in relative blood, plasma and red blood cell volumes can be calculated between two time points (denoted by subscripts) using the following calculations proposed by Dill and Costil (1974):

$$BV_2 = BV_1 \cdot ([Hb]_1 \div [Hb]_2)$$

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

$$PV_2 = BV_2 - CV_2$$

$$\Delta\ BV\ (\%) = 100 \cdot (BV_2 - BV_1) \div BV_1$$

$$\Delta\ CV\ (\%) = 100 \cdot (CV_2 - Hct_1) \div Hct_1$$

$$\Delta\ PV\ (\%) = 100 \cdot (PV_2 - PV_1) \div PV_1$$

Where BV = blood volume, CV = red blood cell volume, PV = plasma volume and BV_1 is assumed to be 100 if not otherwise known.

It should be noted that this calculation was established to calculate blood and plasma volume changes during a single laboratory session when no fluid was ingested, and total Hb was assumed to stay the same (Dill & Costill, 1974). This calculation is based on concentrations and not absolute volumes. For this reason, changes in absolute red cell volume (for example, over a period of weeks; Scoon et al., 2007) could mask or distort changes in plasma volume based on measures of concentrations alone. Thus, although this method is widely used in exercise physiology, there is some nuance associated with using this calculation for multi-day testing in the absence of meticulous control of hydration and measures of total blood volume and plasma protein content. Alternative methods include Evans blue dye (Crooke & Morris, 1942; Farjanel et al., 1997) and carbon monoxide rebreathing (Schmidt & Prommer, 2005), although they carry feasibility constraints with regard to invasiveness, cost and interference with same-day exercise.

3.9.7.4 Blood lactate concentration

Fingertip capillary blood samples were immediately analysed for concentration of blood lactate ($[La^-]$) using a Lactate Plus analyser (Lactate Plus, Nova Biomedical, Waltham, MA, United States) at rest and immediately post-TT in **Chapters 4 and 5**. The Lactate Plus analyser uses an electrochemical lactate oxidase biosensor to measure $[La^-]$ and has an intra-sample typical error of measurement of 0.4 $mmol \cdot L^{-1}$ (Tanner et al., 2010). A two-level quality control test was performed weekly and prior to use of each new test strip vial. Samples were measured in duplicate, and results were averaged to yield a single value for each time-point. Extreme outliers falling outside the typical physiological range were

excluded, and only the rational value was used (Goodwin et al., 2007; *n*=3 outlier incidences).

Fingertip capillary blood samples were also collected for blood lactate measures during the lactate profile test and post-$\dot{V}O_{2max}$ test in **Chapters 6** and **7**. A 20 µL sodium-heparinised capillary tube (EKF Diagnostic Group, Barleben, Germany) was filled end-to-end with the blood sample, combined with a standardised amount of haemolysing solution contained within Eppendorf tubes (EKF Diagnostic Group, Barleben, Germany), shaken until adequately mixed and immediately analysed for [La^-] using a Biosen Lactate Analyser (C-Line, EKF Diagnostic Group, Barleben, Germany). The analyser was calibrated prior to each testing session and every 60 min thereafter using a lactate multi-standard (12 $mmol \cdot L^{-1}$ [La^-], EKF Diagnostic Group, Barleben, Germany). A two-level quality control test was performed on each day of testing. Lactate in the sample reacts enzymatically with the lactate oxidase-coated biosensor, generating an electrical signal via H_2O_2 to be detected by the electrode within the analyser. The electrical signal is proportional to the [La^-] of the sample. This enzymatic method is comparatively more accurate and thus preferred to the photometric detection used in older lactate analysers (Rassaei et al., 2014).

3.9.7.5 Biochemical analysis

Quantifications of serum EPO and plasma VEGF were performed using enzyme-linked immunosorbent assay (ELISA) kits (Human Erythropoietin DuoSet ELISA and Human VEGF DuoSet ELISA, respectively; R&D Systems, Abingdon, UK). For both kits, the 96-well plates were coated with the diluted capture antibody, incubated, and washed according to manufacturer instructions. Samples and serially diluted standards were then added to the wells in duplicate. The plates were washed, so that only the bound protein of interest (i.e. EPO or VEGF) remained on the plate. The diluted detection antibody was then added and plates were washed again. Finally, Streptavidin-horseradish peroxide, and after 20 min, a stop solution was added to each well for colorimetric detection. The signal, which is directly proportional to the amount of protein in the sample, was immediately read at 405 nm. A four-parameter logistic curve-fit was generated from the calibration concentrations and used to convert the raw EPO and VEGF plate reader results into $mU \cdot mL^{-1}$ or $pg \cdot mL^{-1}$, respectively (AssayFit Pro online software). The manufacturer reports the EPO and VEGF assay detection ranges as 3-200 $mIU \cdot mL^{-1}$ and 31-2000 $pg \cdot mL^{-1}$, respectively.

3.10 Perceptual measures

RPE for training sessions (**Chapters 6-8**) was rated on a 1-10-point scale (RPE_{1-10}; **Figure 3.9**) and athletes were instructed to report ratings in isolation following each training session. RPE reported during all other experimental sessions was measured using a 6-20-point scale (RPE_{6-20}; Borg , 1982; **Figure 3.9**). Participants were asked, "How hard do you feel you are exercising?" and pointed to the corresponding number.

Thermal Sensation and Thermal Comfort were measured during experimental sessions using modified 13-point and 10-point scales, respectively (Gagge et al., 1967). Participants were asked, "How does the temperature of your body feel?" and "How comfortable do you feel with the temperature of your body?" to discern Thermal Sensation and Thermal Comfort, respectively (**Figure 3.10**).

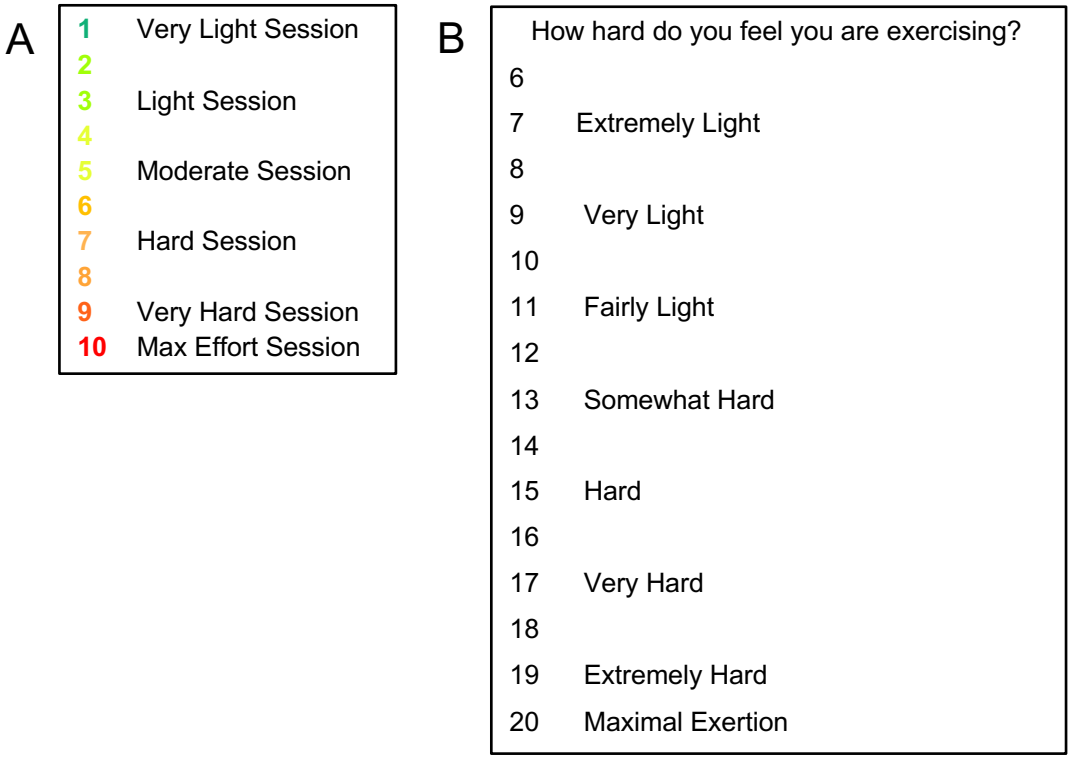


Figure 3.9 Scales employed to measure Rating of Perceived Exertion were a 1-10-point scale (RPE_{1-10}; ***A***) for training diaries and a 6-20-point scale (RPE_{6-20}; ***B***) during experimental trials.

A

How does the temperature of your body feel?	
1	Unbearably Cold
2	Extremely Cold
3	Very Cool
4	Cold
5	Cool
6	Slightly Cool
7	Neutral
8	Slightly Warm
9	Warm
10	Hot
11	Very Hot
12	Extremely Hot
13	Unbearably Hot

B

How comfortable do you feel with the temperature of your body?	
1	Comfortable
2	
3	Slightly Uncomfortable
4	
5	Uncomfortable
6	
7	Very Uncomfortable
8	
9	Extremely Uncomfortable
10	

Figure 3.10 Scales employed to measure Thermal Sensation (***A***) and Thermal Comfort (***B***).

3.11 Calculation of adaptation decay

Decay is presented as a percentage, and calculated as the quotient of the difference between scores at post-intervention and decay and the difference between scores at pre- and post-intervention (**Figure 3.11**), according to Pandolf et al. (1977):

$$\%Decay = 100 \cdot \frac{(\text{post-intervention value} - \text{value following decay period})}{(\text{post-intervention value} - \text{pre-intervention value})}$$

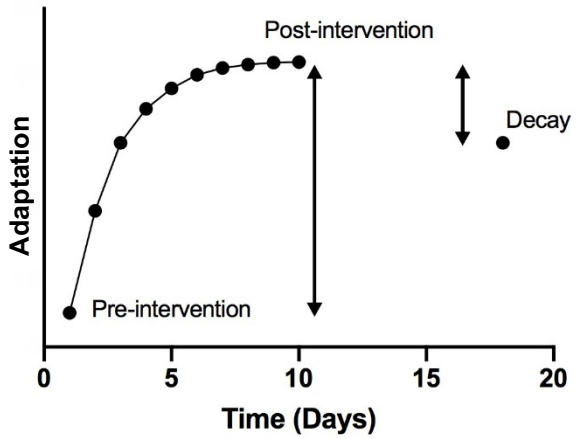


Figure 3.11 Example of heat acclimation decay calculation. Days 1-10 represent a heat acclimation intervention and days 10-18 represent Post-intervention to Decay, when participants are not exposed to heat. %Decay is calculated as (Post-intervention–Decay)/(Post-intervention–Pre-intervention). Reproduced from Daanen et al. (2018).

3.12 Training diaries

Throughout the protocols detailed in **Chapters 6-8**, participants recorded their training type, distance, frequency and session $RPE_{1\text{-}10}$.

3.13 Calculation of area under the curve

Area under the curve (AUC) was calculated using minute averages of power output and 30-s values of T_{rec} during TTs in **Chapters 4 and 5**, according to Pruessner et al. (2003). AUC was calculated using the principle of 'area under the curve with respect to ground', whereby absolute values (from zero, or 'ground') are used:

$$\sum_{i=1}^{n-1} \frac{(m_{i+1} + m_i)}{2}$$

Where m_i denotes individual measurements.

3.14 Statistical analysis

All data were analysed using SPSS statistical software (SPSS version 25.0.0, SPSS, Chicago, IL, United States). Specific statistical analyses are described within each chapter.

4. NINE-, BUT NOT FOUR-DAYS HEAT ACCLIMATION IMPROVES SELF-PACED ENDURANCE PERFORMANCE IN FEMALES

This chapter was published in *Frontiers in Physiology* (Kirby, N. V., Lucas, S. J., & Lucas, R. A. [2019]. Nine-, but not four-days heat acclimation improves self-paced endurance performance in females. *Frontiers in physiology*, *10*, 539) and has undergone minor modifications to adapt to the format of this thesis. Specifically, details for methods outlined in the general methods chapter are referred to using section identifiers.

4.1 Abstract

Background: Although emerging as a cost- and time-efficient way to prepare for competition in the heat, recent evidence indicates that "short-term" heat acclimation (<7 days) may not be sufficient for females to adapt to repeated heat stress. Furthermore, self-paced performance following either short-term, or longer (>7 days) heat acclimation has not been examined in a female cohort. Therefore, the aim of this study was to investigate self-paced endurance performance in hot conditions following 4 and 9 days of high-intensity isothermic heat acclimation in a female cohort.

Methods: Eight female endurance athletes (mean±SD, age 27±5 years, mass 61±5 kg, $\dot{V}O_{peak}$ 47±6 mL · kg · min^{-1}) performed 15-min self-paced cycling time trials in hot conditions (35°C, 30%RH) before (TT_{HOT1}), and after 4 days (TT_{HOT2}) and 9 days (TT_{HOT3}) isothermic heat acclimation (with power output manipulated to increase and maintain rectal temperature (T_{rec}) at ~38.5°C for 90-min cycling in 40°C, 30%RH) with permissive dehydration.

Results: There were no significant changes in distance cycled (p=0.47), mean power output (p=0.55) or cycling speed (p=0.44) following 4 days of heat acclimation (i.e. from TT_{HOT1} to TT_{HOT2}). Distance cycled (+3.2%, p=0.01; +1.8%, p=0.04), mean power output (+8.1%, p=0.01; +4.8%, p=0.05) and cycling speed (+3.0%, p=0.01; +1.6%, p=0.05) were significantly greater in TT_{HOT3} than in TT_{HOT1} and TT_{HOT2}, respectively. There was an increase in active sweat glands per cm^2 in TT_{HOT3} as compared to TT_{HOT1} (+32%; p=0.02) and TT_{HOT2} (+22%; p<0.01), whereas thermal sensation immediately before TT_{HOT3} decreased ("Slightly Warm," p=0.03) compared to ratings taken before TT_{HOT1} ("Warm") in 35°C, 30%RH.

Conclusions: Four-days heat acclimation was insufficient to improve performance in the heat in females as observed following 9 days of heat acclimation.

4.2 Introduction

Hot ambient temperatures and elevated humidity are known to negatively impact endurance exercise performance (Tatterson et al., 2000; Periard et al., 2011). Heat acclimation is an effective strategy to drive favourable physiological adaptations, thereby reducing athletic performance impairments caused by these challenging environments (Sawka et al., 2011; Periard et al., 2015; Racinais et al., 2015b). Heat acclimation typically consists of repeated daily heat stress exposures, with exposure durations commonly lasting between 60 and 90 min. Traditionally, 10 days of heat exposure are undertaken to elicit the heat acclimation phenotype and improve endurance performance in the heat (Armstrong & Maresh, 1991; Lorenzo et al., 2010; Sawka et al., 2011), though 75–80% of physiological adaptations occur in the first 4–7 days of heat acclimation in male cohorts (Pandolf, 1998; Shapiro et al., 1998). Based on this, Garrett et al. (2009) first demonstrated meaningful performance improvements following just 5 days of isothermic heat acclimation, termed "short-term heat acclimation" (STHA).

STHA has since been defined as being <7 days in length (Garrett et al., 2011), and is promoted as a cost and time efficient option for athletes preparing for competition in the heat. Successful STHA lasting 4–7 days in male cohorts has been well documented (Petersen et al., 2010; Fujii et al., 2012; Garrett et al., 2012; Chen et al., 2013; Best et al., 2014; Costa et al., 2014; Garrett et al., 2014; Gibson et al., 2015b; Mee et al., 2015b; Racinais et al., 2015b; Guy et al., 2016; Willmott et al., 2016; James et al., 2017b; Willmott et al., 2017). However, few studies to date have examined STHA effects in female cohorts. It was initially shown that there were no sex differences (4 females *vs* 4 males) in adaptations to 10 days of heat acclimation when aerobic fitness and surface area-to-mass ratios were matched (Avellini et al., 1980a). However, Mee et al. (2015b) more recently reported a significant sex difference in the time course of heat acclimation (i.e. 5 *vs* 10 days), challenging past assumptions. In this study Mee et al. (2015b) found that following STHA, females did not exhibit a lower resting core temperature or an attenuated rise in core temperature and heart rate (HR) when exercising at a fixed workload, which are critical requirements in demonstrating the heat acclimation adaptation. However, the male cohort successfully attained these adaptations following the same protocol. Mee et al. (2018) then demonstrated that a longer daily heat exposure (achieved via 20 min sitting in sauna suits in 50°C, 30%RH immediately before 90-min isothermic heat acclimation) successfully induced heat adaptations in a female cohort following STHA (5 days). The authors therefore concluded that females require either a longer daily heat exposure (Mee et al., 2018), or a greater number of heat exposures (Mee et al., 2015b) to elicit favourable physiological adaptations.

It is unclear if females can achieve meaningful performance improvements following STHA or if a longer heat acclimation period is needed. Sunderland et al. (2008) reported a

33% improvement in distance running during a repeated shuttle run performance test following STHA in a female cohort, as well as a reduced rate of rise in rectal temperature (T_{rec}). The authors attributed performance improvements to the high-intensity intermittent exercise performed during the acclimation sessions; a strategy implemented by Pethick et al. (2018) to successfully induce plasma volume expansion in a female cohort following 5 days of high-intensity heat acclimation. Therefore, the addition of high-intensity interval training (HIIT) may offer a means to increase effectiveness of STHA in a female cohort. However, the performance test employed by Sunderland and colleagues was a time to exhaustion trial, which is a less reliable test and subject to greater variation than self-paced performance tests (Hopkins et al., 2001; Borg et al., 2018). Self-paced performance outcomes following heat acclimation have only been documented in male (Garrett et al., 2012; Keiser et al., 2015; Racinais et al., 2015b; Guy et al., 2016; Wingfield et al., 2016) or mostly male (Lorenzo et al., 2010) cohorts, and remain to be investigated in females. Such information is timely for female athletes competing at upcoming international competitions in hot climates, such as the 2020 Olympic Games in Tokyo and the 2019 IAAF World Athletics Championships in Doha. Currently, female athletes must either depend on conflicting literature or fill knowledge gaps with information inferred from male cohorts.

4.2.1 Aim

The aim of the present study was to investigate self-paced endurance performance in hot conditions following 4 days (STHA) and 9 days of a high-intensity exercise, isothermic heat acclimation protocol in a female cohort.

4.2.2 Hypotheses

It was hypothesised that females would not exhibit performance improvements in self-paced exercise following STHA, as previous studies indicate that 4 days of 90-min heat acclimation is unlikely to be a sufficient stimulus for the thermoregulatory and cardiovascular adaptations necessary for performance improvements in the heat. Therefore, it was further hypothesised that performance improvements in power output and time trial distance would occur following 9 days of heat acclimation.

4.3 Methods

4.3.1 Participants

Eight recreational female endurance athletes aged 21–35 years volunteered for and completed this study. An additional participant volunteered, but dropped out due to relocation after preliminary testing and was not included in the results. All participants were familiar with competitive, race-style endurance events, and trained 5±1 days per week, averaging 9±4 h of weekly endurance exercise training. Participants were eumenorrheic or using various forms of hormonal contraceptives. Individual characteristics are detailed in **Table 4.1**. Participants complied with all criteria and instructions for participation (**Section 3.3** General participant instructions, **Section 3.6** Menstrual cycle questionnaire and self-reported log and **Section 3.9.1** Instructions for normal hydration).

Table 4.1 Participants' characteristics

ID	Age (years)	Height (cm)	$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Body mass (kg)	Menstrual cycle /Contraceptive	Day of menstrual cycle or pill-taking phase on TT_{HOT1}
1	32	168	40	54	OCP (Cilest)	15
2	28	168	45	59	Implant	N/A
3	25	176	43	65	Implant	N/A
4	23	165	43	55	EU	23
5	35	173	42	69	EU	17
6	32	172	53	63	IUD/Coil	N/A
7	23	165	53	61	OCP (Yasmin)	11
8	21	174	54	61	Implant	N/A
Mean	*27*	*170*	*47*	*61*		

OCP, Oral contraceptive pill user (pill brand); IUD/Coil, Copper coil intrauterine device; EU, Eumenhorreic natural cycle; TT_{HOT1}, 15-min cycling time trial in hot conditions at baseline

4.3.2 General overview and design

All experimental procedures were completed in the environmental chamber in the School of Sport, Exercise and Rehabilitation Sciences building at the University of Birmingham (**Section 3.4.1**). Participants' $\dot{V}O_{2peak}$ was determined as described in **Section 3.7.6**. Participants were familiarised with the 15-min time trial performance tests in cool conditions (15°C, 30% RH) on two occasions, plus completed TT_{COOL1} (see **Chapter 5**) 48 h prior to the protocol described in the current chapter. Participants performed the 15-min cycling time trial in hot (35°C, 30% RH) conditions (i.e. TT_{HOT}; **Section 3.7.2**) pre-acclimation (TT_{HOT1}), following 4 days (STHA; TT_{HOT2}) and following 9 days of isothermic heat acclimation (TT_{HOT3}). An overview of the protocol used in the current chapter, as well as in

Chapter 5, is presented in **Figure 4.1**. This experiment was conducted in the United Kingdom during the months of February, April, May, and June, when mean ambient temperatures were below 20°C (exclusive of 3 days where the mean daily temperatures were 23, 24, and 27°C).

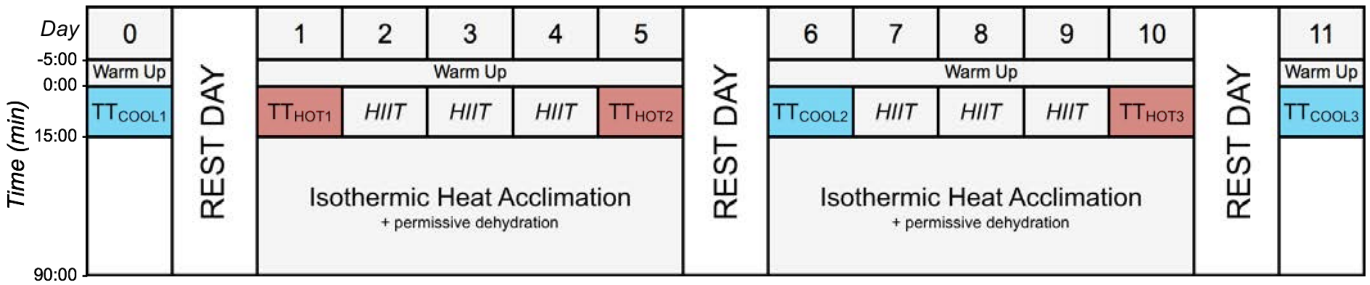


Figure 4.1 Schematic of the time trials and heat acclimation sessions. Time trials were conducted in hot (TT_{HOT}; 35°C, 30%RH) or cool (TT_{COOL}; 15°C, 30%RH) conditions, pre-acclimation (TT_{COOL1} and TT_{HOT1}), after 4 days (TT_{HOT2}), 5 days (TT_{COOL2}) 9days (TT_{HOT3}) and 10days (TT_{COOL3}) of heat acclimation. On days 2–4 and 7–9, participants completed 15-min of high-intensity intervals (HIIT), where maximum effort was given for 15 s, with 45-s active recovery. Participants then undertook 75-min isothermic heat acclimation (where exercise intensity was manipulated to increase and maintain rectal temperature at ~38.5°C; 40°C, 30%RH) with permissive dehydration. Rest days separated any consecutive time trials. Data related to TT_{COOL} are detailed in **Chapter 5**.

4.3.3 Experimental procedures

4.3.3.1 Active heat acclimation

Participants completed a 5-min warm-up, plus 90-min active heat acclimation sessions in 40°C, 30%RH with power output manipulated to increase and maintain T_{rec} at ~38.5°C and permissive dehydration (**Section 3.7.1)**. Resting T_{rec} was measured in the anti-chamber (i.e. room temperature). Power output, HR, T_{rec} and T_{sk} were recorded continuously. Sweat loss was measured via towel-dried NBM, with fluid consumption (295±235 mL each session) recorded to 0.001 L (Oertling, United Kingdom) and considered in total body sweat loss calculations. Thermal Sensation, Thermal Comfort and RPE_{6-20} were recorded at 15-min intervals during active heat acclimation sessions. Power output, HR, T_{rec} responses across heat acclimation sessions during STHA (days 1–4) and days 5–9 are depicted in **Figure 4.2**.

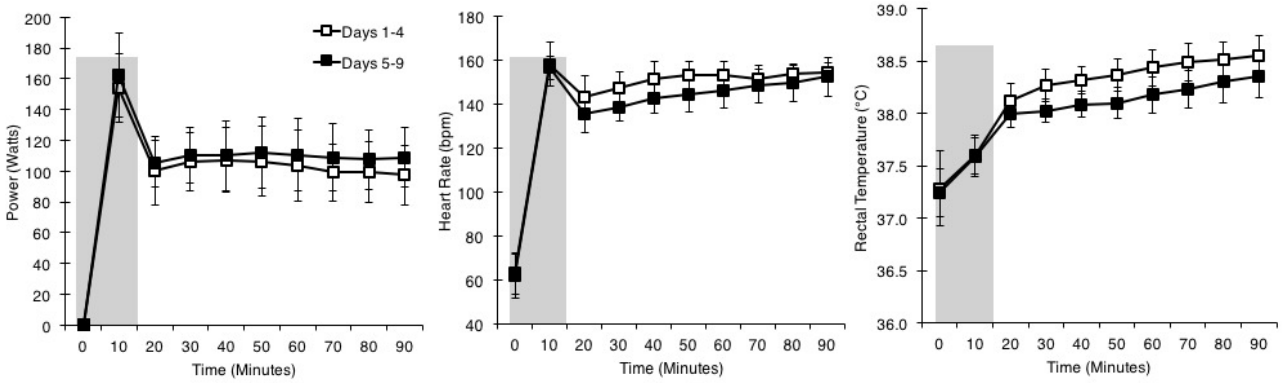


Figure 4.2 Power output (*left*), heart rate (*middle*), and rectal temperature (*right*), at 10-min intervals during the heat acclimation protocol. Data from days 1–4 (*open squares*) and days 5–9 (*solid squares*) are presented as mean±SD. Shaded area represents a 15-min time trial or 15-min high-intensity intervals.

4.3.3.2 Hot time trials

Participants lay supine for 10 min of stabilisation at room temperature prior to TT_{HOT} to collect resting measures of T_{rec} and [La^-]. Following confirmation of normal hydration, participants commenced the 5-min warm-up and 15-min self-paced cycling time trial (**Section 3.7.2)**. Power output, speed, distance cycled, HR, T_{rec} and T_{sk} were recorded continuously. Sweat gland activity (**Section 3.9.6**) and [La^-] (**Sections 3.9.7.2** and **3.9.7.4**) were recorded immediately following TT_{HOT}. Ratings of thermal comfort and thermal sensation were reported inside the environmental chamber preceding the warm-up and immediately post-trial. Following TT_{HOT}, participants completed 5 min of self-paced active recovery before proceeding with the acclimation session for that visit.

4.3.4 Data analyses

Peak T_{rec}, $T_{recRISE}$, T_{rec75} (mean T_{rec} for the final 75 min of active heat acclimation sessions) and weighted T_{sk} were calculated as described in **Section 3.9.3**. Percentage of age-estimated maximal heart rate (%HR_{max}) was calculated as described in **Section 3.9.4.** Sweat loss (change in NBM with consideration for water consumed) was expressed as a percentage of body mass. Sweat rate (SR_{BSA}) was calculated as absolute sweat loss normalised by body surface area, as described in **Section 3.9.6**.

Area under the curve (**Section 3.12**) was calculated for power output and T_{rec} during TT_{HOT}.

4.3.5 Statistical approach

For analysis of performance and physiological differences during heat acclimation days 1–4 vs. days 5–9, mean values were analysed using paired-samples *t*-tests. Mean performance values during TT_{HOT} (i.e. power output and speed), AUC comparisons (power output and T_{rec}), distance cycled, and physiological measures between TT_{HOT1}, TT_{HOT2}, and TT_{HOT3}, were analysed using repeated-measures one-way ANOVAs. Additionally, 1-min averages of power output, HR, T_{rec} and T_{sk} were analysed using a two-way repeated-measures ANOVA (3 trials × 15 time-intervals; *time* × *interval*). When a significant main effect was found, Dunn-Bonferroni-corrected *post hoc* comparisons were made. Main effect sizes for both one-way and two-way ANOVAs were calculated using partial eta-squared (η_p^2). Normality of the data was assessed using the Shapiro-Wilk Test (not violated on any occasion). Sphericity was assessed using were assessed using Mauchly's test of sphericity, and Greenhouse–Geisser corrections were applied where assumptions of sphericity were violated.

For analysis of ordinal data (i.e. $RPE_{6\text{-}20}$, Thermal Sensation and Thermal Comfort) differences during HA days 1–4 vs. days 5–9, and between TT_{HOT1}, TT_{HOT2}, and TT_{HOT3}, Friedman's test was performed with *post hoc* analyses by Wilcoxon sign-rank tests.

A power analysis indicated that eight participants was a sufficient sample size to detect an 8–10% difference in power output during time trial performance (as observed by Lorenzo et al., 2010 and Keiser et al., 2015). This analysis used an accepted parameter of power ($\beta \geq 0.80$) at an α level of 0.05.

Absolute data and within-subject changes are expressed as mean ± standard deviation (SD), and 95% confidence limits ([95% CL: lower limit, upper limit]) are presented with mean between-group differences. Significance was set at $p<0.05$ for each analysis.

4.4 Results

4.4.1 Active heat acclimation

Mean T_{rec75} (−0.2°C [−0.1, −0.3]; $p<0.01$), peak T_{rec} (−0.1°C [−0.1, −0.2]; $p=0.01$), and peak T_{sk} (−0.4°C [−0.1, −0.7]; $p=0.01$) were lower during heat acclimation sessions on days 5–9 as compared to days 1–4. Mean HR and $\%HR_{max}$ were also lower during heat acclimation sessions on days 5–9 as compared to days 1–4 (−5 beats·min^{-1} [−1, −10]; $p=0.03$, and −3% [−1, −5]; $p=0.02$, respectively). These physiological changes were present in spite of a significantly higher workload (i.e. power output) on days 5–9 as compared to days 1–4 (+9 W [+3, +14], $p=0.01$). Participants' mean $RPE_{6\text{-}20}$, Thermal

Sensation and Thermal Comfort ratings across all heat acclimation sessions were not different (p=0.16-0.48) and equalled 15±2 ("Hard"), 10±1 ("Hot"), and 5±2 ("Uncomfortable"), respectively. There were no changes in $T_{recRISE}$ (p=0.12), sweat loss (p=0.73) or SR_{BSA} (p=0.74). Results of heat acclimation sessions are summarised in **Table 4.2**.

Table 4.2 Performance and physiological responses across heat acclimation sessions averaged across Days 1-4 and Days 5-9. Data are presented as mean±SD

	Days 1-4	Days 5-9
Mean power output (W)	108±17	117±21*
Mean T_{rec75} (°C)	38.4±0.2	38.2±0.2*
Resting T_{rec} (°C)	37.3±0.4	37.2±0.2
Peak T_{rec} (°C)	38.6±0.2	38.5±0.2*
$T_{recRISE}$ (°C)	1.4±0.4	1.2±0.3
Peak T_{sk} (°C)	35.5±0.9	35.0±1.0*
Mean HR (beats·minute^{-1})	150±6	145±6*
Mean $\%HR_{max}$	78±3	75±4*
SR_{BSA} (g·h^{-1}·m^{-2})	694±105	705±143
Sweat loss (%BM)	2.9±0.5	2.9±0.6

T_{rec}, rectal temperature; $T_{rec75,}$ mean rectal temperatures recorded during the final 75 minutes of the session; $T_{recRISE}$, maximal change in rectal temperature during exercise from rest; HR, heart rate; $\%HR_{max}$, percentage of age-estimated maximum heart rate; SR_{BSA}, estimated sweat rate relative to body surface area; %BM, percentage of body mass. *Significantly different from Days 1-4 (p<0.05)

4.4.2 Hot time trials

There was a large (η_p^2=0.55) and significant (p<0.01) main effect of acclimation on distance cycled during TT_{HOT} (**Figure 4.3**). *Post hoc* analyses indicated that distance increased by 3.2% (+240 m [+70, +420]; p=0.01) from TT_{HOT1} to TT_{HOT3}, and by 1.8% (+140 m [+10, +270]; p=0.04) from TT_{HOT2} to TT_{HOT3} (**Table 4.3**). There was no difference in distance cycled from TT_{HOT1} to TT_{HOT2} (+100 m [−100, +300]; p=0.47).



Figure 4.3 Distance cycled (***A***) and mean power output (***B***) during time trials in hot conditions (35°C, 30% RH) performed pre-acclimation (TT_{HOT1}; *white*), following 4 days of heat acclimation (TT_{HOT2}; *light grey*) and following 9 days of heat acclimation (TT_{HOT3}; *dark grey*). Bars represent group mean and filled circles represent individual participant data. *Significant difference between indicated time-points (p<0.05)

Table 4.3 Performance measures during time trials in the heat (35°C, 30% RH). Data are presented as mean±SD

	TT_{HOT1}	TT_{HOT2}	TT_{HOT3}
Distance (km)	7.96±0.58	8.06±0.55	8.20±0.59*†
Mean Power (W)	180±34	185±32	195±36*†
Mean Speed ($km \cdot h^{-1}$)	31.9±2.3	32.3±2.2	32.8±2.4*†

TT_{HOT1}, time trial pre-acclimation; TT_{HOT2}, time trial following 4 days of heat acclimation; TT_{HOT3}, time trial following 9 days of heat acclimation. *Significant increase from TT_{HOT1}; †Significant increase from TT_{HOT2}, (p<0.05).

These results were matched by the comparison of minute averages of power output. A two-way ANOVA yielded a large (η_p^2=0.59) and significant main effect of acclimation (p<0.01), but no significant *time* × *interval* interaction (p=0.20; **Figure 4.4**). *Post hoc* analyses of *time* indicated that power output during the 15-min trial increased by 8.1% (+14 W [+4, +24]; p=0.01) from TT_{HOT1} to TT_{HOT3}, and by 4.8% (+9 W [0, +18]; p=0.05) from TT_{HOT2} to TT_{HOT3} (**Table 4.3**). There was no difference in mean power output from TT_{HOT1} to TT_{HOT2} (+5 W [−6, +16]; p=0.55). Additionally, AUC calculated from minute averages of power output yielded a large (η_p^2=0.58) and significant (p<0.01) main effect of acclimation. *Post hoc* comparisons revealed that power output AUC during TT_{HOT3} was greater than in TT_{HOT1} (+7.6% [+2.1, +12.8]; p=0.01) and showed a trend toward increases from TT_{HOT2} (+4.4% [−0.4, +7.7]; p=0.07). Power output AUC was not different between TT_{HOT1} to TT_{HOT2} (+3.2% [−3.0, +7.0]; p=0.53).

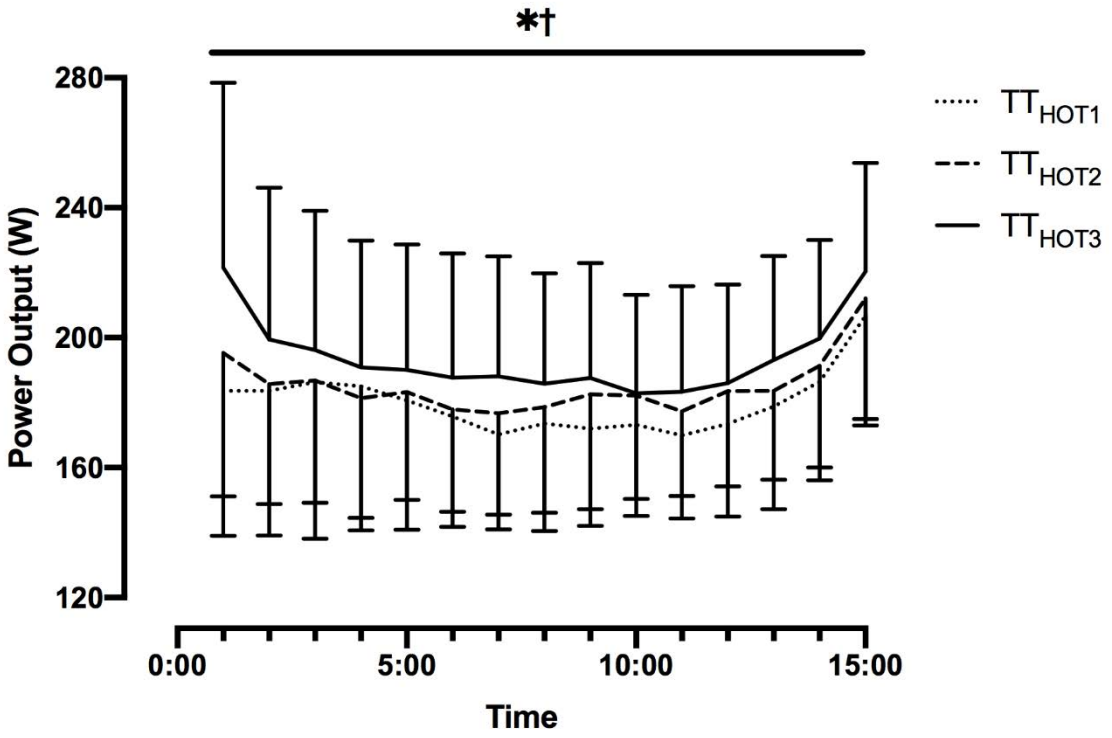


Figure 4.4 Power output during 15-min time trials in hot conditions (35°C, 30% RH) performed pre-acclimation (TT_{HOT1}; *dotted line*), following 4 days of heat acclimation (TT_{HOT2}; *dashed line*), and following 9 days of heat acclimation (TT_{HOT3}; *solid line*). Each data point is an average of the preceding minute, and presented as mean±SD. Significant effect of acclimation: *TT_{HOT3} > TT_{HOT1} (p<0.05), †TT_{HOT3} > TT_{HOT2} (p<0.05)

There was a large (η_p^2=0.57) and significant (p<0.01) main effect of acclimation on mean cycling speed during time trials in hot conditions. *Post hoc* analyses indicated that mean cycling speed increased by 3.0% (+0.9 km·h^{-1} [+0.2, +1.7]; p=0.01) from TT_{HOT1} to TT_{HOT3}, and by 1.6% (+0.5 km·h^{-1} [0, +1.1]; p=0.05) from TT_{HOT2} to TT_{HOT3} (Table **4.3**). There was no difference in distance cycled from TT_{HOT1} to TT_{HOT2} (+0.4 km·h^{-1} [−0.4, +1.2]; p=0.44).

Mean (p=0.63), peak (p=0.97), rise (p=0.46) or AUC (p=0.39) of T_{rec} during TT_{HOT} were not affected by heat acclimation (**Table 4.4**). There was an average reduction in T_{rec} at rest, although this was not significant (p=0.07; **Table 4.4**). Mean (p=0.26) and peak (p=0.13) T_{sk} during TT_{HOT} were not affected by acclimation (**Table 4.4**). Mean (p=0.45) and peak (p=0.38) %HRmax during TT_{HOT} was not different between trials (**Table 4.4**). Two-way ANOVAs of minute averages of T_{rec}, T_{sk} and HR showed no main effects of *time* (p=0.37-0.56) or *time* × *interval* interactions (p=0.39-0.84) during TT_{HOT} (**Figure 4.5**).

Table 4.4 Physiological and psychophysical measures recorded during time trials in hot conditions. Data are presented as mean±SD

	TT_{HOT1}	TT_{HOT2}	TT_{HOT3}
Thermoregulatory			
Resting T_{rec} (°C)	37.2±0.4	37.2±0.3	37.0±0.4
Mean T_{rec} (°C)	37.7±0.2	37.7±0.3	37.6±0.3
Peak T_{rec} (°C)	38.1±0.3	38.1±0.3	38.1±0.4
$T_{recRISE}$ (°C)	0.9±0.5	0.9±0.3	1.0±0.6
Mean T_{sk} (°C)	34.6±0.6	34.1±0.7	34.5±0.9
Peak T_{sk} (°C)	35.0±0.5	34.5±0.5	35.0±0.5
Cardiovascular			
Mean HR (beats · minute^{-1})	168±14	165±9	168±12
Mean $\%HR_{max}$	87±7	86±4	87±6
Peak $\%HR_{max}$	94±5	93±5	95±4
Sudomotor response			
Active sweat glands per cm^2	58±23	63±24	75±25*†
Blood lactate			
Pre-test (mmol · L^{-1})	1.2±0.7	1.0±0.4	1.0±0.5
Post-test (mmol · L^{-1})	11.1±4.1	11.3±1.7	12.8±2.7
Thermal comfort (10-point scale)			
Pre-test	3±2	3±1	2±1
Post-test	5±1	5±2	5±2
Thermal sensation (13-point scale)			
Pre-test	9±1	9±1	8±0*
Post-test	10±1	10±1	10±1

TT_{HOT1}, pre-acclimation trial; TT_{HOT2}, trial following 4 days of heat acclimation; TT_{HOT3}, trial following 9 days of heat acclimation. T_{rec}, rectal temperature; $T_{recRISE}$, change in rectal temperature during time trial; T_{sk}, weighted mean skin temperature; HR, heart rate; $\%HR_{max}$, percentage of age-estimated maximum heart rate. *Significant difference from TT_{HOT1}; †Significant difference from TT_{HOT2}, (p<0.05)

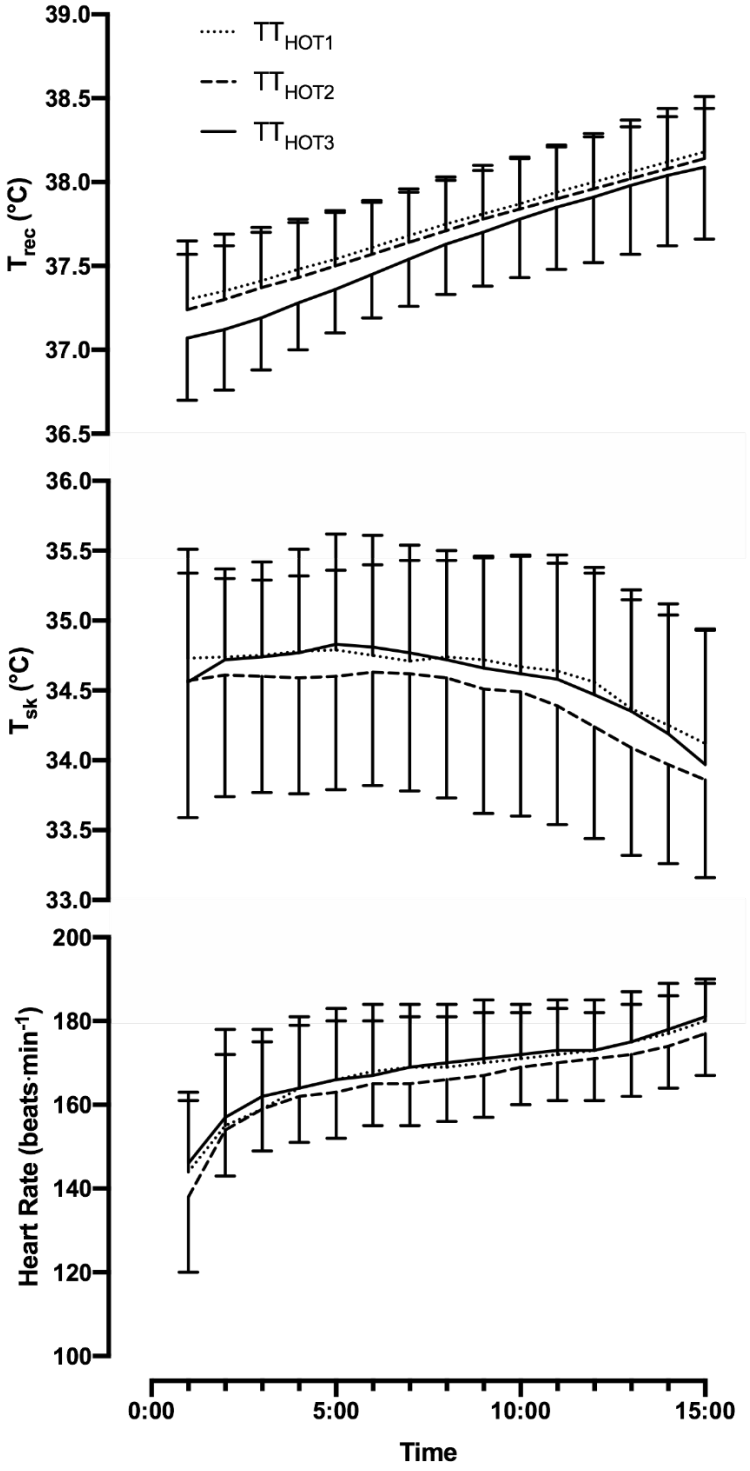


Figure 4.5 Physiological responses during 15-min time trials in hot conditions (35°C, 30% RH) performed pre-acclimation (TT_{HOT1}; *dotted line*), following 4 days of heat acclimation (TT_{HOT2}; *dashed line*), and following 9 days of heat acclimation (TT_{HOT3}; *solid line*). Rectal temperatures (T_{rec}; *top*), skin temperatures (T_{sk}; *middle*) and heart rate (*bottom*) are presented as an average of the preceding minute. Data points are presented as mean±SD.

There was a main effect of acclimation on number of active sweat glands immediately following TT_{HOT} (*time*: p=0.01, η_p^2=0.64). *Post hoc* analyses indicated that number of active sweat glands increased by 33% (+17 active sweat glands per cm^2 [+3,

+30]; p=0.02) from TT_{HOT1} to TT_{HOT3}, and by 22% (+12 active sweat glands per cm^2 [+6, +17]; p<0.01) from TT_{HOT2} to TT_{HOT3} (**Table 4.4**). There was no difference in sweat glands activity from TT_{HOT1} to TT_{HOT2} (+5 active sweat glands per cm^2 [−7, +17]; p=0.62). An example of sweat gland activity recorded following TT_{HOT} is depicted in **Figure 4.6**.

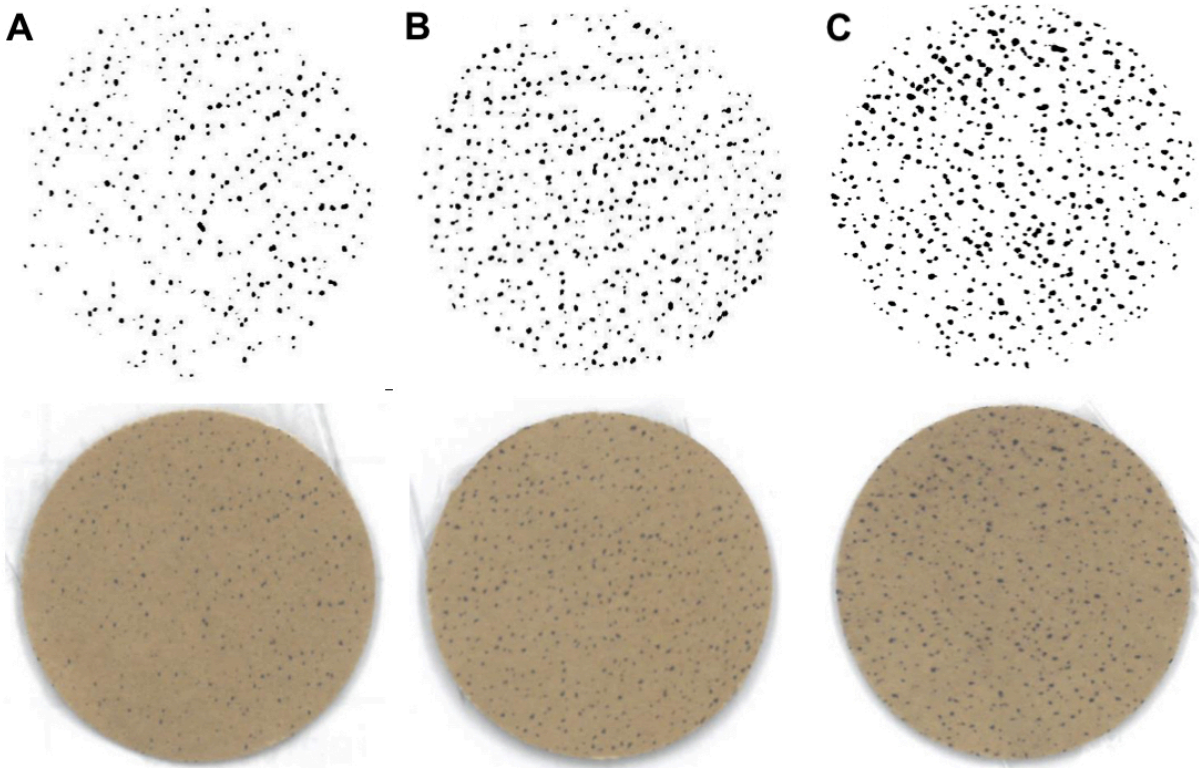


Figure 4.6 Example of sweat gland activity measured immediately following time trials **(*A*)** pre-acclimation (TT_{HOT1}), **(*B*)** following 4 days of heat acclimation (TT_{HOT2}), and **(*C*)** following 9 days of heat acclimation (TT_{HOT3}). Bottom row images are scanned copies of iodine-cotton paper applied to participant's forearm. Top row of images are the same samples following computer processing (ImageJ; Gagnon et al., 2012).

There was no significant difference in SR_{BSA} during TT_{HOT} and including the 75-min heat acclimation that followed (main effect of *time*: p=0.08). There were no differences in blood lactate at rest (immediately preceding TT_{HOT}; p=0.34) or immediately following TT_{HOT} (p=0.41; **Table 4.4**). Ratings of Thermal Sensation taken in the environmental chamber immediately before exercise were significantly different between trials (main effect of *time*: p=0.02; **Table 4.4**), and on average, corresponded to "Warm" (TT_{HOT1}), "Warm" (TT_{HOT2}) and "Slightly Warm" (TT_{HOT3}). *Post hoc* pairwise comparisons indicated that differences were between TT_{HOT1} and TT_{HOT3} (p=0.03). There were no significant differences in Thermal Sensation between TT_{HOT1} and TT_{HOT2} (p=0.10) or between TT_{HOT2} and TT_{HOT3} (p=0.08). Ratings of Thermal Comfort taken in the environmental chamber immediately before exercise were not significantly different between trials (average ratings corresponded to ratings between "Comfortable" and "Slightly Uncomfortable"; p=0.39; **Table 4.4**). Ratings of

Thermal Sensation and Thermal Comfort taken at the end of TT_{HOT} were not significantly different between trials ("Hot", p=0.25; and "Uncomfortable", p=0.53, respectively; **Table 4.4**).

4.5 Discussion

This study was designed to determine whether STHA (4 days) is sufficient to improve self-paced endurance performance in hot conditions in females, as has been observed in males, or whether a longer heat acclimation stimulus (i.e. 9 days) is required. In this study's female cohort, STHA did not significantly improve time-trial performance in the heat; however, 9 days of heat acclimation did. These results were consistent with the study hypothesis, which predicted that STHA would be insufficient to improve self-paced performance in females, and that a longer heat acclimation stimulus would be required to induce the physiological adaptations needed for performance improvements in the heat.

4.5.1 Self-paced endurance performance

Following STHA, female participants showed no significant performance improvements in distance cycled, mean power output, or speed during TT_{HOT2}, as compared to TT_{HOT1}. This is in direct contrast to a number of studies in male cohorts, where males have shown meaningful physiological adaptions *and* improved endurance performance in the heat following STHA (Garrett et al., 2009; Garrett et al., 2012; Chen et al., 2013; Racinais et al., 2015b; Guy et al., 2016; Willmott et al., 2016; Wingfield et al., 2016; James et al., 2017b; Willmott et al., 2017). Thus, it appears that STHA using 90-min daily exercise heat stress is insufficient to improve endurance performance in females, reflecting the lack of physiological adaptation to heat acclimation previously demonstrated in females following STHA (Mee et al., 2015b). The current study's performance results following STHA differ from those observed by Sunderland et al. (2008), who reported a 33% improvement in distance run during a repeated shuttle run performance test (Loughborough Intermittent Shuttle Test) following STHA (4 days) in a female cohort. Of note, time to exhaustion is the main outcome measure of the Loughborough Intermittent Shuttle Test. This outcome is influenced by technique (i.e. ability to change direction and accelerate; Mendez-Villanueva & Buchheit, 2013), making it less reliable and subject to greater variation than the self-paced performance trial used in the current study (Hickey et al., 1992; Gosens et al., 2015; Borg et al., 2018). Furthermore, the behavioural regulation of performance possible in a self-paced time trial is not available in a time to exhaustion protocol (Schlader et al., 2011b). Indeed, the

lower pre-exercise Thermal Sensation reported by participants after 9 days of heat acclimation may be an indication of perceptual changes contributing to behavioural regulation (i.e. pacing). Thus, the self-paced performance test used in the current study is a more reliable and holistic assessment of performance than a time-to-exhaustion test. Despite efforts in the current study to create an "intense" heat stimulus by combining isothermic heat acclimation, HIIT, and permissive dehydration, it still appears that females require either a longer daily heat exposure (Mee et al., 2018), or a greater number of heat exposures (as observed in the current study and by Mee et al., 2015) to improve exercise performance in the heat.

This is the first study to quantify improvements in self-paced time-trial performance following a longer (i.e. 9 days) heat acclimation stimulus in a female cohort. The ~8% mean improvement in mean power output in TT_{HOT3} as compared to TT_{HOT1} is comparable to performance improvements observed in male, or mostly male cohorts following similar heat acclimation protocols. Keiser et al. (2015) showed that male participants experienced a ~10% improvement in power output during a 30-min self-paced time trial following 10 days of heat acclimation (daily bouts: 90-min cycling at 50% $\dot{V}O_{2max}$ in 38°C, 30%RH). Lorenzo et al. (2010) also found that participants (10 males and 2 females) had an 8% mean improvement in power output during their 1-h self-paced time trial following 10 days of heat acclimation (daily bouts: 90-min cycling at 50% $\dot{V}O_{2max}$ in 40°C, 30%RH). In the current study, improvements in mean power output coincided with improvements in mean cycling speed and distance covered from TT_{HOT1} to TT_{HOT3}. These data demonstrate that 9, but not 4 days of heat acclimation, improves endurance performance outcomes in females.

4.5.2 Physiological measures

Participants exhibited reduced markers of physiological strain (i.e., T_{rec}, T_{sk} and HR) during days 5–9 of HA, as compared to days 1–4. These physiological changes occurred in spite of an increased mean power output during days 5–9 of heat acclimation. Although these data indicate a reduction in the desired stimulus across the heat acclimation protocol, it also indicates that the greatest heat stimulus was administered during STHA. Furthermore, the improved performance in TT_{HOT3} as compared to the previous trials indicates that this reduced stimulus during days 5–9 was still effective in producing heat acclimation-related performance improvements. Also, this acclimation protocol produced a sufficient dehydration stimulus, as the ~3% body mass loss achieved across heat acclimation days 1–4 and 5–9 in addition to permissive dehydration presumably exceeded the osmotic threshold required for compensatory fluid regulatory responses (i.e. 2% body mass loss; Cheuvront and Kenefick,

2014). However, as changes in plasma volume were not measured here, it is unknown whether participants experienced the fluid regulatory responses typically associated with heat acclimation.

There was a trend for a lower T_{rec} at rest before TT_{HOT3}, which appeared to influence T_{rec} during the initial minutes of TT_{HOT3} (albeit not significantly). Menstrual cycle phase and associated changes in female sex hormones influence resting T_{rec} (Inoue et al., 2005) and the overall thermoregulatory set point range (Charkoudian & Stachenfeld, 2016). This may have contributed to the non-significant change in resting T_{rec} observed in the current study. By the end of each TT_{HOT}, T_{rec} reached similar values (~38.1°C). This is perhaps unsurprising as a previous study has shown that heat acclimation does not change the maximal T_{rec} reached (40.1–40.2°C) during a 43.4-km time trial in the heat, despite a lower T_{rec} for the first 80% of the post-acclimation time trial (Racinais et al., 2015b).

In the current study, there was an observed increase of active sweat glands at the end of TT_{HOT3}. This contrasts with findings in male cohorts, where sweat gland activation did not increase following 8–10 days of heat acclimation (Inoue et al., 1999; Lee et al., 2010; Poirier et al., 2016). In the current study, the number of active sweat glands (75±25 per cm^2) at the end of TT_{HOT3} were lower than values previously reported in acclimated males (~96–108 per cm^2; Inoue et al., 1999; Lee et al., 2010; Poirier et al., 2016) and unacclimated females (~93 per cm^2; Knip, 1969). Therefore, changes observed following the 15-min TT_{HOT} may not indicate improved maximal sweat gland activation *per se*, but rather earlier activation of the sweat glands. Although there is large intra-subject coefficient variation associated with this measure, the 33% and 22% mean improvements following TT_{HOT3} in comparison to TT_{HOT1} and TT_{HOT2}, respectively, surpass the ~11% coefficient of variation reported by Gagnon et al. (2012).

4.5.3 Perspectives and considerations

These results contribute to the limited research that informs the expected performance outcomes of heat acclimation for female athletes. The results of this study indicate that while heat acclimation can be an effective training component in preparation for competition in the heat, female athletes may require up to 9 days of 90-min heat acclimation sessions before experiencing performance improvements. However, there will be individual variation in how athletes (male or female) respond to heat acclimation (Racinais et al., 2012). In the current study, three participants' performance deteriorated in TT_{HOT2} as compared to TT_{HOT1}, whereas four participants showed improvements and one participant showed no change. Thus, some female athletes may achieve meaningful performance

benefits after 4 days of heat acclimation, while others could require longer than 9 days of. A heat acclimation protocol lasting longer than 9 days has yet to be initiated in a female cohort, which would be hypothesised to further stabilize adaptions and improve performance (Racinais et al., 2015b). It is also unclear how different phases of the menstrual cycle/hormonal contraception may affect heat adaption during acclimation (as outlined in **Section 2.2.2.2**). Future research is also needed to clarify the impact of mixed-intensity heat acclimation on longer performance tests in both male and female athletes.

Despite the absence of a control group, it is unlikely that performance improvements in TT_{HOT3} were due to learning or training effects. After preliminary testing and familiarisations, TT_{HOT1} was the fourth time that participants would have completed the 15-min time trial, minimising learning effects. Furthermore, performance improvements in the current study are similar to previous studies (Lorenzo et al., 2010; Keiser et al., 2015), where control groups showed no improvements.

It is possible that the combined high-intensity exercise, isothermic heat acclimation protocol used in the current study may have caused a general fatigue that impaired performance during TT_{HOT2} and TT_{HOT3} (Schmit et al., 2018; Reeve et al., 2019). However, this is a negative bias as fatigue-related performance impacts would presumably have been greatest at TT_{HOT3}. A further consideration is that heat acclimation adaptions are specific to the type/intensity of exercise employed (Wingfield et al., 2016). Therefore, the 15 min of HIIT undertaken at the beginning of each heat acclimation session may have facilitated specific adaptations. Whether this type of mixed-intensity heat acclimation (15-min HIIT + 75-min isothermic heat acclimation) would be equally or more effective than steady-state isothermic heat acclimation protocols typically reported in the literature remains unknown.

This study did not control for menstrual cycle. Recent data has shown that performance under heat stress is not affected by menstrual cycle or oral contraceptive pill (OCP) use in trained female athletes (Lei et al., 2017; Lei et al., 2019), nor does menstrual cycle affect whole-body heat loss (Notley et al., 2019b). Eumenorrheic participants and OCP users did not cross over phases between TT_{HOT1} and TT_{HOT2}. Participants were counterbalanced in their phases in TT_{HOT3}, with both eumenorrheic participants being in opposite phases and both OCP users being in opposite phases (i.e., pill-taking, or non-pill-taking). None of the other four participants (contraceptive implant or copper intrauterine devices) were menstruating during the protocol, mitigating concerns of premenstrual symptoms that could affect performance (Giacomoni et al., 2000). Despite this, variable hormonal states may have affected the degree of relative heat stimulus administered when targeting an absolute core temperature of 38.5°C during heat acclimation sessions.

Finally, it should be noted that measures of sweat gland activity were taken from sites on the forearm and are not a precise indication of whole-body sweat gland adaptations

given the regional heterogeneity of sweat gland activity. While an increased sweat gland activity may imply a better use of body surface area to dissipate heat, sweat gland activation is not directly proportional to local sweat output of the area (Poirier et al., 2016). In future, measures of local sweat output should be combined with measures of sweat gland activation to fully understand sex differences in peripheral sudomotor adaptations.

4.6 Conclusions

This study was the first to document performance outcomes during self-paced time trials in a female cohort following STHA (4 days) and 9 days of a high-intensity exercise, isothermic heat acclimation protocol. In the current study, females did not show an improvement in self-paced endurance performance following STHA. This differs from the well-documented performance improvements previously observed in male cohorts following STHA. However, following 9 days of heat acclimation, females achieved meaningful improvements in self-paced endurance performance. These improvements included an ~8% increase in mean power output, a ~3% increase in distance cycled, and a ~3% increase in speed when performing a 15-min self-paced time trial in hot conditions. These data offer a reference for the changes that female athletes can expect when undergoing heat acclimation with the aim of improving self-paced endurance exercise performance in hot conditions, and provides further evidence that STHA may be insufficient for female athletes.

5. IS ACTIVE HEAT ACCLIMATION ERGOGENIC FOR PERFORMANCE IN COOL CONDITIONS IN FEMALES?

5.1 Abstract

Background: Chronic exposure to exercise-heat stress elicits adaptations that may be beneficial for exercise performance in cool conditions, though the efficacy of active heat acclimation to improve self-paced exercise performance in a female cohort is unknown. This study aimed to investigate self-paced endurance performance in cool conditions following 5 and 10 days of isothermic heat acclimation in a female cohort.

Methods: Eight female endurance athletes (mean±SD, age 27±5 years, mass 61±5 kg, $\dot{V}O_{peak}$ 47±6 mL · kg · min^{-1}) performed 15-min self-paced cycling time trials in cool conditions (15°C, 30%RH) before (TT_{COOL1}), and after 5 days (TT_{COOL2}) and 10 days (TT_{COOL3}) of isothermic heat acclimation (with power output manipulated to increase and maintain rectal temperature (T_{rec}) at ~38.5°C for 90-min cycling in 40°C, 30%RH) with permissive dehydration.

Results: There were no significant changes in distance cycled, mean power output or cycling speed (p=0.85-0.87) following 5 days of heat acclimation. Distance cycled (+2.5%), mean power output (+5.3%) and cycling speed (+2.4%) were significantly greater in TT_{COOL3} than in TT_{COOL1} (p<0.04); but not TT_{COOL2} (p=0.27-0.31). Improvements in distance cycled from TT_{COOL1} to TT_{COOL3} showed a strong negative correlation with distance cycled at baseline (r=-0.86, p<0.01), whereby a greater improvement was associated with a shorter distance cycled at baseline. Resting T_{rec} was 0.3°C lower at TT_{COOL3} (p=0.01), but not TT_{COOL2} (p=0.23), compared to TT_{COOL1}.

Conclusions: Five-days heat acclimation was insufficient to improve performance in cool conditions in females. Ten-days heat acclimation improved performance in cool conditions in females, with the greatest improvements shown in participants who covered the shortest distance at baseline.

5.2 Introduction

Heat acclimation has historically been used to improve tolerance and overall exercise or work capacity in hot conditions. However, the array of physiological adaptations that have been observed following chronic heat exposure, including thermoregulatory, cardiovascular, haematological, fluid-regulatory and metabolic adaptations, may also benefit exercise performance in cool conditions (Corbett et al., 2014). Lorenzo and colleagues (2010) were the first to demonstrate improvements in a wide range of performance outcomes (i.e. $\dot{V}O_{2max}$, lactate threshold and time trial performance) in cool conditions (13°C, 30%RH) following 10 days of 90-min heat acclimation (40°C, 30%RH). Since then, there has been conflicting evidence and some debate as to whether heat acclimation is indeed ergogenic (Minson & Cotter, 2016; Nybo & Lundby, 2016). However, these studies in trained participants have been in male or mostly male cohorts.

There is also some evidence that short-term heat acclimation (<7 days; STHA; Garrett et al., 2011) could be a sufficient stimulus to improve performance in cool or temperate conditions. Neal and colleagues (2016a) observed some performance improvements (i.e. improved lactate threshold and peak power output during a graded exercise test, and a trend for a 2% improvement in mean power during a 20-km time trial) in temperate conditions (22°C, 60%RH) alongside thermoregulatory and cardiovascular adaptations in males following 5 days of isothermic heat acclimation with permissive dehydration. Similarly, Tebeck et al. (2019) observed a reduction in heart rate at lactate threshold power in temperate conditions (21°C, 53%RH) following 5 days of isothermic heat acclimation. Other studies (Morrison et al., 2002; Chen et al., 2013; Chalmers et al., 2016; Zurawlew et al., 2016; Philp et al., 2017; Mackay et al., 2018) have shown no or trivial effects of STHA on performance in cool or temperate conditions. However, these studies did not use an isothermic protocol, which might not have provided a sustained thermal stimulus throughout (Taylor, 2014). Moreover, Garrett and colleagues (2011; 2014) have suggested that permissive dehydration may accelerate the induction of heat acclimation via an augmented fluid-regulatory hormone response and subsequent plasma volume expansion. As with longer heat acclimation protocols, studies examining STHA have primarily been in male cohorts. One exception is that by Pethick et al. (2018), who reported a small (1.5%) but meaningful improvement in 30-15 intermittent running test performance (20°C) in trained female soccer players following 5 days of 90-min soccer-specific circuit training in the heat (35°C, 37%RH). However, this 30-15 intermittent running test is associated with greater variability and relatively reduced reliability as compared to a time trial (Borg et al., 2018), and thus the effects of STHA on self-paced performance responses in a female cohort is unknown. Additional investigation is warranted, given sex differences in the temporal

patterning of thermoregulatory and cardiovascular markers of heat acclimation (i.e. reduced body temperatures and heart rate; Mee et al., 2015b) thought to be indicative of adaptations contributing to performance improvements in cool conditions.

Overall, it is unknown if heat acclimation can be ergogenic for self-paced endurance performance in cool conditions in females, and if so, if improvements can be observed following STHA.

5.2.1 Aim

The aim of the present study was to investigate self-paced endurance performance in cool conditions following 5 and 10 days of a high-intensity exercise, isothermic heat acclimation protocol with permissive dehydration in a female cohort.

5.2.2 Hypotheses

It was hypothesised that females would exhibit performance improvements in self-paced exercise following 10, but not 5 days of heat acclimation, as previous literature (Mee et al., 2015b) indicates that 5 days of 90-min heat acclimation is unlikely to be a sufficient stimulus for the thermoregulatory and cardiovascular adaptations that could underpin performance improvements in cool conditions.

5.3 Methods

5.3.1 General overview and design

These data were collected alongside data in the previous chapter, with the same eight female participants (**Section 4.3.1**), and detailed methods have been described in the General Methods chapter and/or in **Chapter 4** (referred herein with section markers to **Chapters 3 & 4**). All experimental procedures were completed in the environmental chamber in the School of Sport, Exercise and Rehabilitation Sciences building at the University of Birmingham (**Section 3.4.1**). Participants performed the 15-min cycling time trial in cool (15°C, 30% RH) conditions (i.e. TT_{COOL}; **Section 3.7.2**) pre-acclimation (TT_{COOL1}), following 5 days (TT_{COOL2}) and following 10 days of isothermic heat acclimation (TT_{COOL3}). An overview of the experiment is presented in **Figure 4.1**.

5.3.2 Experimental procedures

Isothermic heat acclimation sessions were 90-min cycling in 40°C, 30%RH with power output manipulated to increase and maintain T_{rec} at ~38.5°C and permissive dehydration (**Sections 3.7.1** and **4.3.3).**

Cool time trials followed confirmation of normal hydration and 10-min stabilisation at room temperature, and consisted of 5-min warm-up and 15-min self-paced cycling time trial (**Section 3.7.2)**. Power output, speed, distance cycled, HR, T_{rec} and T_{sk} were recorded continuously. Blood lactate concentration was measured immediately following TT_{COOL} (**Sections 3.9.7.2** and **3.9.7.4)**.

5.3.3 Data analyses and statistical approach

Data analyses of peak T_{rec}, $T_{recRISE}$, T_{rec75}, T_{sk} $\%HR_{max}$, and sweat loss were as described in the previous chapter (**Section 4.3.4**).

For analysis of performance and physiological differences during heat acclimation days 1–5 vs. days 6–10, mean values were analysed using paired-samples *t*-tests. Single time-point performance values during TT_{COOL} were analysed using repeated-measures one-way ANOVAs. Additionally, 1-min averages of power output, T_{rec} and HR were analysed using a two-way repeated-measures ANOVA (3 trials × 15 time-intervals; *time* × *interval*). When a significant main effect was found, Dunn-Bonferroni-corrected *post hoc* comparisons were made. Main effect sizes for both one-way and two-way ANOVAs were calculated using partial eta-squared (η_p^2). Normality of the data was assessed using the Shapiro-Wilk Test (not violated on any occasion). Sphericity was assessed using were assessed using Mauchly's test of sphericity, and Greenhouse–Geisser corrections were applied where assumptions of sphericity were violated.

Pearson's correlations were performed *posteriori* to examine possible relationships between baseline performance/training variables (i.e. $\dot{V}O_{2peak}$, training hours and TT_{COOL1} performance) and changes in distance cycled from TT_{COOL1} to TT_{COOL3}.

A power analysis indicated that eight participants was a sufficient sample size to detect a 6% difference in power output during time trial performance (as observed by Lorenzo et al., 2010). This analysis used an accepted parameter of power ($\beta \geq 0.80$) at an α level of 0.05.

Absolute data and within-subject changes are expressed as mean ± standard deviation (SD), and 95% confidence limits ([95% CL: lower limit, upper limit]) are presented with mean between-group differences. Significance was set at $p<0.05$ for each analysis.

5.4 Results

5.4.1 Active heat acclimation

Heat acclimation data are summarised in **Table 5.1**. Mean T_{rec75} (−0.2°C [−0.1, −0.3]; p<0.01) was lower and there was a trend for peak T_{rec} (−0.1°C [−0.0, −0.2]; p=0.09) to be lower during heat acclimation sessions on days 6–10 as compared to days 1–5. Resting T_{rec} (p=0.44) and $T_{recRISE}$ (p=0.38) were not different between heat acclimation sessions on days 6–10 as compared to days 1–5. Peak T_{sk} was lower during heat acclimation sessions on days 6–10 as compared to days 1–5 (−0.5°C [−0.1, −1.0]; p=0.02). There was a trend for $\%HR_{max}$ to be lower during heat acclimation sessions on days 6–10 as compared to days 1–5 (−2% [0, −5]; p=0.08). Neither estimated sweat rate (p=0.27), total sweat loss (p=0.22) nor power output (p=0.11) were different between heat acclimation sessions on days 6–10 as compared to days 1–5.

Table 5.1 Performance and physiological responses across heat acclimation sessions averaged across Days 1-5 and Days 6-10. Data are presented as mean±SD

	Days 1-5	Days 6-10
Mean power output (W)	110±18	117±21
Mean T_{rec75} (°C)	38.3±0.1	38.1±0.1*
Resting T_{rec} (°C)	37.3±0.4	37.2±0.2
Peak T_{rec} (°C)	38.6±0.2	38.5±0.2
$T_{recRISE}$ (°C)	1.3±0.3	1.3±0.3
Peak T_{sk} (°C)	35.4±1.0	34.9±1.1*
Mean $\%HR_{max}$	78±3	76±5
SR_{BSA} ($g \cdot h^{-1} \cdot m^{-2}$)	677±129	703±121
Sweat loss (%BM)	2.9±0.5	3.0±0.5

T_{rec}, rectal temperature; $T_{rec75,}$ mean rectal temperatures recorded during the final 75 minutes of the session; $T_{recRISE}$, maximal change in rectal temperature during exercise from rest; T_{sk}, mean weighted skin temperature; HR, heart rate; $\%HR_{max}$, percentage of age-estimated maximum heart rate; SR_{BSA}, estimated sweat rate relative to body surface area; %BM, percentage of body mass. *Significantly different from Days 1-5 (p<0.05)

5.4.2 Cool time trials

There was a large (η_p^2=0.40) and significant (p=0.03) main effect of acclimation on distance cycled during TT_{COOL} (**Figure 5.2**). *Post hoc* analyses indicated that distance increased by 2.5% (+193 m [+13, +372]; p=0.04) from TT_{COOL1} to TT_{COOL3} (**Table 5.2**). There was no difference in distance cycled from TT_{COOL1} to TT_{COOL2} (+84 m [−143, +310]; p=0.86), nor from TT_{COOL2} to TT_{COOL3} (+109 m [-71, +288]; p=0.30).

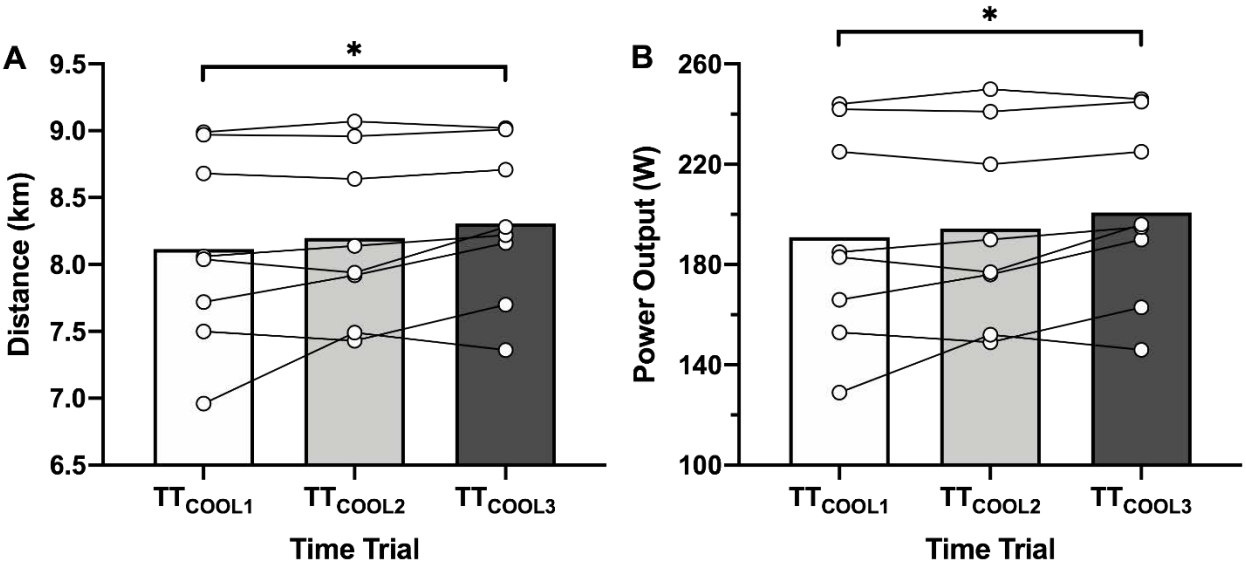


Figure 5.1 Distance cycled (***A***) and power output (***B***) during time trials in cool conditions (15°C, 30% RH) performed pre-acclimation (TT_{COOL1}; *white*), following 5 days of heat acclimation (TT_{COOL2}; *light grey*), and following 10 days of heat acclimation (TT_{COOL3}; *dark grey*). Bars represent group mean and filled circles represent individual participant data. *Significant difference between TT_{COOL1} and TT_{COOL3} (p<0.05)

Table 5.2 Performance measures during time trials in cool conditions. Data are presented as mean±SD

	TT_{COOL1}	TT_{COOL2}	TT_{COOL3}
Distance (km)	8.11±0.73	8.20±0.63	8.31±0.59*
Mean power (W)	191±42	194±39	201±36*
Mean speed (km·h^{-1})	32.5±2.9	32.9±2.5	33.3±2.4*

TT_{COOL1}, time trial pre-acclimation; TT_{COOL2}, time trial following 5 days of heat acclimation; TT_{COOL3}, time trial following 10 days of heat acclimation. *Significant increase from TT_{COOL1} (p<0.05)

There was a large (η_p^2=0.41) and significant (p=0.03) main effect of acclimation on mean cycling speed during the TT_{COOL} (**Table 5.2**). *Post hoc* analyses indicated that mean cycling speed increased by 2.4% (+0.8 km·h^{-1} [+0.1, +1.5]; p=0.03) from TT_{COOL1} to TT_{COOL3}. There was no difference in mean cycling speed from TT_{COOL1} to TT_{COOL2} (+0.3 km·h^{-1} [−0.6, +1.2]; p=0.85), nor from TT_{COOL2} to TT_{COOL3} (+0.4 km·h^{-1} [−0.3, +1.1]; p=0.31).

These results were matched by the comparison of minute averages of power output. A two-way ANOVA yielded a large (η_p^2=0.44) and significant main effect of acclimation (p=0.02), but no significant *time* × *interval* interaction (p=0.18; **Figure 5.3**). *Post hoc* analyses of *time* indicated that power output during the 15-min trial increased by 5.3% (+10 W [+2, +19]; p=0.02) from TT_{COOL1} to TT_{COOL3} (**Table 5.2**). There was no difference in mean power output from TT_{COOL1} to TT_{COOL2} (+4 W [−7, +15]; p=0.87) nor from TT_{COOL2} to TT_{COOL3} (+6 W [−2, +19]; p=0.27).

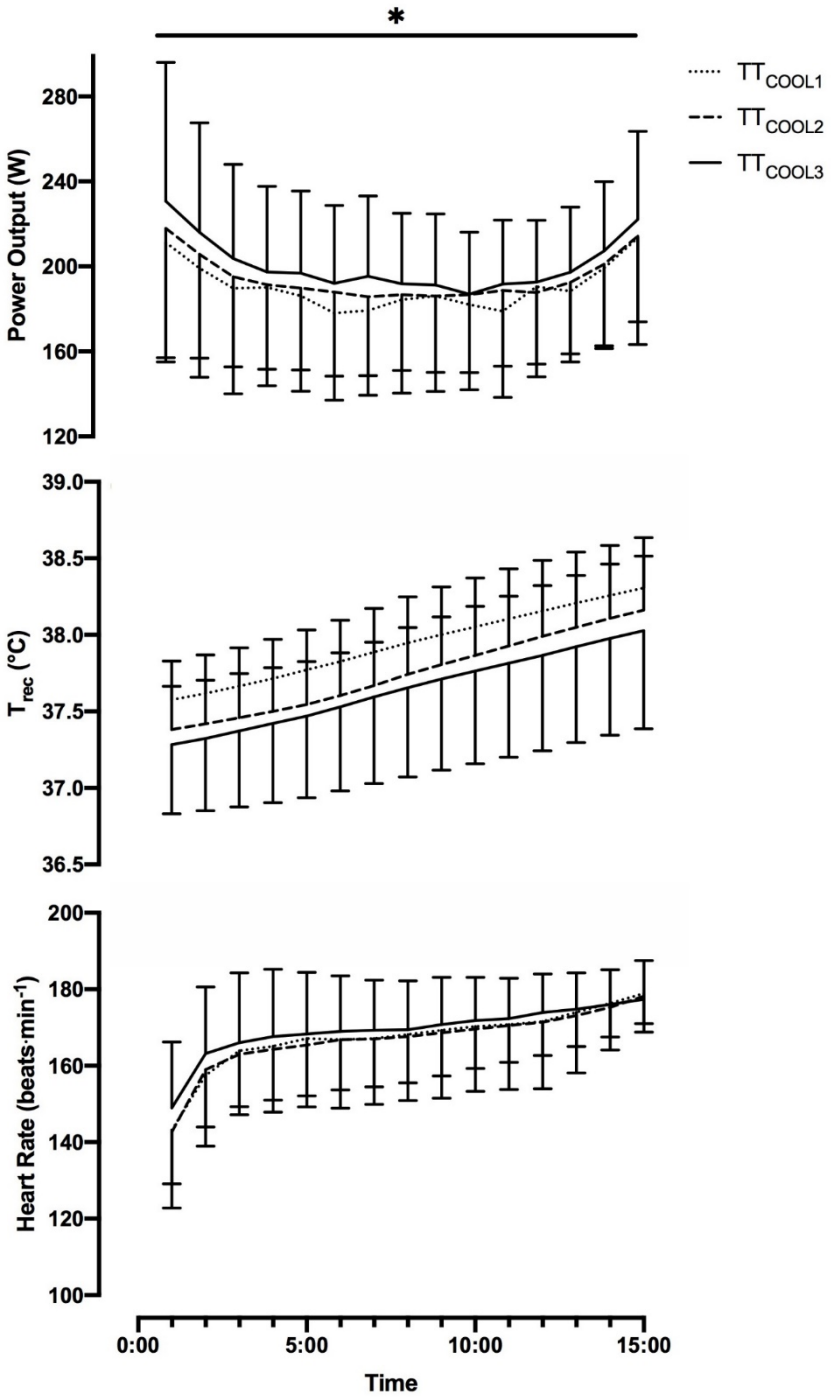


Figure 5.2 Physiological responses during 15-min time trials in cool conditions (15°C, 30% RH) performed pre-acclimation (TT_{COOL1}; *dotted line*), following 5 days of heat acclimation (TT_{COOL2}; *dashed line*), and following 10 days of heat acclimation (TT_{COOL3}; *solid line*). Power output (*top*), rectal temperatures (T_{rec}; *middle*) and heart rate (*bottom*) are presented as an average of the preceding minute. Data points are presented as mean±SD. *Significant effect of acclimation: TT_{COOL3} > TT_{COOL1} (p<0.05)

Mean (p=0.11), peak (p=0.41) and rise (p=0.88) of T_{rec} during TT_{COOL} were not affected by heat acclimation (**Table 5.3**). However, there was a large (η_p^2=0.51) and significant (p=0.02) main effect of acclimation on resting T_{rec} before TT_{COOL}. *Post hoc* analyses indicated that resting T_{rec} was reduced by 0.3°C [-0.1, -0.4]; p=0.01) from TT_{COOL1} to TT_{COOL3}. There was no difference in resting T_{rec} from TT_{COOL1} to TT_{COOL2} (-0.2°C [+0.1, -0.5]; p=0.23), nor from TT_{COOL2} to TT_{COOL3} (-0.1°C [+0.1, −0.2]; p=0.73). Peak T_{sk} during TT_{COOL} was not affected by acclimation (p=0.53; **Table 5.3**). Mean (p=0.42) and peak (p=0.50) $\%HR_{max}$ during TT_{COOL} were not different between trials (**Table 5.3**). Two-way ANOVAs of minute averages of T_{rec} and HR showed no main effects of *time* (p=0.15-0.49) or *time* × *interval* interactions (p=0.52-0.73) during TT_{COOL} (**Figure 5.3**). Blood lactate concentration immediately following TT_{COOL} was not affected by acclimation (p=0.89; **Table 5.3**).

Table 5.3 Physiological and psychophysical measures recorded during time trials in cool conditions. Data are presented as mean±SD

	TT_{COOL1}	TT_{COOL2}	TT_{COOL3}
Thermoregulatory			
Resting T_{rec} (°C)	37.5±0.2	37.3±0.3	37.2±0.3*
Mean T_{rec} (°C)	37.9±0.3	37.7±0.3	37.7±0.4
Peak T_{rec} (°C)	38.3±0.3	38.2±0.4	38.0±0.6
$T_{recRISE}$ (°C)	0.8±0.4	0.9±0.3	0.8±0.4
Peak T_{sk} (°C)	29.2±1.0	28.5±1.0	28.9±0.7
Cardiovascular			
Mean HR (beats · minute^{-1})	167±14	167±11	169±11
Mean $\%HR_{max}$	87±7	87±5	88±5
Peak $\%HR_{max}$	95±5	94±4	95±5
Blood lactate			
Post-test (mmol· L^{-1})	12.6±2.3	12.8±2.6	12.2±3.3

TT_{COOL1}, pre-acclimation trial; TT_{COOL2}, trial following 5 days of heat acclimation; TT_{COOL3}, trial following 10 days of heat acclimation. T_{rec}, rectal temperature; $T_{recRISE}$, change in rectal temperature during time trial; T_{sk}, weighted mean skin temperature; HR, heart rate; $\%HR_{max}$, percentage of age-estimated maximum heart rate. *Significant difference from TT_{COOL1} (p<0.05)

5.4.3 Relationship between baseline variables and improvements in cool time trial performance

There was a strong negative correlation between distance cycled at TT_{COOL1} and the improvement in distance cycled from TT_{COOL1} to TT_{COOL3} (r=-0.86, p<0.01), whereby those with a shorter distance cycled at baseline experienced a greater improvement at TT_{COOL3} (**Figure 5.4**). The improvement in distance cycled from TT_{COOL1} to TT_{COOL3} was not

significantly correlated with $\dot{V}O_{2peak}$ (r=-0.40, p=0.33) or pre-intervention training hours (r=-0.63, p=0.09).

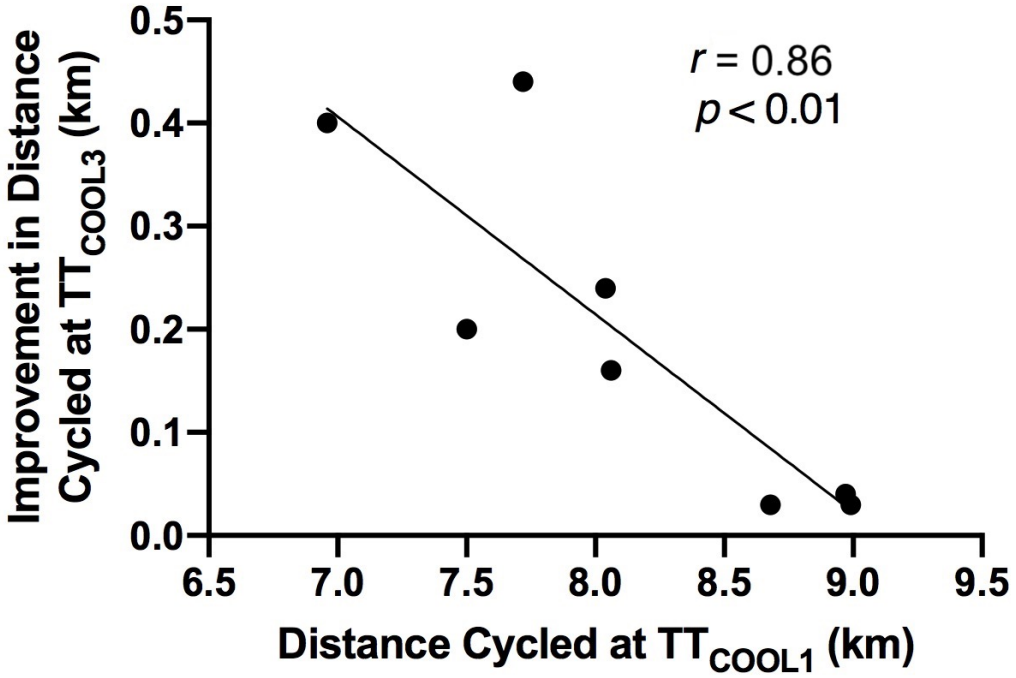


Figure 5.3 Relationship between baseline distance cycled during the 15-min time trial in cool conditions (15°C, 30%RH; TT_{COOL1}) *vs* improvement from baseline following 10 days of heat acclimation (TT_{COOL3}).

5.5 Discussion

This study was designed to determine whether a high-intensity exercise, isothermic heat acclimation protocol with permissive dehydration improves self-paced endurance performance in cool conditions in a female cohort, and if so, whether improvements could be observed following both 5 and 10 days of heat acclimation. In this study's female cohort, 5 days of this high-intensity exercise, isothermic heat acclimation protocol did not significantly improve time trial performance in cool conditions; however, 10 days of heat acclimation did. These results were consistent with the study's hypothesis, which predicted that 5 days of high-intensity exercise, isothermic heat acclimation would be insufficient to improve self-paced performance in females and that a longer heat acclimation stimulus would be required to induce the physiological adaptations needed to be an ergogenic aid.

5.5.1 Self-paced endurance performance following 10 days of heat acclimation

Following 10 days of high-intensity exercise, isothermic heat acclimation with permissive dehydration, females improved all markers of time trial performance. The ~5% improvement in mean power output is comparable to the 6% increase in work done reported by Lorenzo and colleagues (2010) during a 30-min time trial in cool conditions following 10 days of heat acclimation (10M, 2F). However, this improvement contrasts with the observations by Keiser et al. (2015), who did not observe any significant differences in average power during a 30-min time trial following 10 days of heat acclimation in 7 males (240±15 *vs* 246±14 at pre- and post-intervention, respectively).

As expected, the improvement in distance cycled during TT_{COOL3} following 10 days of heat acclimation (+2.5%) was less pronounced than the improvement in mean power, as cycling speed is influenced by both power and cadence. Whilst the improvement in distance cycled was marginal, it does surpass the 0.8% coefficient of variation for recreational cyclists familiarised with a time-trial lasting ~15 min (Borg et al., 2018). Conversely, it does not surpass the ~4% minimum difference required for a worthwhile change (Borg et al., 2018). In fact, only two participants in the current study surpassed this 4% improvement threshold, creating some uncertainty as to the meaningfulness of these results.

Improvements in performance at TT_{COOL3} were accompanied by a lower resting T_{rec}, though there were no other physiological differences between time trials. However, we also did not observe any thermoregulatory or cardiovascular differences during time trials in hot conditions (**Chapter 4**), so this was somewhat expected. Various mechanisms have been proposed to underpin performance improvements following heat acclimation, including hypervolemia-mediated increases in stroke volume (Nielsen et al., 1993) and cardiac output (Lorenzo et al., 2010), which contribute to a lower heart rate during exercise; better maintenance of perfusion pressure via improved skin blood vessel compliance (Roberts et al., 1977); improved metabolic efficiency (Young et al., 1985); heat shock protein-mediated (Henstridge et al., 2014; Ohno et al., 2015) improvements in skeletal muscle strength (Racinais et al., 2017b); and improved defence of body temperatures to the thermal challenge of exercise (even in cool or temperate conditions; Ely et al., 2009). However, as the performance test was self-paced instead of at a fixed-workload, the mechanisms for improved time trial performance in cool conditions could not be elucidated in the current study.

Although a higher fitness has previously been associated with greater heat acclimation adaptations (Cheung & McLellan, 1998), and recently, greater improvements in $\dot{V}O_{2max}$ in temperate conditions following 10 days of heat acclimation (Mang et al., 2020), this was not reflected in performance in cool conditions in the current study. Instead, there was a

negative correlation between distance cycled at TT_{COOL1} and the improvement in distance cycled from TT_{COOL1} to TT_{COOL3}, whereby the participants that performed best at baseline showed the least improvement. This phenomenon was not apparent in data reported by Lorenzo et al. (2010), perhaps because of the homogeneity of fitness and training regime in their cohort (who were all part of the same cycling club).

5.5.2 Self-paced endurance performance following 5 days of heat acclimation

Following 5 days of high-intensity exercise, isothermic heat acclimation with permissive dehydration, females showed no significant improvements in performance, including distance cycled, mean power output nor speed, during TT_{COOL2}, as compared to TT_{COOL1}. Thus, it appears that 5 days of heat acclimation is insufficient to improve endurance performance in females in cool conditions. This reflects the lack of physiological adaptations previously demonstrated in females following 5 days of heat acclimation (Mee et al., 2015b).

The current study was conducted before the publication of the investigations by Pethick et al. (2018) and Perrotta et al. (2018). Both studies showed improved performance (i.e. 30–15 Intermittent Fitness Test and speed at second ventilatory threshold) in temperate conditions (20-23°C) in elite level female soccer players following short-term (5-6-days) heat acclimation. Pethick et al. (2018) and Perrotta et al. (2018) also reported a 9.5% and 5.0% expansion in plasma volume, respectively, a marker of heat acclimation (Sawka et al., 2011). Although plasma volume was not measured in the current study, participants in this thesis did not demonstrate other markers of heat acclimation (e.g. no change in resting T_{rec} or exercise capacity in the heat at TT_{HOT2} in **Chapter 4**) following 4-5 days of heat acclimation. Thus, a number of the physiological adaptations hypothesised to contribute to temperate performance improvements were unlikely to have developed by TT_{COOL2}. The reason for the contrasting findings between the current data and that of other female cohorts is unclear, especially as Pethick and colleagues also implemented a 90-min daily isothermic heat acclimation protocol with permissive dehydration and began with 20-min HIIT in similar conditions (35°C, 36-46%RH). They did however, employ a sample size of 18 athletes, making their study better powered to detect their small (1.5%) change in performance. Pethick et al. (2018) also used a more thermally-challenging performance test, as evidenced by: i) the 20°C air temperature (as opposed to the 13 °C in the current study), and ii) the nature of the Intermittent Fitness Test employed, where the indoor environment likely did not allow for the same convective and evaporative cooling as a front-facing fan (as employed in the current study). Thus, participants may have experienced a greater benefit from heat acclimation-mediated improvements in thermoregulation in the study by Pethick et al. (2018).

Additionally, data from studies investigating short-term active heat acclimation for cool or temperate exercise performance in male or mostly male cohorts is equivocal. Data in the current thesis contrasts with positive findings by Neal et al. (2016a) and Tebeck et al. (2019), who reported improvements in exercise performance markers (including lactate threshold and mean time trial power) following 5 days of isothermic heat acclimation. However, the improvement in mean time trial power output observed by Neal et al. (2016a) was small (~2%) and did not translate to an improved 20-km time trial completion time. Neither Neal et al. (2016a) nor Tebeck et al. (2019) observed an increase in $\dot{V}O_{2max}$ as has been observed alongside cool or temperate performance improvements following 10 days of heat acclimation (Lorenzo et al., 2010; Mang et al., 2020) or following ~4 days of recovery post-acclimation (Waldron et al., 2019), which may be an important determinant of time trial performance (Périard & Racinais, 2015). Moreover, the number of studies that did not observe a difference in temperate exercise performance following short-term heat acclimation (albeit using different acclimation protocols; Morrison et al., 2002; Chen et al., 2013; Chalmers et al., 2016; Zurawlew et al., 2016; Philp et al., 2017; Mackay et al., 2018) indicates that detectable improvements could be somewhat elusive following short-term heat acclimation.

5.5.3 Perspectives and considerations

Anecdotally, participants reported severe fatigue and "heavy legs" at TT_{COOL3}. The fatigue reported by participants in the current study is in line with impairments to exercise capacity sometimes reported in the days immediately following a heat acclimation protocol (Waldron et al., 2019), especially when exercise intensity during heat acclimation is high (Schmit et al., 2018; Reeve et al., 2019). After the current study had commenced, Reeve et al. (2019) reported that high-intensity heat acclimation (5 consecutive days of 30-min HIIT in 35°C, 50%RH) impaired cycling capacity in the heat (as quantified using time-to-exhaustion) at both 3-4 days and 10-13 days post-intervention in well-trained cyclists/triathletes ($\dot{V}O_{2max}$ ~61 $mL \cdot kg^{-1} \cdot min^{-1}$). A lower-intensity protocol, like those implemented by Lorenzo et al. (2010; cycling at 60% $\dot{V}O_{2max}$) and Mang et al. (2020; walking at 30-40% $\dot{V}O_{2max}$) may be preferred to elicit improvements in exercise capacity, especially in fitter participants (Mang et al., 2020). Indeed, Schmit and colleagues (2018) observed that a 5-day heat acclimation protocol where athletes completed their self-described "low-intensity" sessions in the heat resulted in favourable performance improvements in a 20-km time trial in the heat (35°C, 50%RH), as compared to a protocol of the same duration where athletes completed their self-described "high-intensity" sessions in the heat. A low-intensity protocol may also be

preferred by athletes, as anecdotally, participants in the current study often found the protocol overly difficult and unenjoyable.

Furthermore, the timing of TT_{COOL2} and TT_{COOL3}, and specifically their temporal proximity to the heat acclimation sessions, may have contributed to the lack of widespread *meaningful* performance improvements in the current dataset. Waldron et al. (2019) reported that $\dot{V}O_{2max}$ in temperate conditions was acutely decreased during, and in the 2 days following the 5 days 0f heat acclimation (daily 60-min cycling at 50% $\dot{V}O_{2max}$), and that $\dot{V}O_{2max}$ was not elevated compared to baseline until 4 days post-heat acclimation intervention. Together with data from the current study, this indicates that implementing a heat acclimation protocol for performance aims requires careful consideration in terms of protocol intensity and allotting adequate time for recovery before performance tests. Whilst significant improvements in temperate exercise performance were observed following 10 days of heat acclimation in the current study, this was mostly driven by participants with lower baseline scores, and therefore the current protocol and/or timing may not be appropriate for a high-performing cohort.

This study did not control for menstrual cycle or hormonal contraceptives; however, these factors are unlikely to affect exercise performance in temperate conditions (Mattu et al., 2020). Despite this, variations in body temperature and thermoregulation during the menstrual cycle (Giersch et al., 2020) may have modified the heat or exercise stimulus experienced by individual participants when targeting a 38.5°C T_{rec} during heat acclimation sessions. Cyclical variations may have also obscured potential differences in physiological measures of heart rate or T_{rec} during time trials.

5.6 Conclusions

This study was the first to document performance outcomes during self-paced time trials in a female cohort following 5 and 10 days of high-intensity exercise, isothermic heat acclimation. In the current study, females did not show an improvement in self-paced endurance performance following 5 days of high-intensity exercise, isothermic heat acclimation. Conversely, following 10 days of high-intensity exercise, isothermic heat acclimation, females improved self-paced endurance performance; namely, a ~5.3% increase in mean power output and a ~2.5% increase in distance cycled during a 15-min self-paced time trial in cool conditions. However, these improvements were largely driven by participants with lower baseline scores. Thus, practitioners should exercise caution when applying the current protocol in a high-performance female cohort.

Note on Chapters 4 & 5

Anecdotally, participants found the interventions detailed in the previous two chapters to be difficult and often overly unpleasant. Such perceptions and experiences could have implications for adherence and implementation. Furthermore, results from **Chapters 4** and **5** indicated that for females, 9-10 days of acclimation was required for meaningful physiological and performance benefits to occur. This represents a great deal of training time spent undergoing an intervention that requires consecutive days of physically exhaustive exercise.

Moreover, access to an environmental chamber is often cited as a barrier to implementing heat acclimation strategies, and it should be acknowledged that barriers related to cost and accessibility are exponentially greater for female athletes (Emmonds et al., 2019). Elite females often experience a vastly different sporting “environment”, which includes less support personnel, resources and funding than their male counterparts, as well as relatively little budget allotment for what might be considered auxiliary costs (Emmonds et al., 2019). Thus, for heat acclimation interventions to accrue widespread use by female athletes, they must be accessible and cost-effective. Considering these factors, the next three chapters investigate a possible solution.

6. INTERMITTENT POST-EXERCISE SAUNA BATHING IMPROVES MARKERS OF EXERCISE CAPACITY IN HOT AND TEMPERATE CONDITIONS IN TRAINED MIDDLE-DISTANCE RUNNERS

This chapter was published in the *European Journal of Applied Physiology* (Kirby, N. V., Lucas, S. J., Armstrong, O. J., Weaver, S. R., & Lucas, R. A. [2020]. Intermittent post-exercise sauna bathing improves markers of exercise capacity in hot and temperate conditions in trained middle-distance runners. *European Journal of Applied Physiology*, 1-15) and has undergone minor modifications to adapt to the format of this thesis. Specifically, details for methods outlined in the general methods chapter are referred to using section identifiers.

6.1 Abstract

Purpose: This study investigated whether intermittent post-exercise sauna bathing across 3 weeks of endurance training improves exercise-heat tolerance and exercise performance markers in temperate conditions, compared to endurance training alone. The subsidiary aim was to determine whether exercise-heat tolerance would further improve following seven-weeks post-exercise sauna bathing.

Methods: Twenty middle-distance runners (13 female; mean±SD, age 20±2 years, $\dot{V}O_{2max}$ 56.1±8.7 mL·kg^{-1}·min^{-1}) performed a running heat tolerance test (30-min, 9 km·h^{-1}/2% gradient, 40°C/40%RH; HTT) and temperate (18°C) exercise tests (maximal aerobic capacity [$\dot{V}O_{2max}$], speed at 4mmol · L^{-1} blood lactate concentration ([La^{-}]) before (Pre) and following 3 weeks (3-Weeks) normal training (CON; *n*=8) or normal training with 28±2 min post-exercise sauna bathing (101-108°C, 5-10%RH) 3±1 times per week (SAUNA; *n*=12). Changes from Pre to 3-Weeks were compared between groups using an analysis of co-variance. Six SAUNA participants continued the intervention for seven weeks, completing an additional HTT (7-Weeks; data compared using a one-way repeated-measures analysis of variance).

Results: During the HTT, SAUNA reduced peak rectal temperature (T_{rec}; -0.2°C), skin temperature (-0.8°C), and heart rate (-11 beats·min^{-1}) more than CON at 3-Weeks compared to Pre (all p<0.05). SAUNA also improved $\dot{V}O_{2max}$ (+0.27 L·min^{-1}; p=0.02) and speed at 4 mmol · L^{-1} [La^{-}] (+0.6 km·h^{-1}; p=0.01) more than CON at 3-Weeks compared to Pre. Only peak T_{rec} (-0.1°C; p=0.03) decreased further from 3-Weeks to 7-Weeks in SAUNA (other physiological variables p>0.05).

Conclusions: Three weeks of post-exercise sauna bathing is an effective and pragmatic method of heat acclimation, and an effective ergogenic aid. Extending the intervention to seven weeks only marginally lowered T_{rec} further.

6.2 Introduction

Heat acclimation improves exercise performance in the heat (Sawka et al., 2011). The most common model studied in the literature is “medium-term” active heat acclimation (Tyler et al., 2016), where individuals exercise in a climatic/environmental chamber for 60-120 min for 7-14 consecutive days (Garrett et al., 2009). This model of heat acclimation carries considerable barriers related to financial and temporal costs, as well as accessibility to climatic/environmental chambers. Consequently, heat acclimation is not widely used by athletes (recently recorded prevalence of ~15%; Périard et al., 2017) despite its effectiveness in improving exercise performance in hot environments. With major athletic events in extreme heat fast approaching, such as the Tokyo 2020 Olympic Games (postponed to 2021; Gerrett et al., 2019) and the 2022 FIFA World Cup in Doha, it is imperative that athletes are prepared to compete in such challenging environments. Post-exercise sauna bathing presents a practical and accessible heat acclimation alternative to active heat acclimation that could be implemented without disruption to an athlete’s training programme. Repeated bouts of sauna bathing have been observed to elicit some heat acclimation adaptations (e.g. reduced resting core temperature with sauna independent of exercise [Leppäluoto et al., 1986] and plasma volume expansion using post-exercise sauna bathing [Scoon et al., 2007; Stanley et al., 2015]). However, its efficacy to elicit hallmark heat acclimation adaptations during exercise-heat stress (e.g. reduced exercising heart rate and body temperatures, etc.; Sawka et al., 2011) has not previously been investigated (Casadio et al., 2017). Despite the relatively sparse scientific evidence, post-exercise sauna bathing is already being recommended to athletes preparing for competition in the heat (Racinais et al., 2019a). Therefore, the first aim of this study was to assess exercise-heat tolerance following repeated bouts of intermittent sauna bathing using a fixed-workload exercise-heat stress test.

The myriad of physiological adaptations attained through heat acclimation may improve exercise performance in cool or temperate conditions (Minson & Cotter, 2016). Similar improvements may result from post-exercise sauna bathing, as a case study of a young female tennis player showed improvements in performance outcomes of time-to-exhaustion (TTE), maximal aerobic capacity ($\dot{V}O_{2max}$), and lactate threshold after 12 sauna sessions following exercise spread across 3 weeks (Novak et al., 2018). Similarly, Scoon and colleagues (2007) observed that running TTE increased in six male athletes after approximately 12 intermittent post-exercise sauna sessions. However, the effects of intermittent post-exercise sauna bathing on more objective markers of temperate exercise performance (i.e. $\dot{V}O_{2max}$ and blood lactate profile) have not been assessed in a larger cohort. Therefore, the second aim of this study was to assess whether integrating

intermittent post-exercise sauna bathing (~3 sessions·week^{-1}) into an endurance training programme further improves temperate exercise performance markers ($\dot{V}O_{2max}$ and running speed at 4 mmol·L^{-1} blood lactate concentration [La^{-}]), as compared to an endurance training programme alone.

Finally, long-term heat acclimation interventions (i.e. >14 days) are expected to maximise exercise capacity in the heat (Tyler et al., 2016). These longer protocols may also allow time for the increased production of erythropoietin (EPO) and vascular endothelial growth factor (VEGF), as observed following hypoxia exposure (Girard et al., 2013), both primary factors driving favourable adaptations to oxygen carrying capacity and delivery. Notably, ten consecutive days of active heat acclimation elicits increases in hypoxia inducible factor 1-alpha (HIF-1a; the master regulator of downstream oxygen-modulating genes such as EPO and VEGF) in males (Lee et al., 2016). Therefore, the subsidiary aim of this study was to assess exercise heat tolerance and changes in EPO and VEGF in response to long-term (i.e. more than 14 heat exposures) intermittent post-exercise sauna bathing.

6.2.1 Aim

The aim of this study was to determine the efficacy of adding intermittent post-exercise sauna bathing to normal endurance training in order to induce heat acclimation adaptations in trained athletes, and to determine the impact of these adaptations on maximal and submaximal exercise performance markers in temperate conditions. The subsidiary aim was to determine the efficacy of a long-term intermittent post-exercise sauna bathing protocol to further improve heat acclimation adaptations.

6.2.2 Hypotheses

It was hypothesised that intermittent post-exercise sauna bathing would: i) elicit hallmark heat acclimation adaptations and improve thermoregulation during exercise-heat stress more than endurance training alone, and ii) improve physiological markers of exercise performance in temperate conditions to a greater extent than endurance training alone.

6.3 Methods

6.3.1 General overview and design

An overview of the protocol is depicted in **Figure 6.1**. Participants took daily iron tablets from two weeks prior to the first experimental trial until the completion of the protocol (**Section 3.3.1**). A battery of experimental trials was completed within a 1-week timeframe at baseline (Pre), following 3 weeks (3-Weeks) and following 7 weeks (7-Weeks) of either normal endurance training (CON) or normal endurance training with the post-exercise sauna bathing intervention (SAUNA). Experimental trials included a running heat tolerance test (HTT), temperate exercise tests (a lactate profile test to determine running speed at 4 $mmol \cdot L^{-1}$ [La^{-}] and a $\dot{V}O_{2max}$ test) and a resting venous blood sample. The temperate exercise tests were conducted at Pre and 3-Weeks only. Throughout the interventions, participants were asked to keep a training diary (**Section 3.12**).

Experimental trials at Pre, 3-Weeks and 7-Weeks were arranged ~28 days apart in order to test female participants during the same menstrual cycle phase, as per a typical menstrual cycle. Participants self-allocated into groups prior to Pre testing. This experiment was conducted in the United Kingdom between the months of October and March to minimise any natural heat acclimatisation.

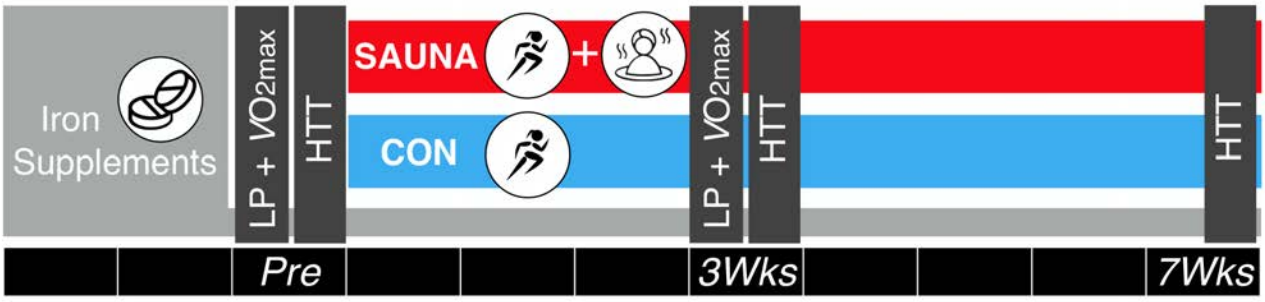


Figure 6.1 Schematic of the experimental design. Post-exercise sauna bathing intervention group (SAUNA) and control group (CON) completed temperate exercise tests (18°C) consisting of lactate profile (LP) and maximal aerobic capacity ($\dot{V}O_{2max}$) tests, and a running heat tolerance test (HTT; 30-min, 9 $km \cdot h^{-1}$/2% gradient, 40°C/40%RH), at baseline (Pre), and following 3 weeks (3Wks) of intervention or control. A subgroup of participants completed an additional HTT following seven-weeks intervention (7Wks). Lower black bars indicate weeks.

6.3.2 Participants

Fifty-one trained middle-distance and cross-country runners were recruited from the university's athletics club to participate in this study. However, due to various reasons (injury, scheduling, etc.) only twenty athletes (SAUNA, *n*=12 [F, *n*=9; M, *n*=3]; CON, *n*=8 [F, *n*=4; M, *n*=4]) completed experimental trials at 3-Weeks. Two female participants 'crossed-over', meaning they completed both the SAUNA and CON interventions (in opposite order, with ~5 weeks washout). One 'cross-over' participant only completed the lactate profile test at both Pre and 3-Weeks whilst undergoing the CON intervention. Eight athletes completed experimental trials at 7-Week, though only the six athletes in the SAUNA group (F, *n*=3; M, *n*=3) will be reported in the HTT at the final time-point (7-Weeks). The two athletes in the CON group who completed experimental trials at 7-Weeks were not included in the 7-Weeks analysis. An overview of the progression of athletes through the study is detailed in **Figure 6.2**. Participants in the SAUNA and CON group at 3-Weeks had similar baseline characteristics (age 19±1 and 20±2 years, height 169±7 and 172±6 cm, $\dot{V}O_{2max}$ 59.3 and 58.4±7.2 mL·kg^{-1}·min^{-1}, respectively; all p>0.05). SAUNA participants who completed experimental trials at 7-Weeks were 19±1 years of age, 171±6 cm tall and had a baseline $\dot{V}O_{2max}$ of 60.2±10.1 mL·kg^{-1}·min^{-1}. Female participants included in analyses at 3-Weeks were eumenorrheic (*n*=7), oligomenorrheic (*n*=1), amenorrheic (*n*=1), or using various forms of hormonal contraceptives (monophasic oral contraceptive pill, *n*=1; progestin-only oral contraceptive pill, *n*=1; contraceptive coil, *n*=1). Of these, female participants included in analysis at 7-Weeks were either eumenorrheic (*n*=2) or amenorrheic (*n*=1). Participants complied with all criteria and instructions for participation (for full details, see **Section 3.3** General participant instructions, **Section 3.6** Menstrual cycle questionnaire and self-reported log and **Section 3.9.1** Instructions for normal hydration).

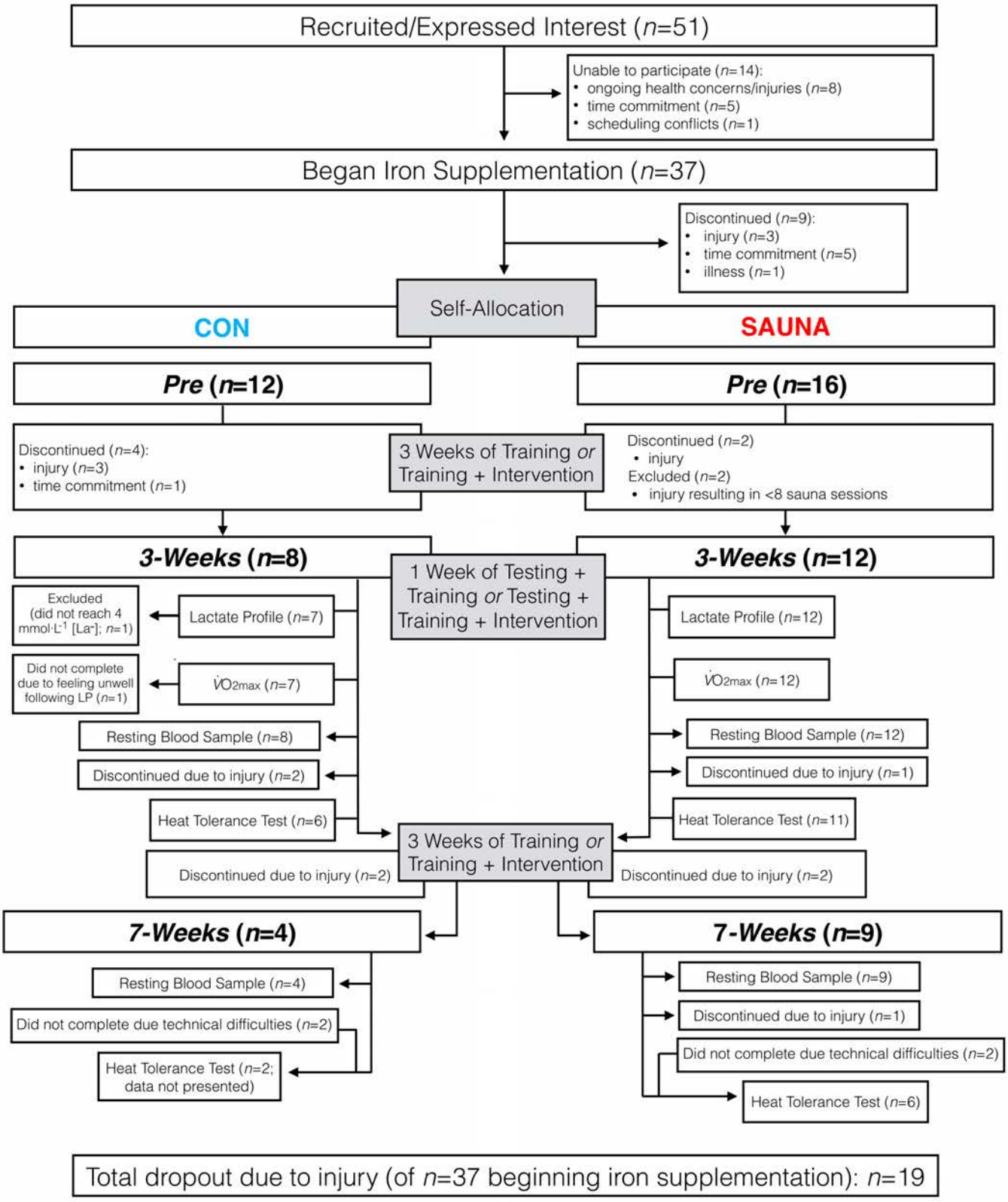


Figure 6.2 Progression of participants through phases of the study. LP, lactate profile test; [La^-], blood lactate concentration; $\dot{V}O_{2max}$, maximal aerobic capacity

6.3.3 Experimental procedures

6.3.3.1 Post-exercise sauna bathing

Participants entered the sauna (101-108°C, 5-10%RH at ~1.8 m; **Section 3.4.3**) within ~5 min of cessation of outdoor exercise and were asked to remain in the sauna for 30 min or until they could no longer tolerate the discomfort (**Section 3.7.3)**. Sauna bathing typically followed scheduled low-intensity, continuous exercise training sessions (i.e. 'easy runs' or 'long runs'). Peak heart rate (HR; **Section 3.9.4**) was recorded during the final minute of exposure.

6.3.3.2 Running heat tolerance test (HTT)

The running heat tolerance test (HTT) was performed in hot conditions (40°C, 40%RH) in the environmental chamber (**Sections 3.4.1** and **3.7.4)**. Briefly, participants ran on a treadmill at 9 $km \cdot h^{-1}$ and 2% gradient for 30 min (Mee et al., 2015a). Rectal temperature (T_{rec}), skin temperature (T_{sk}), and HR were measured continuously (**Sections 3.9.3.1**, **3.9.3.2** and **3.9.4**, respectively). Perceptual measures, including ratings of perceived exertion (RPE), thermal comfort and thermal sensation (**Section 3.10**), were obtained in the final minute of the HTT. Sweat gland activity of the forearm and upper back (**Section 3.9.6**) were recorded immediately following the HTT. Sweat loss was measured via changes in towel-dried, nude body mass (**Section 3.9.6**).

6.3.3.3 Lactate profile test

The lactate profile test was performed in temperate conditions (~18°C) in the Human Performance Laboratory (**Sections 3.4.2** and **3.7.5)**. Briefly, participants completed a light 10-min warm-up before commencing the step-style incremental test on the treadmill (3-min stages increased by 1 $km \cdot h^{-1}$ until [La^{-}] exceeded 4$mmol \cdot L^{-1}$; **Sections 3.9.7.2 and 3.9.7.4**). HR and respiratory gas exchange were continuously measured.

6.3.3.4 Maximal aerobic capacity ($\dot{V}O_{2max}$)

Approximately 10 min after completing the lactate profile test, participants performed a ramp-style $\dot{V}O_{2max}$ test, as described in **Section 3.7.6**. Briefly, the test consisted of 1-min stages that increased in speed (+1 $km \cdot h^{-1}$) for the first two stages, and then in gradient (+1%) until volitional exhaustion. When participants were re-tested at 3-Weeks, the starting

speed and progression of the protocol was repeated to allow for measurement of time-to-exhaustion (TTE). HR and respiratory gas exchange were continuously measured. Capillary [La^-] was measured 5-min from the time of exhaustion (**Sections 3.9.7.2 and 3.9.7.4**).

6.3.3.5 Resting blood sampling and analysis

On a separate visit (between the hours of 9:00-11:00 h), participants rested in a supine position for a minimum of 10-min, at which time resting HR was assessed and venous blood was drawn from the antecubital vein (**Section 3.9.7.1)**. Samples were analysed for measurement of plasma VEGF and serum EPO (**Section 3.9.7.5)**

6.3.4 Data analyses

Peak T_{rec}, $T_{recRISE}$ and weighted T_{sk} were calculated as described in **Section 3.9.3**. Peak HR was calculated as described in **Section 3.9.4.** Respiratory gas exchange data were measured via open-circuit spirometry and used to calculate $\dot{V}O_{2max}$, RER and running economy (**Section 3.9.2**). During the lactate profile test, submaximal RER, running economy and HR data were averaged across the final minute of the stage where participants exceeded 4 $mmol \cdot L^{-1}$ [La^-] at baseline, with the same running speed compared at 3-Weeks. For individual T_{sk} sites, if a time-point was lost, the missing data were modelled according to the slope/pattern of other data points from that participant during the previous test. Data at multiple T_{sk} sites was lost for two SAUNA group participants in the HTT at 7-Weeks, and therefore SAUNA T_{sk} at 7-Weeks (n=4) was deemed incomplete and not reported herein. Training data and resting HR were not collected in n=1 SAUNA participant for both 3- and 7-Weeks.

6.3.5 Statistical approach

To address the main aims, physiological data from HTT, lactate profile and $\dot{V}O_{2max}$ tests were compared between SAUNA and CON groups using a one-way analysis of co-variance (ANCOVA), with change scores (Δ; difference between Pre and 3-Weeks) entered as the dependent variable and pre-intervention absolute data entered as a co-variate. This allowed all changes to be covariate-adjusted for the baseline values (Vickers & Altman, 2001) and yielded a single p-value to represent the between-group comparison. This p-value reflects the effect of the *intervention*. Because sex affects cardiorespiratory fitness, sex as a variable was tested for violations related to collinearity and entered as co-variate in analyses

of $\dot{V}O_{2max}$ and running speed at 4 mmol·L^{-1} [La^-]. Effect sizes derived from between-group comparisons are presented as Cohen's *d*, whereby *d*>0.8 are considered large, 0.5–0.8 moderate and 0.2–0.5 small (Cohen, 1988). Normality of these data were assessed using Levene's Test of Equality of Error Variances, which was not violated on any occasion.

To address the subsidiary aim, a one-way repeated-measures analysis of variance (ANOVA) was used to compare HTT physiological data from the SAUNA group participants at Pre, 3-Weeks and 7-Weeks (*time [3 levels]*).

Total training frequency and average weekly running distance were compared either between groups (3-Weeks) using an independent *t*-test (*group [2 levels]*) or within-group (7-Weeks) using a one-way repeated-measures ANOVA (*week [7 levels]).* Average weekly frequency of each type of training session (i.e. easy run, tempo training, interval training, and long run) were compared using a two-way ANOVA (3-Weeks: between groups, *type [4 levels] x group [2 levels]*; 7-Weeks: repeated-measures, *type [4 levels] x week [7 levels]*).

Normality of data analysed by ANOVA using the Shapiro-Wilk Test (not violated on any occasion). Sphericity was assessed using were assessed using Mauchly's test of sphericity, and Greenhouse–Geisser corrections were applied where assumptions of sphericity were violated. When a significant main effect was found, Dunn-Bonferroni-corrected *post hoc* comparisons were made. Main effect sizes for ANOVAs were calculated using partial eta-squared (η_p^2), whereby η_p^2 >0.14 are considered large, 0.06-0.14 moderate and <0.06 small (Cohen, 1988).

Ordinal data, including average running session RPE_{1-10}, and peak RPE_{6-20}, thermal comfort and thermal sensation during HTTs, were compared between groups (3-Weeks) using a Mann-Whitney U test or within-group (7-Weeks) using a Friedman's test with *post hoc* analysis by Wilcoxon sign-rank tests.

Two sample size calculations (G*Power 3.1.2) were performed using mean data taken from a 5-day heat acclimation study (Garrett et al., 2009) and a 5-km treadmill TT reliability study (Laursen et al., 2007). For a one-tailed t-test with alpha level set at 0.05 and power set at 0.8 a sample size of nine participants was calculated to detect a meaningful heat acclimation induced difference in final exercising Tre (0.3 °C). To detect a meaningful improvement in 5-km treadmill TT performance (set at 3%) it was estimated that a sample size of seven participants was needed. To ensure adequate power for both key variables, and allowing for dropout, a sample size of 10 participants was used for HWI.

Power analyses were performed for heat acclimation adaptations using data from a heat acclimation study by Mee et al. (2015b) and for temperate exercise adaptations using data from Lorenzo et al. (2010). These studies were chosen to inform the power analyses because they included both female and male participants. For a one-way ANCOVA with one covariate, an α level of 0.05 and power set at ≥0.8, sample sizes of 12 and 14 total

participants were calculated to be sufficient to detect a 0.4 °C reduction in peak T_{rec} and a 6% increase in $\dot{V}O_{2max}$, respectively. Given the long duration of the intervention and the high prevalence of injury in distance runners (up to ~85% over a single season; van Gent et al., 2007), the recruitment strategy was to recruit the maximum number of participants from the university running club at each intake period.

Absolute data and within-subject changes are expressed as mean ± standard deviation (SD), and 95% confidence limits ([95% CL: lower limit, upper limit]) are presented with mean between-group differences. Significance was set at $p<0.05$ for each analysis.

6.4 Results

6.4.1.1 Training

3-Weeks: Training sessions were performed 7±2 times per week (SAUNA: 6±1; CON: 7±2) in the weeks between Pre and 3-Weeks testing. Average weekly running distances were 53.3±23.3 km and 54.5±28.2 km in SAUNA and CON, respectively. Training was not different in type, frequency, running distance or perceived exertion between the SAUNA and CON groups at 3-Weeks (all $p>0.05$; **Figure 6.3**).

7-Weeks: Participants in the SAUNA group trained 6±1 times per week, and ran 59.1±21.8 km·week^{-1}. Training was not different in type, frequency, running distance or perceived exertion across the seven weeks prior to 7-Weeks testing in the SAUNA group (all $p>0.05$).

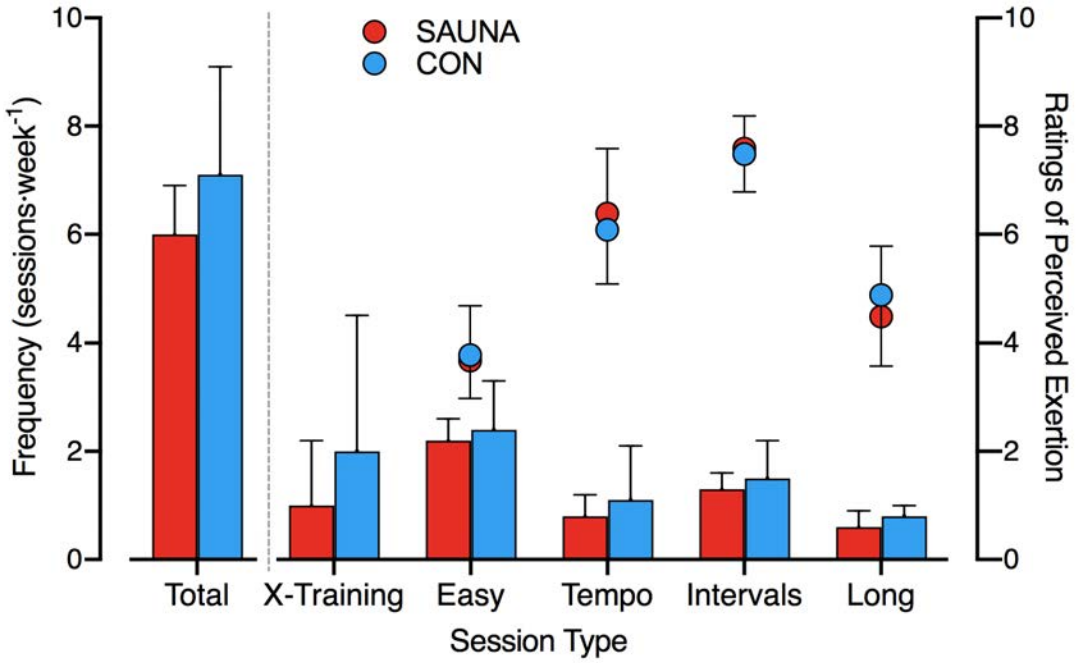


Figure 6.3 Training frequency, type (*bars, left axis*) and session perceived exertion (10-point scale; *circles, right axis*) for 3 weeks of training during the post-exercise sauna intervention (SAUNA, *red*) and control (CON, *blue*) groups. X-Training, cross-training. Data are presented as mean±SD

6.4.2 Post-exercise sauna bathing sessions

3-Weeks: Participants in the SAUNA group attended sauna sessions 3±1 times per week, accumulating 9±1 sauna sessions before completing the lactate profile and $\dot{V}O_{2max}$ tests, and 10±1 sauna sessions before completing the HTT. Participants remained in the sauna for 28±2 min each session, totalling 290±48 min of sauna exposure. Peak HR reached 127±10 beats·min^{-1} whilst in the sauna.

7-Weeks: Participants in the SAUNA group attended sauna sessions 3±1 times per week, accumulating 19±1 sauna sessions before completing the final HTT at 7-Weeks. These participants remained in the sauna for 28±1 min each session, totalling 528±29 min of sauna exposure. Peak HR reached 120±14 beats·min^{-1} whilst in the sauna. Frequency, duration, and peak HR were not different between the sauna sessions completed before 3-Weeks testing as compared to those completed between 3-Weeks and 7-Weeks testing (all p>0.05).

6.4.3 Menstrual cycle

Female participants completed all experimental trials at Pre, 3-Weeks and 7-Weeks 28±2 days apart. Normally menstruating participants (*n*=8) reported menstrual cycles ranging between 26-31 days and five completed all tests in the same phase of their menstrual cycle. Of the remaining three, one SAUNA participant completed all Pre tests in the follicular phase and all 3-Weeks tests in the luteal phase, one SAUNA participant completed temperate exercise tests at Pre in the luteal phase and at 3-Weeks in the follicular phase (though HTTs were completed in the same phase), one CON participant completed temperate exercise tests at Pre in the follicular phase and at 3-Weeks in the luteal phase (HTTs completed in the same phase). Participants using oral contraceptives (*n*=2) completed all tests in the active pill-taking phase.

6.4.4 Resting measures

3-Weeks: Changes in resting HR were not different between groups at 3-Weeks as compared to Pre (SAUNA: 58±6 *vs* 55±9 beats·min^{-1}; CON: 54±6 *vs* 51±4 beats·min^{-1}, at Pre *vs* 3-Weeks, respectively; *p*=0.90, *d*=0.06). Changes in body mass were not different between groups (SAUNA: 61.0±6.3 *vs* 61.2±6.4 kg; CON: 63.8±5.5 *vs* 63.5±5.4 kg, at Pre *vs* 3-Weeks, respectively; *p*=0.43, *d*=0.45). Serum EPO was detectable in 17 out of 20 samples (SAUNA, *n*=12; CON, *n*=5). Changes in resting serum EPO were not different (*p*=0.82, *d*=0.24) between groups (SAUNA: 3.8±2.7 *vs* 3.1±1.6 mIU·mL^{-1}; CON: 2.8±2.3 *vs* 2.6±1.9 mIU·mL^{-1}, at Pre *vs* 3-Weeks, respectively). VEGF was detectable in 10 out of 20 samples (SAUNA, *n*=5; CON, *n*=5). Changes in resting plasma VEGF were not significantly different (*p*=0.09, *d*=0.35) between groups (SAUNA: 128.5±121.9 *vs* 117.9±106.4 pg·mL^{-1}; CON: 183.0±234.4 *vs* 179.8±274.7 pg·mL^{-1}, at Pre *vs* 3-Weeks, respectively).

7-Weeks: Participants in the SAUNA group showed a trend (*p*=0.06) for a decrease in resting HR across *time*, though this was not significant (57±6, 53±10 and 53±7 beats·min^{-1} at Pre, 3-Weeks and 7-Weeks, respectively). Serum EPO did not significantly change across *time* (3.6±1.6, 3.1±1.8 and 4.4±2.8 mIU·mL^{-1} at Pre, 3-Weeks and 7-Weeks, respectively; *p*=0.18). VEGF was detectable in *n*=3 samples and therefore not statistically compared (77.9±55.6, 77.5±39.4 and 76.1±51.6 pg·mL^{-1} at Pre, 3-Weeks and 7-Weeks, respectively).

6.4.5 Running Heat Tolerance Test

6.4.5.1 Body temperatures

3-Weeks: Body temperature responses at Pre and 3-Weeks are detailed in **Table 6.1**. The SAUNA group exhibited a 0.2 °C [0.0, 0.4] greater reduction in peak T_{rec} than the CON group at 3-Weeks as compared to Pre (p=0.04, d=0.67; Figure **6.4A**). However, changes in $T_{recRISE}$ were not different between groups (p=0.14; d=0.54; Figure **6.4B**). The SAUNA group exhibited a 0.8 °C [0.1, 1.5] greater reduction in peak T_{sk} than the CON group at 3-Weeks as compared to Pre (p=0.03, d=0.99).

Table 6.1 Physiological and perceptual responses during the running Heat Tolerance Test. Data are presented as mean±SD

	SAUNA		CON	
	Pre	*3-Weeks*	*Pre*	*3-Weeks*
Peak T_{rec} (°C)	38.6±0.5	38.3±0.3*	38.6±0.4	38.5±0.3
$T_{recRISE}$ (°C)	1.4±0.4	1.2±0.3	1.5±0.5	1.5±0.5
Peak T_{sk} (°C)	36.4±1.3	35.7±0.8*	36.3±1.4	36.5±1.2
Peak HR (beats·min^{-1})	163±24	152±19*	157±16	158±21
Sweat Loss (kg)	0.8±0.2	0.8±0.2*	0.8±0.3	0.7±0.1
Forearm active sweat glands (per cm^2)	51±24	70±20*	45±20	44±24
Upper back active sweat glands (per cm^2)	64±15	70±24	71±16	63±26
Peak RPE_{6-20}	12±2	10±2	11±2	11±2
Peak thermal sensation	10±1	9±1*	9±1	9±0
Peak thermal comfort	5±2	4±2*	3±2	4±2

HR, heart rate; T_{rec}, rectal temperature; $T_{recRISE}$, change in rectal temperature during exercise; RPE_{6-20}, rating of perceived exertion (6-20 point scale). Thermal sensation and thermal comfort were measured on 13- and 10-point scales, respectively. *Change from Pre to 3-Weeks in SAUNA group significantly different from CON (p<0.05).

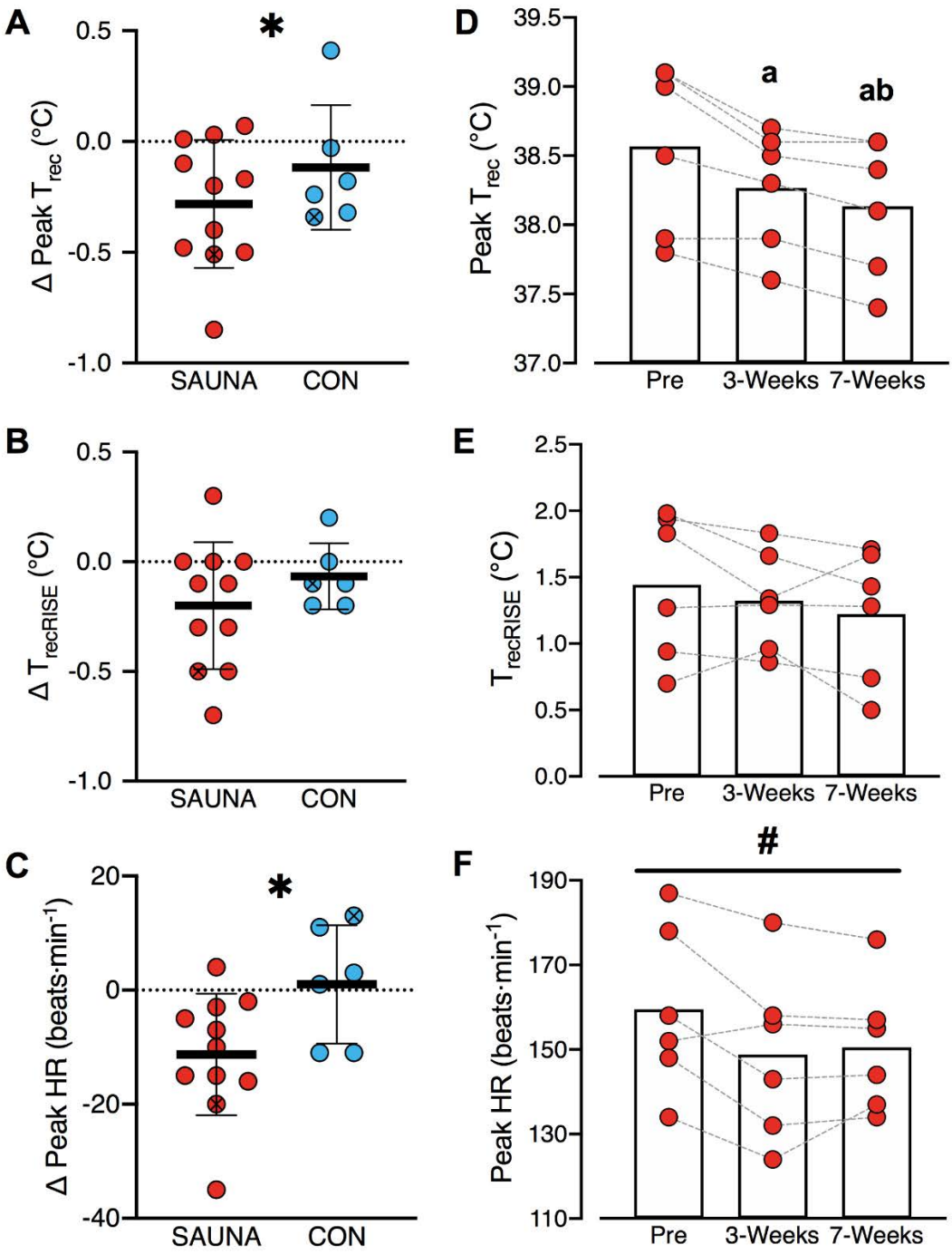


Figure 6.4 Delta (Δ) peak rectal temperature (T_{rec}; ***A***), rise in rectal temperature ($T_{recRISE}$; ***B***) and peak heart rate (***C***) during the running heat tolerance test (HTT; 40°C, 40%RH) in the SAUNA (*red*) and CON (*blue*) groups from baseline (Pre) to following 3 weeks of post-exercise sauna bathing intervention or control (3-Weeks). Absolute peak T_{rec} (***D***), $T_{recRISE}$ (***E***) and peak heart rate (***F***) during the HTT at Pre, 3-Weeks and following seven-weeks intervention (7-Weeks). Horizontal black lines (***A***-***C***) or bars (***D***-***F***) and error bars represent the group mean±SD. Circles represent individual participant data. The participant who completed both SAUNA and CON interventions is indicated by an *X*. *Significant difference in SAUNA *vs* CON; #Significant main effect of *time*; [a]Significant difference from Pre; [b]Significant difference from 3-Weeks, (p<0.05)

7-Weeks: Peak T_{rec} in the SAUNA group showed a large and significant main effect of *time* (p<0.01, η_p^2=0.84; **Figure 6.4D**). *Post hoc* analysis revealed that peak T_{rec} was 0.3 °C [0.0,

0.6] lower in SAUNA at 3-Weeks (p=0.04) and 0.4 °C [0.2, 0.6] lower at 7-Weeks (p<0.01), as compared to Pre. Furthermore, peak T_{rec} was 0.1 [0.0, 0.3] °C lower at 7-Weeks as compared to 3-Weeks (p=0.03).

6.4.5.2 Heart Rate

3-Weeks: The SAUNA group exhibited an 11 beats·min^{-1} [0, 22] greater reduction in peak HR during the HTT than the CON group at 3-Weeks as compared to Pre (p<0.05, d=1.16; **Figure 6.4C**; **Table 6.1**).

7-Weeks: Peak HR in the SAUNA group showed a large and significant main effect of *time* (p=0.02, η_p^2=0.54; **Figure 6.4F**). *Post hoc* analysis showed no significant differences, however there was a trend for peak HR to be lower at 3-Weeks as compared to Pre (-11 beats·min^{-1} [+2, -23]; p=0.08).

6.4.5.3 Sweating

3-Weeks: Absolute sweating data at Pre and 3-Weeks are detailed in **Table 6.1**. The CON group showed an attenuated sweat loss of 0.2 kg [0.0, 0.3] during the HTT, as compared to the SAUNA group at 3-Weeks *vs* Pre (p=0.02, d=0.95). The SAUNA group exhibited a 54±59% increase in sweat gland activity on the forearm, equating to a 22 active glands per cm^2 [7, 36] greater increase than the CON group's 6±17% reduction, at 3-Weeks as compared to Pre (p<0.01, d=1.40). Changes in sweat gland activity on the upper back were not different between groups (p=0.59, d=0.35).

7-Weeks: Sweat loss in the SAUNA group was not different between any of the HTTs (0.9±0.2, 0.8±0.1 and 0.8±0.2 kg at Pre, 3-Weeks and 7-Weeks, respectively; p=0.21, η_p^2=0.27). Sweat gland activity on the forearm showed a large and significant main effect of *time* (48±17, 66±14 and 69±13 active glands per cm^2 at Pre, 3-Weeks and 7-Weeks, respectively; p=0.01, η_p^2=0.57). *Post hoc* analysis showed no significant differences, however there was a trend for sweat gland activity on the forearm to be greater at 3-Weeks as compared to Pre (p=0.07). Sweat gland activity on the upper back was not different between any of the HTTs (72±6, 77±20 and 75±7 active glands per cm^2 at Pre, 3-Weeks and 7-Weeks, respectively; p=0.33, η_p^2=0.20).

6.4.5.4 Perceptual measures

3-Weeks: Perceptual data at Pre and 3-Weeks are detailed in **Table 6.1**. The SAUNA group exhibited a 1±1 scale point greater reduction in peak thermal sensation (p=0.02) and a 2±2 scale point greater reduction in peak thermal comfort (p<0.01) during the HTT than the CON group at 3-Weeks as compared to Pre. There was also a trend (p=0.08) for a 2±2 scale point greater reduction in RPE_{6-20} during the HTT in the SAUNA group than the CON group at 3-Weeks as compared to Pre.

7-Weeks: Peak RPE in the SAUNA group was significantly different between the HTTs (main effect: p=0.04; 12±3, 11±2 and 10±2 at Pre, 3-Weeks and 7-Weeks, respectively). *Post hoc* analysis revealed peak RPE was lower at 7-Weeks as compared to Pre (p=0.04) and as compared to 3-Weeks (p=0.03). There was a trend (p=0.08) for a reduction in peak RPE at 3-Weeks as compared to Pre. Thermal sensation was not different between HTTs (main effect: p=0.12; 10±1, 9±1 and 9±1 at Pre, 3-Weeks and 7-Weeks, respectively). Similarly, thermal comfort was not significantly different between HTTs (main effect: p=0.09; 5±2, 4±2 and 3±1 at Pre, 3-Weeks and 7-Weeks, respectively).

6.4.6 Lactate profile test

Physiological responses during the lactate profile test at Pre and 3-Weeks are detailed in **Table 6.2**. The SAUNA group exhibited a 4±3% improvement in running speed at 4 $mmol \cdot L^{-1}$ [La^-], equating to a 0.6 $km \cdot h^{-1}$ [0.1, 1.0] greater improvement than the CON group (0±3%) at 3-Weeks as compared to Pre (p=0.01, d=1.19; **Figure 6.5B**).

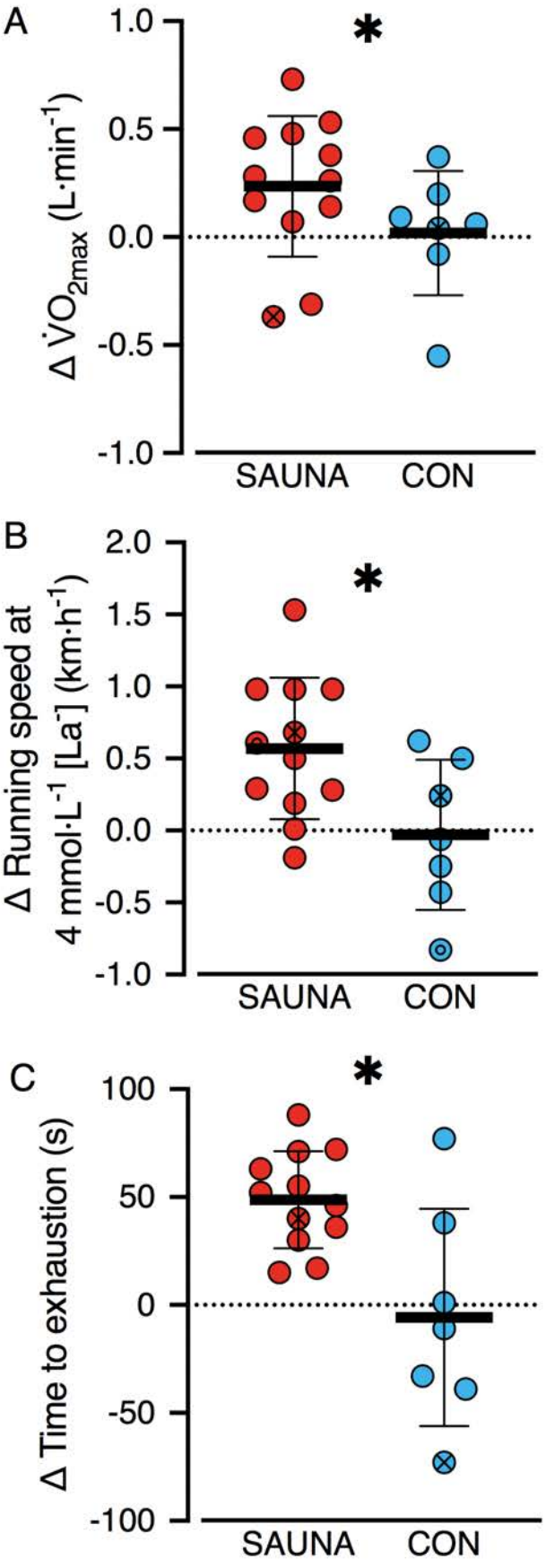


Figure 6.5 Delta (Δ) exercise performance markers in temperate conditions (18°C) from baseline (Pre) to following a 3-week post-exercise sauna bathing intervention (SAUNA; *red, left panel*) or control (CON; *blue, right panel*). Mean±SD (Horizontal lines and error bars) plus individual participant data (*circles*) presented for delta (Δ) maximal aerobic capacity ($\dot{V}O_{2max}$, ***A***), running speed at 4 mmol·L^{-1} blood lactate concentration ([La^{-}]) (***B***) and running time to exhaustion (***C***). The two participants who completed experimental tests following both SAUNA and CON interventions as indicated by an X and (in ***B*** only) an O. *Significant difference in SAUNA *vs* CON (p<0.05)

Table 6.2 Physiological responses to temperate exercise tests. Data are presented as mean±SD

	SAUNA		CON	
	Pre	*3-Weeks*	*Pre*	*3-Weeks*
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	3.42±0.81	3.65±0.75*	3.72±0.76	3.73±0.55
TTE (s)	426±57	474±59*	439±62	433±79
Running speed at 4 $mmol \cdot L^{-1}$ [La^-] ($km \cdot h^{-1}$)	15.6±1.7	16.2±1.7*	16.8±1.5	16.8±1.4
Submaximal HR ($beats \cdot min^{-1}$)	188±9	183±8	178±12	182±12
Submaximal RER	1.01±0.06	1.00±0.05	1.02±0.03	1.04±0.03
Submaximal RE ($mL \cdot kg^{-1} \cdot min^{-1}$)	189±14	195±11	187±25	194±22

$\dot{V}O_{2max}$, maximal aerobic capacity; TTE, time to exhaustion; [La^-], blood lactate concentration; HR, heart rate; RER, respiratory exchange ratio; RE, running economy. *Change from Pre to 3-Weeks in SAUNA group significantly different from CON (p<0.05).

During the lactate profile test, there was a trend (p=0.06; d=1.40) for a greater reduction in submaximal HR in the SAUNA group than in the CON group at 3-Weeks as compared to Pre (-7 $beats \cdot min^{-1}$ [0, -14]). Neither changes in submaximal running economy (p=0.73, d=0.27) nor RER (p=0.14, d=0.30) were different between groups.

6.4.7 Maximal aerobic capacity ($\dot{V}O_{2max}$) test

The SAUNA group exhibited an 8±12% improvement in $\dot{V}O_{2max}$, equating to a 0.27 $L \cdot min^{-1}$ [0.05, 0.49] greater improvement than the CON group (+2±8%) at 3-Weeks as compared to Pre (p=0.02, d=0.69; **Figure 6.5A**; **Table 6.2**). Similarly, the SAUNA group exhibited a 12±6% improvement in TTE during the $\dot{V}O_{2max}$ test, equating to a 54 s [17, 90] greater improvement than the CON group (-1±11%) at 3-Weeks as compared to Pre (p<0.01, d=1.56; **Figure 6.5C**; **Table 6.2**).

6.5 Discussion

The main findings of this study are that 3 weeks of post-exercise sauna bathing induced heat acclimation adaptations (reduced HR, T_{rec} and T_{sk}, and increased sweat gland activity), and improved markers of exercise performance in temperate conditions (i.e. $\dot{V}O_{2max}$, running speed at 4 $mmol \cdot L^{-1}$ [La^-] and TTE), to a greater extent than endurance training alone. These improvements were consistent with the study hypothesis. Following seven weeks of post-exercise sauna bathing, T_{rec} was the only physiological variable measured during the HTT that showed further improvements. However, the additional 0.1°C reduction in peak T_{rec} from 3-Weeks to 7-Weeks may not be physiologically meaningful. Whilst

participants did adhere well to the intervention, the dropout rate was high (51%; mainly due to injuries acquired during normal training in both groups). This drop-out rate was an important finding in-and-of-itself as it highlights the difficulty of implementing a seven-week long heat acclimation protocol into the training programme of competitive endurance runners.

6.5.1 Exercise heat tolerance

The mean 0.3°C reduction in peak T_{rec} observed in the SAUNA group at 3-Weeks surpasses the coefficient of variation of the HTT (0.34%, corresponding to 0.1°C in the current dataset) as validated by Mee and colleagues (2015). Furthermore, the 0.2°C difference in the change in peak T_{rec} between the SAUNA and the CON groups meets the physiologically meaningful threshold identified in a meta-analysis by Tyler and colleagues (2016). This reduction in peak T_{rec} in the SAUNA group may have resulted from an increased heat dissipation, possibly by means of increased skin blood flow combined with greater peripheral sweat gland activation to improve evaporative heat loss efficiency (Taylor, 2014; Smith & Havenith, 2019), given we did not observe increased whole-body sweat loss. The 0.8°C greater reduction in peak T_{sk} observed in the SAUNA group at 3-Weeks as compared to the CON group is consistent with other heat acclimation interventions, as Tyler et al. (2016) reported a 0.5±0.5 °C reduction in peak T_{sk} across the 67 interventions assessed within their meta-analysis. Similarly, the ~11 beats·min^{-1} greater reduction in peak HR observed in the SAUNA group at 3-Weeks as compared to the CON group is consistent with other heat acclimation interventions, which report a 16±6 beats·min^{-1} reduction in peak HR across 118 interventions (Tyler et al., 2016). This reduction in peak HR in the SAUNA group was likely mediated by increased plasma and/or blood volume, as was demonstrated using a similar post-exercise sauna bathing protocol by Scoon et al. (2007), though these variables were not measured in the current study. Though endurance training may (Nadel et al., 1974, Ichinose et al., 2009; untrained cohorts, 10 days to ~3-months training) or may not (McGarr et al., 2014; moderately-trained cohort, 2-weeks training) improve thermoregulation during exercise-heat stress, the changes in T_{rec}, T_{sk} and HR in the SAUNA group were greater than those observed in the CON group. Overall, this indicates that the improvements observed in the SAUNA group during the HTT were as a result of the post-exercise sauna intervention as opposed to normal training. Therefore, intermittent post-exercise sauna bathing appears to induce thermoregulatory and cardiovascular adaptations that are superior to 3 weeks of normal training alone, and of a similar magnitude to those observed in other heat acclimation

protocols in the literature (Tyler et al., 2016). These improvements occurred despite using only ten 30-min exposures and did not cause any disruptions to normal training.

Sweat loss during the HTT differed between groups. In the CON group, there was a reduction in sweat loss during the HTT at 3-Weeks. Conversely, there was no change in sweat loss in the SAUNA group during the HTT at 3-Weeks, despite their lower body temperature (i.e. T_{re} and T_{skin}). This indicates a possible seasonal change in sweat rate for the CON group (Matsumoto et al., 1990), whereas the SAUNA group showed an enhanced evaporative heat loss for a given body temperature. Further, at 3-Weeks the SAUNA group exhibited a substantially greater increase (+54%) in sweat gland activation on the forearm than the CON group (-6%). Though sweat gland activation is not indicative of sweat gland output, these findings are supported by a recent study that showed a greater relative sweat output on the limbs following heat acclimation (Smith & Havenith, 2019). Notably, the increase in sweat gland activation on the forearm without increases in total sweat loss (as exhibited by the SAUNA group in the current study) may be a more efficient adaptation for athletes competing in hot-humid environments, as it would improve evaporative capacity without exacerbating dehydration (Alber-Wallerström & Holmer, 1985).

Besides the further ~0.1°C reduction in peak T_{rec} at 7-Weeks in the SAUNA group, physiological adaptations appeared to plateau by 3-Weeks, despite nearly doubling the participants' total sauna exposures by 7-Weeks. This plateau is consistent with data from active heat acclimation interventions, whereby changes in heart rate and skin and core body temperatures are nearly maximised by ~7 days (Griefahn, 1997; Periard et al., 2015). Interestingly, $RPE_{6\text{-}20}$ was only decreased at 7-Weeks. Whether this would coincide with the anticipated steady increase in self-paced exercise capacity in the heat with a long-term intervention (Periard et al., 2015; Racinais et al., 2015b) is unknown, as the HTT was completed at a fixed workload.

6.5.2 Temperate exercise performance

Our data demonstrate that 3 weeks of intermittent post-exercise sauna bathing is ergogenic for exercise performance in temperate conditions in well-trained participants. The training status of the athletes in the current study make these results especially meaningful. The 8% mean improvement in $\dot{V}O_{2max}$ observed in the current study (~6% more than the CON group) is comparable to the 5% observed by Lorenzo and colleagues (2010) when using a 10-day active heat acclimation protocol. Possible mechanisms facilitating this improvement in aerobic capacity with heat acclimation include a greater maximum cardiac output, as demonstrated by Lorenzo et al. (2010) in their study, or increases in total

haemoglobin mass, which are possible in an intervention stretching over multiple weeks, as more recently demonstrated by Rønnestad and colleagues (2020). The iron supplements given to participants in the current study may have helped to facilitate improvements in oxygen carrying capacity, though participants were not given iron supplements by Rønnestad et al. (2020) or by Scoon et al. (2007), who both observed haematological adaptations following heat acclimation.

Three weeks of post-exercise sauna bathing also increased running speed at 4 $mmol \cdot L^{-1}$ $[La^-]$ by ~4%, equating to a 0.6 $km \cdot h^{-1}$ greater improvement than in the CON group. These results were similar to the 5% improvement in cycling power output at 4 $mmol \cdot L^{-1}$ $[La^-]$ reported by Lorenzo et al. (2010), and are supported by other observations of reduced lactate accumulation during exercise following heat acclimation (Young et al., 1985). Although this improvement in running speed at 4 $mmol \cdot L^{-1}$ $[La^-]$ was accompanied by a trend for a greater reduction in submaximal HR in the SAUNA group, running economy was not different during the lactate profile test at 3-Weeks. Thus, changes in $[La^-]$ may have been due to short-term adaptations such as a decreased rate of glycogenolysis (Kirwan et al., 1987; Febbraio et al., 1994a), or due to better splanchnic perfusion (and thus improved lactate removal) and haemodilution via increased blood and plasma volume (Scoon et al., 2007, Lorenzo et al., 2010). Alternatively, the multi-week design of the current intervention might have allowed time for heat acclimation-induced mitochondrial biogenesis to manifest (Tamura et al., 2014; Hafen et al., 2018).

The SAUNA group's ~12% increase in TTE following 3 weeks of post-exercise sauna bathing was superior to the CON group's ~1% reduction in TTE. The mean improvement in the SAUNA group was lower than the 32% increase observed by Scoon et al. (2007), however there were important differences in the TTE tests used, making comparisons difficult. Scoon and colleagues implemented a ~15-min TTE test at the runners' best 5-km race pace and 0% gradient. In the current study, the TTE test increased in either speed or gradient each minute and on average participants reached volitional exhaustion at ~8-min, but at a running speed at which they had reached 4 $mmol \cdot L^{-1}$ $[La^-]$ and at a 4% incline. Despite using a different style of TTE test, this study builds on evidence for the ergogenic benefits of post-exercise sauna bathing provided by Scoon and colleagues, with the additions of the gold-standard test of aerobic capacity, a robust submaximal exercise test and a larger, mixed-sex cohort.

6.5.3 Training and intervention

Of the 37 participants who began the experiment, 19 athletes succumbed to an injury (ranging from minor injuries that prevented training for ~1 week, to more severe injuries such as stress fractures or sprains) and did not complete the study. This is consistent with data reported in a review by van Gent et al. (2007), whereby incidences of lower extremity running injuries ranged between 19-79% in distance runners, with the highest rates observed in athletes reporting weekly running distances and training frequencies similar to those in the current study (i.e. females running 48-63 km·week^{-1}, males running 64+ km·week^{-1}, and athletes training 6-7 days·week^{-1}). For those who completed the intervention, training was consistent across the seven weeks of post-exercise sauna bathing. Importantly, unlike active heat acclimation protocols that require changes to an athlete's training in order to accommodate heat acclimation sessions, there were no differences in training between the CON group and SAUNA group in this study. This included similar perceived training intensity and training volume (i.e. frequency and type of sessions and weekly running distance) across the intervention period.

6.5.4 Blood markers

Serum EPO changes in the SAUNA group were not significantly different from the CON group at 3-Weeks, and did not significantly change in the SAUNA group by 7-Weeks. As EPO is inherently variable in response to environmental stimuli (Chapman, 2013; Płoszczyca et al., 2018), additional data may be required to fully understand this effect. Furthermore, serum EPO reflects a balance between renal EPO production and EPO consumption in the bone marrow (Rusko et al., 2004), making it difficult to discern the influence of either production or consumption by measurement of circulating EPO levels alone. Although the subsequent haematological responses of either increased consumption or production of EPO (i.e., increased red blood cell production) would coincide with the improved aerobic capacity observed at 3-Weeks in the current study and the increased red cell volume observed by Scoon et al. (2007), we were unable to measure red blood cell mass in the current study. VEGF was largely undetectable across the study, as a number of participant samples fell outside the ELISA kit's limits of detection for the measurement, providing little insight into any possible angiogenesis with post-exercise sauna bathing.

6.5.5 Perspectives and considerations

These results contribute to the growing body of research supporting favourable health and performance outcomes following passive heating interventions. The investigation of the efficacy of post-exercise sauna bathing to induce heat acclimation is especially timely as the Tokyo 2020 Olympic games (though postponed to 2021) are predicted to be the hottest Summer Olympic Games to date (Gerrett et al., 2019). We observed that heat acclimation was nearly maximised by 3 weeks of intermittent post-exercise sauna bathing and seven weeks did not induce any further meaningful physiological improvements. Thus, athletes need only incorporate a post-exercise sauna regime for 3 weeks to achieve beneficial adaptations.

This study did not exclude participants based on menstrual cycle or hormonal contraceptive use, and instead included a random sample of female athletes in random phases of their menstrual cycle. The diverse menstrual cycle patterns and contraceptive choices observed in this cohort is representative of the female athlete population. Whilst menstrual cycle was self-reported, the study design successfully allowed for tests and re-tests to be in the same phase for most female participants, making it unlikely that results were skewed or biased by menstrual cycle.

Participants self-selected into the SAUNA or CON groups. However, participants in both groups were given performance feedback following temperate exercise tests, which they discussed with their coach and used to inform their training. Given the highly competitive nature of the athletes recruited and the training ethos of the running club they were recruited from, the SAUNA and CON groups were equally invested in their training and performance during the exercise tests. This is supported by the similar SAUNA and CON training profiles (intensity, frequency, type and duration). Furthermore, the objective nature of the $\dot{V}O_{2max}$ and lactate profile tests reduces the impact of extrinsic, motivational factors on temperate performance results.

Although alternative methods of lactate profile testing may be preferred for determining lactate threshold (i.e. maximal lactate steady-state, or the D-max method described by Cheng et al., 1992), these methods significantly underestimate “race pace” for ~5-km races (Palmer et al., 1999; Chalmers et al., 2015). The training aims of the running club that participants were recruited from for the current study were largely centred around cross-country races (typically ranging between 4-6 km). Thus, running speed at 4 $mmol \cdot L^{-1}$ [La^-], observed to closely reflect 5-km race pace (Fay et al., 1989), had important ecological relevance for this cohort. In addition, though the results of this study certainly imply an

improved exercise capacity, the extent at which the observed adaptations would impact self-paced performance in either the heat or in temperate conditions is unknown.

Finally, the repeated-measures aspect of the two participants who undertook both the SAUNA and CON intervention are not statistically accounted for. However, as depicted in figures where individual data from the cross-over participant(s) are indicated, this exerted a negative bias for most measures. A sensitivity analysis was undertaken whereby the cross-over data for the two cross-over participants was removed (e.g. participants' data from the intervention they completed first was only included, and data from their cross-over intervention was removed; see **Appendix 2**).

6.6 Conclusions

This study is the first to demonstrate that post-exercise sauna bathing can induce thermoregulatory adaptations during exercise at an absolute fixed-workload, which supports current recommendations to use post-exercise sauna bathing as an alternative to active heat acclimation (Racinais et al., 2019a). These adaptations occur following ~10 intermittent exposures across 3 weeks. Extending the intervention to seven weeks appeared to have little additional benefit. Further, this study supports the efficacy of heat acclimation as an ergogenic aid for exercise performance in temperate conditions. Specifically, intermittent post-exercise sauna bathing on average improved $\dot{V}O_{2max}$ by ~8%, running speed at 4 $mmol \cdot L^{-1}$ [La^{-}] by ~4%, and time to exhaustion by ~12%, all of which were significantly greater improvements than those exhibited by the CON group. This intermittent-style intervention is especially attractive as the flexible nature and minimal impact on normal training circumvent common challenges associated with more traditional active heat acclimation approaches.

7. SEX DIFFERENCES IN ADAPTATION TO INTERMITTENT POST-EXERCISE SAUNA BATHING IN TRAINED MIDDLE-DISTANCE RUNNERS

This chapter is in-prep for publication (Kirby, N. V., Lucas, S. J., Cable, T.G., Armstrong, O. J., Weaver, S. R., & Lucas, R. A. Sex differences in adaptation to intermittent post-exercise sauna bathing in trained middle-distance runners) and has undergone minor modifications to adapt to the format of this thesis. Specifically, details for methods outlined in the general methods chapter are referred to using section identifiers.

7.1 Abstract

Purpose: The purpose of this study was to investigate the effect of sex on the efficacy of intermittent post-exercise sauna bathing to induce heat acclimation and improve markers of temperate exercise performance in trained athletes.
Methods: Forty-one trained runners (23 female; mean±SD, age 19±2 years, $\dot{V}O_{2max}$ F: 52.7±6.1 mL·kg^{-1}·min^{-1}, M: 64.2±2.7 mL·kg^{-1}·min^{-1}) performed a running heat tolerance test (30-min, 9 km·h^{-1}/2% gradient, 40°C/40%RH; HTT) and temperate (18°C) exercise tests (maximal aerobic capacity [$\dot{V}O_{2max}$] and lactate profile) pre- and post-3 weeks of normal training (CON; *n*=15, 7F) or normal training with 29±1 min post-exercise sauna bathing (101-108°C) 3±1 times per week (SAUNA; *n*=26, 16F).
Results: SAUNA females and males exhibited similar reductions (interactions $p>0.05$) in peak rectal temperature (-0.3°C; $p<0.001$), skin temperature (-0.9°C; $p<0.001$) and heart rate (-10 beats·min^{-1}; $p=0.001$) during HTT at post- *vs* pre-intervention. Only SAUNA females exhibited a reduced rise in rectal temperature during the HTT (F: -0.2°C, $p<0.001$; M: +0.1°C, $p=0.17$), and an increase in active sweat glands on the forearm (F: +57%, $p<0.001$; M: +1%, $p=0.47$). Conversely, only SAUNA males increased forearm blood flow (F: +31%, $p=0.61$; M: +123%; $p<0.001$). SAUNA females and males showed similar (interactions $p>0.05$) improvements in $\dot{V}O_{2max}$ (+5%; $p=0.02$) and running speed at 4 mmol·L^{-1} blood lactate concentration (+0.4 km·h^{-1}; $p=0.001$). CON showed no improvements in any physiological variable (all $p>0.05$).
Conclusions: Three weeks of post-exercise sauna bathing effectively induces heat acclimation in females and males, though amid different thermoeffector adaptations. Post-exercise sauna bathing is also an effective ergogenic aid for both sexes.

7.2 Introduction

Hot ambient temperatures adversely impact endurance performance (Galloway & Maughan, 1997). Athletes can use repeated bouts of heat stress (i.e. heat acclimation) to improve exercise performance in the heat, as heat acclimation induces a myriad of integrative adaptations that improve cardiovascular and thermoeffector function and lowers skin and core body temperatures (Sawka et al., 2011). There is growing interest in using passive forms of heat acclimation to drive these favourable physiological adaptations, on account of their practical and accessible nature (Heathcote et al., 2018). Specifically, post-exercise passive heating (e.g. post-exercise sauna bathing) is recommended (Racinais et al., 2019; World Athletics, 2019), as athletes begin passive heating bouts at elevated body temperatures (a key driver of heat adaptation) due to the preceding exercise bout (Heathcote et al., 2019). Indeed, data from **Chapter 6** and the literature (Scoon et al., 2007; Stanley et al., 2015) demonstrates that repeated bouts of post-exercise sauna bathing elicit heat acclimation adaptations.

A post-exercise sauna bathing protocol may elicit different acute and/or adaptive responses in females compared to males for a number of reasons. Acutely, females have been observed to exhibit a higher heart rate despite a similar rise in (sublingual) temperature to males during 30-min dry sauna exposure independent of exercise (Jezova et al., 1994). Females also exhibit reduced sudomotor sensitivity (Gagnon & Kenny, 2011) and a lower sweat rate overall (Frye & Kamon, 1981) when exposed to dry heat. It stands to reason that the extreme heat experienced in a dry sauna (typically >80°C), where ambient temperatures far exceed skin temperatures, could pose an additional thermoregulatory challenge for females because of their greater surface area-to-mass ratio and consequently greater dry heat gain (Shapiro et al., 1980). Moreover, females exhibit even greater elevations in post-exercise sweating and vasodilation onset thresholds than males (Kenny et al., 2006; Kenny & Jay, 2007). Thus, when severe heat stress is superimposed onto exercise recovery, such as during post-exercise sauna bathing, females may experience comparatively greater thermoregulatory strain.

Despite possibly inducing greater thermoregulatory strain in females, a post-exercise sauna bathing intervention may not be as effective for inducing adaptation in females as compared to males. Females may demonstrate a slower adaptive response to heat acclimation (Mee et al., 2015b), even when females experience comparatively greater thermoregulatory and cardiovascular strain during the initial heat exposures (Wyndham et al., 1965). Notably, Mee et al. (2015b) reported that 5 consecutive days of 90-min isothermic active heat acclimation (i.e. 450 min total heat exposure and total ~255 min with rectal temperature >38.5°C) was not sufficient to reduce females' body temperatures or heart rate

during exercise-heat stress. This is supported by findings in **Chapter 4**, whereby females did not show any physiological or performance improvements following 4 days of 90-min isothermic heat acclimation. The three-week intermittent post-exercise sauna bathing intervention used in **Chapter 6** only consisted of ~300 min of heat exposure (10 x 30 min sauna), a seemingly lesser thermal impulse than that reported by Mee et al. (2015b) and in **Chapter 4**. Thus, 3 weeks of intermittent post-exercise sauna bathing may be more effective for inducing heat acclimation in males than in females. The first aim of the current study was to investigate the effects of sex on the acute and adaptive response to repeated bouts of intermittent post-exercise sauna bathing.

Though there is conflicting evidence as to whether the physiological adaptations attained through heat acclimation improve exercise performance in cool or temperate conditions (Minson & Cotter, 2016; Nybo & Lundby, 2016), studies investigating active heat acclimation's ergogenic potential have not directly compared its efficacy in both sexes. Moreover, studies investigating the ergogenic effects of post-exercise hot water immersion (Zurawlew et al., 2016) as well as sauna bathing post- (Scoon et al., 2007) and independent of exercise (Glazachev et al., 2020), have been either in male or mixed-sex (**Chapter 6**) cohorts. Considering the aforementioned potential sex differences in adaptive response to heat stress and females' comparatively reduced aerobic capacity trainability (Diaz-Canestro & Montero, 2019), investigating the ergogenic potential of post-exercise sauna bathing in both sexes is warranted. Therefore, the second aim of this study was to investigate the effect of sex on markers of temperate exercise performance following repeated bouts of intermittent post-exercise sauna bathing.

7.2.1 Aim

The overall aim of this study was to investigate the effect of sex on the efficacy of intermittent post-exercise sauna bathing to induce heat acclimation and improve markers of maximal and submaximal exercise performance in temperate conditions in trained athletes.

7.2.2 Hypotheses

It was hypothesised that an acute bout of post-exercise sauna bathing would be more physiologically challenging in females. However, despite this greater thermoregulatory strain, it was further hypothesised that repeated post-exercise sauna bathing would be more effective in males for inducing desirable adaptations during both exercise-heat stress and temperate exercise.

7.3 Methods

7.3.1 General overview and design

This study expands on findings from **Chapter 6**, and as such, data from participants in the previous chapter are included in these analyses. The research questions of the current chapter (i.e. the influence of sex on the acute response to and the efficacy of post-exercise sauna bathing) were contrived after first determining the effectiveness of 3 and 7 weeks of the post-exercise sauna bathing intervention as compared to a control group. Despite a subset of participant data in this chapter being previously reported in **Chapter 6**, it was deemed important and worthwhile to present some of these data again in the current chapter because: i) the study has a distinctly different primary aim (i.e. examining the effects of sex), ii) this study reports additional outcome measures not previously reported following the post-exercise sauna bathing intervention, and iii) the additional participants in this chapter more than doubles the sample size of the previous chapter.

The overall protocol and experimental tests in the current study matched the 3-Weeks protocol in **Chapter 6** (**Figure 6.1**). A battery of experimental trials was completed within a one-week timeframe at baseline (Pre) and following three weeks (Post) of either normal endurance training (CON) or normal endurance training with the post-exercise sauna bathing intervention (SAUNA). Experimental trials included a running heat tolerance test (HTT), a temperate exercise test (consisting of a lactate profile test and test of maximal aerobic capacity [$\dot{V}O_{2max}$]) and a morning resting heart rate and finger-prick blood sample. Participants took daily iron tablets from 2 weeks prior to the first experimental trial until the completion of the protocol (**Section 3.3.1**).

Experimental trials were arranged ~28 days apart to test female participants during the same menstrual cycle phase, as per a typical menstrual cycle. Participants self-allocated into groups prior to baseline testing. This experiment was conducted in the United Kingdom between the months of October and March to minimise any natural heat acclimatisation.

7.3.2 Participants

Eighty trained middle-distance and cross-country runners were recruited from the university's athletics club to participate in this study. However, due to various reasons (injury, scheduling, etc.) only 41 athletes completed experimental trials at Post. Participant characteristics are summarised in **Table 7.1**. Of these 41 athletes, 1 female CON participant did not complete the Post lactate profile and $\dot{V}O_{2max}$ tests and 3 SAUNA (F: *n*=2; M: *n*=1) and 2 CON (F: *n*=1; M: *n*=1) participants did not complete the Post HTT. A subset of SAUNA

group participants was sampled at the first post-exercise sauna bathing session to quantify the acute physiological response to post-exercise sauna bathing (F: age 19±1 years, body mass 54.4±6.7 kg, height 167±7 cm, body surface area 1.61±0.10 m^2, body surface area-to-mass ratio 297±21 $cm^2 \cdot kg^{-1}$, $\dot{V}O_{2max}$ 52.0±7.2 $mL \cdot kg^{-1} \cdot min^{-1}$, *n*=7; M: age 20±1 years, body mass 66.4±8.6 kg, height 177±7 cm, body surface area 1.82±0.15 m^2, body surface area-to-mass ratio 276±16 $cm^2 \cdot kg^{-1}$, $\dot{V}O_{2max}$ 64.3±1.9 $mL \cdot kg^{-1} \cdot min^{-1}$, *n*=8). In this subset, females were shorter, lighter and had a lower body surface area, body surface area-to-mass ratio and $\dot{V}O_{2max}$ than males (all $p<0.05$). Females and males were of a similar age ($p=0.14$).

Table 7.1 Participants' characteristics. Data are presented as mean±SD

	SAUNA		CON	
Session Type	*Females*	*Males*	*Females*	*Males*
Participants (*n*)	16	10	7	8
Age (years)	19±1	20±1	19±2	20±2
Height (cm)	166±7#	177±6	166±8#	178±7
Body mass (kg)	54±9#	66±7	56±7#	64±6
BSA (m^2)	1.59±0.11#	1.82±0.13	1.62±0.14#	1.80±0.09
BSA-mass ratio ($cm^2 \cdot kg^{-1}$)	298±28#	275±15	289±13	283±16
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	52.6±6.9#	64.6±2.4	52.9±4.2#	63.6±3.2

SAUNA, post-exercise sauna bathing intervention group; CON, control group; BSA, body surface area; $\dot{V}O_{2max}$, maximal aerobic capacity. #Significantly different from males within group ($p<0.05$)

Female participants included in the SAUNA and CON groups were eumenorrheic (regular menstrual cycles lasting between 25-36 days; *n*=13), oligomenorrheic (menstrual cycles >40 days; *n*=2), amenorrheic (>3 months without menstruation; *n*=2), or using various forms of hormonal contraceptives (monophasic oral contraceptive pill, *n*=2; progestin-only oral contraceptive pill, *n*=1; implant, *n*=1; contraceptive coil, *n*=2). The subset of female participants sampled at the first post-exercise sauna bathing session were eumenorrheic (follicular phase, *n*=3; luteal phase, *n*=1), amenorrheic (*n*=1) or using a contraceptive coil (*n*=2). Participants complied with all criteria and instructions for participation (**Section 3.3** General participant instructions, **Section 3.6** Menstrual cycle questionnaire and self-reported log and **Section 3.9.1** Instructions for normal hydration).

7.3.3 Experimental procedures

7.3.3.1 Post-exercise sauna bathing

In the subset of participants sampled in greater detail at the first post-exercise sauna session (*n*=15), participants ended their outdoor run (7-11°C, 85-93%RH) at the University of

Birmingham's Sport and Fitness Centre between 19:00-20:00 h. Participants entered the sauna 17±12 min after their run, following measurement of nude body mass and instrumentation. Rectal temperature (T_{rec}) was recorded immediately before entering the sauna. Participants were asked to remain in the sauna (101-108°C, 5-10%RH at ~1.8 m; **Section 3.4.3**) for 30 min or until they could no longer tolerate the discomfort (**Section 3.7.3)**. *Ad libitum* water consumption was recorded. Heart rate (HR) and T_{rec} were recorded during the final minute of exposure. Nude body mass was measured ~5-10 minutes following sauna exposure.

For the remaining post-exercise sauna sessions when participants were not instrumented (i.e. in all SAUNA participants), participants entered the sauna within ~5 minutes of finishing outdoor exercise. Sauna bathing typically followed low-intensity, continuous exercise training sessions (i.e. "easy runs" or "long runs"). Only heart rate (HR) was recorded during the final minute of exposure.

7.3.3.2 Running heat tolerance test (HTT)

The running heat tolerance test (HTT) was performed in hot conditions (40°C, 40%RH) in the environmental chamber (**Sections 3.4.1** and **3.7.4)**. Briefly, participants ran on a treadmill at 9 $km \cdot h^{-1}$ and 2% gradient for 30-min (Mee et al., 2015a). Two CON males opted to wear a t-shirt and one CON male opted to wear a sleeveless running top for both Pre and Post tests. Rectal temperature (T_{rec}), skin temperature (T_{sk}), and HR were measured continuously (**Sections 3.9.3.1**, **3.9.3.2** and **3.9.4**, respectively). Perceptual measures of ratings of perceived exertion (RPE_{6-20}), thermal comfort and thermal sensation (**Section 3.10**), were obtained in the final minute of the HTT. Sweat gland activity of the forearm and upper back (**Section 3.9.6**) were recorded immediately following the HTT (within 2-10 s). Participants then sat in a chair with their left arm supported at heart-level, and forearm blood flow (FBF; **Section 3.9.5**) was recorded at 4:26±1:42 min post-exercise. FBF measurement times post-exercise differed by 26±45 s within-participant, between tests at Pre and Post. Sweat loss was measured via changes in towel-dried, nude body mass (**Section 3.9.6**).

7.3.3.3 Lactate profile test

The lactate profile test was performed in temperate conditions (~18°C) in the Human Performance Laboratory (**Sections 3.4.2** and **3.7.5)**. Briefly, participants completed a light 10-min warm-up before commencing the step-style incremental test on the treadmill (3-min stages increased by 1 $km \cdot h^{-1}$ until $[La^-]$ exceeded 4 $mmol \cdot L^{-1}$; **Sections 3.9.7.2** and **3.9.7.4**).

HR and respiratory gas exchange (**Section 3.9.2**) were continuously measured. Participants were then seated on a chair 30 s from the cessation of exercise, and sat quietly until 3-min post-exercise, whilst HR recovery (**Section 3.9.4.1**) was measured continuously.

7.3.3.4 Maximal aerobic capacity ($\dot{V}O_{2max}$) test

Approximately 10 min after completing the lactate profile test, participants performed a ramp-style $\dot{V}O_{2max}$ test (**Section 3.7.6)**. Briefly, the test consisted of 1-min stages that increased in speed (+1 $km \cdot h^{-1}$) for the first two stages, and then in gradient (+1%) until volitional exhaustion. When participants were re-tested at Post, the starting speed and progression of the protocol was repeated to allow for measurement of time-to-exhaustion (TTE). HR and respiratory gas exchange were continuously measured. Capillary [La^-] was measured 5-min from the time of exhaustion (**Sections 3.9.7.2** and **3.9.7.4**).

7.3.3.5 Resting heart rate and plasma volume visit

On a separate visit (between 9:00-11:00 h), participants rested in a supine position for a minimum of 10 minutes, at which time resting HR and plasma volume were assessed. Resting plasma volume changes were calculated via measurement of haemoglobin and haematocrit (**Section 3.9.7.3**) from a finger prick capillary sample (**Section 3.9.7.2**). Plasma volume was measured in 18 SAUNA (F: *n*=9; M: *n*=9) and 10 CON (F: *n*=4; M: *n*=6) participants due to technical difficulties, issues with scheduling or aversion to blood.

7.3.4 Data analyses

Height and body surface area were measured and calculated as described in **Section 3.5**. Peak T_{rec}, $T_{recRISE}$ and weighted T_{sk} were calculated as described in **Section 3.9.3**. For individual T_{sk} sites, if a time-point was lost, the missing data were modelled according to the slope/pattern of other data points from that participant during the previous test. Peak HR was calculated as described in **Section 3.9.4.** Upper back sweat gland activity was not recorded in the two male CON participants who wore a t-shirt during the HTTs. FBF was not recorded in two SAUNA (F: *n*=1; M: *n*=1) and two CON (M: *n*=2) participants due to technical difficulties. Respiratory gas exchange data were measured via open-circuit spirometry and used to calculate $\dot{V}O_{2max}$, RER and running economy (**Section 3.9.2)**. During the lactate profile test, submaximal RER, running economy and HR data were averaged

across the final minute of the stage where participants exceeded 4 mmol·L^{-1} [La^{-}] at baseline, with the same running speed compared at Post.

7.3.5 Statistical approach

Data collected during the first post-exercise sauna session were compared between-sex using independent *t*-tests. Pre- to post-intervention, the CON group was treated as a time-control, with *n*=15 participants deemed to provide sufficient power to detect any favourable changes as a result of normal training (as determined using changes in the SAUNA group in **Chapter 6**). Therefore, the effect of sex on physiological and perceptual data at Pre and Post was compared within each of the SAUNA and CON groups using a two-way (*time* × *sex*) analysis of variance (ANOVA). HR recovery at each post-exercise one-minute interval was compared using a three-way (*time* × *sex* × *interval*) ANOVA.

Average weekly training frequency and running distance were compared between-sexes within each of the SAUNA and CON groups using independent *t*-tests. Average weekly frequency and perceived exertion of each type of training session (i.e. easy runs, tempo sessions, high-intensity sessions and long runs) were compared using a two-way (*type* × *sex*) ANOVA.

Normality of the data was assessed using the Shapiro-Wilk Test (not violated on any occasion). Sphericity was assessed using Mauchly's Test of Sphericity, and Greenhouse–Geisser corrections were applied where assumptions of sphericity were violated. When a significant main effect was found, a Dunn-Bonferroni-corrected *post hoc* analysis was used to compare pairwise differences. Significance was set at $p \leq 0.05$. Absolute data are expressed as mean ± standard deviation (SD).

Power analyses were performed for sex differences in heat acclimation adaptations using data from a heat acclimation study by Mee et al. (2015b) and for temperate exercise adaptations using data from a meta-analysis of sex differences in $\dot{V}O_{2max}$ trainability from Diaz-Canestro and Montero (2019). For a two-way ANOVA, an α level of 0.05 and power set at ≥0.8, sample sizes of 16 and 17 total participants were calculated to be sufficient to detect a 0.3 °C sex difference in the reduction peak T_{rec} and a 1.9 L·min^{-1} sex difference in increase in $\dot{V}O_{2max}$, respectively. Given the high drop-out rate observed in the previous chapter, the recruitment strategy was again to recruit the maximum number of participants from the university running club at each intake period.

Partial correlations (controlling for sex) were performed *posteriori* to examine the relationships between adaptations (i.e. change scores from Pre to Post), as well as between adaptations, training volume and baseline fitness (i.e. relative $\dot{V}O_{2max}$) in the SAUNA group.

7.4 Results

7.4.1 Training

In the weeks between pre- and post-intervention testing, average weekly running distance in the SAUNA group was less in females (42.8±17.1 km) than males (69.5±13.0 km; *sex*: $p<0.001$). Similarly, in the CON group, average weekly running distance was less in females (42.9±12.8 km) than males (63.2±27.3 km; *sex*: $p<0.001$). There were no sex differences in either the SAUNA or CON groups in training session frequency, type or the associated perceived exertion (all $p>0.05$; **Table 7.2**).

Table 7.2 Weekly training type, frequency and mean perceived exertion during the intervention period. Data are presented as mean±SD

		SAUNA		CON	
Session Type		*Frequency (weekly)*	*RPE_{1-10}*	*Frequency (weekly)*	*RPE_{1-10}*
Easy	*F*	2±1	4±1	2±1	4±1
	M	2±1	3±1	2±1	4±1
Tempo	*F*	1±0	5±3	1±1	6±1
	M	1±0	6±1	1±0	7±1
High-Intensity	*F*	2±1	8±1	2±1	8±1
	M	2±0	8±1	2±1	8±0
Long	*F*	1±0	5±1	1±0	4±2
	M	1±0	5±1	1±0	5±1

SAUNA, post-exercise sauna bathing intervention group; CON, control group; RPE_{1-10}, mean rating of perceived exertion on a 1-10-point scale.

7.4.2 Post-exercise sauna bathing intervention

Immediately before the first post-exercise sauna bathing session, the subset of female and male participants sampled ran for a similar duration outdoors (F: 42±12 min, M: 55±9 min; $p=0.09$), with females exhibiting a higher mean HR during the run (F: 168±12; M: 143±8 beats·min^{-1}; $p<0.01$). Participants' T_{rec} was 37.6±0.2°C upon entering the sauna (F: 37.6±0.4°C, M: 37.6±0.2°C; $p=0.96$), and reached 38.5±0.4°C when they exited the sauna (F: 38.4±0.5°C, M: 38.6±0.2°; $p=0.23$), 29±3 minutes later (F: 29±3 min, M: 29±3 min; $p=0.74$). Peak HR in the sauna reached 132±19 beats·min^{-1} (F: 133±21 beats·min^{-1}, M: 131±18 beats·min^{-1}; $p=0.76$). Participants lost 1.2±0.7% of their body mass due to sweating (F: 1.2±0.2%, M: 1.2±0.6%; $p=0.94$), but drank 910±244 mL of water (F: 886±260 mL, M:

931±237 mL; *p*=0.73), therefore replacing 164±93% of water lost (F: 172±67%, M: 158±106%; *p*=0.98).

For the remainder of the study, all females and males in the SAUNA group (*n*=16) attended a similar (*p*=0.59) amount of sauna sessions (3±1 sessions·week^{-1}), accumulating 9±1 sauna sessions before completing temperate exercise tests at Post, and 10±1 sauna sessions before completing the HTT at Post. Participants remained in the sauna for 29±1 min each session, totalling 275±40 min of sauna exposure, which did not differ by sex (*p*=0.40 and *p*=0.68, respectively). Across all sauna sessions, peak HR was similar between female and male participants (F: 123±10, M: 123±8 beats·min^{-1}; *p*=0.92).

7.4.3 Menstrual cycle

Female participants completed all Pre and Post experimental trials 28±2 days apart. Eleven of the eumenhorreic female participants completed HTTs in the same phase of their menstrual cycle and nine completed temperate exercise tests in the same phase. The remainder completed Pre and Post tests in the following phases, HTTs: follicular then luteal (SAUNA, *n*=1), luteal then follicular (SAUNA, *n*=1); temperate exercise tests: follicular then luteal (SAUNA, *n*=1; CON, *n*=1), luteal then follicular (SAUNA, *n*=2). Participants using oral contraceptives (*n*=3) completed all tests in the active pill-taking phase.

7.4.4 Resting measures

Resting measures are detailed in **Table 7.3**. Resting HR and resting T_{rec} were similar between SAUNA females and SAUNA males (*sex*: *p*=0.30 and *p*=0.73, respectively). Post-exercise sauna bathing reduced resting HR (*time*: *p*<0.01; *time* × *sex:* *p*=0.58) and resting T_{rec} (*time*: *p*<0.01; *time* × *sex:* *p*=0.49) to a similar extent in female and male SAUNA participants.

Resting HR (*sex:* *p*=0.90; *time* × *sex:* *p*=0.64) and resting T_{rec} (*sex:* *p*=0.85; *time* × *sex:* *p*=0.54) were similar between CON females and CON males and did not change from Pre to Post (*time*: *p*=0.11 and *p*=0.43, respectively).

Although plasma volume was 6% higher in SAUNA females than SAUNA males (*sex*: *p*<0.01), plasma volume was not affected by post-exercise sauna bathing (*time*: *p*=0.65; *time* × *sex*: *p*=0.49). In the CON group, plasma volume was not different between sexes (*p*=0.50) and did not change from Pre to Post (*time*: *p*=0.47; *time* × *sex:* *p*=0.47).

Table 7.3 Physiological responses at rest and during the Running Heat Tolerance Test. Data are presented as mean±SD

		SAUNA		CON	
Rest		*Pre*	*Post*	*Pre*	*Post*
Resting T_{rec} (°C)	*F*	37.2±0.1	37.0±0.3*	37.2±0.4	37.4±0.6
	M	37.2±0.2	36.9±0.2*	37.3±0.2	37.3±0.3
Resting HR (beats·min^{-1})	*F*	57±8	53±9*	54±6	51±6
	M	53±8	50±7*	53±6	51±5
Resting Plasma Volume (%)	*F*	62±2$^{\#}$	62±4$^{\#}$	61±2	61±3
	M	57±2	56±5	58±3	61±7
Running Heat Tolerance Test					
Peak T_{rec} (°C)	*F*	38.8±0.4$^{\#}$	38.4±0.3*$^{\#}$	38.5±0.5	38.7±0.5
	M	38.3±0.3	38.1±0.4*	38.6±0.3	38.4±0.3
$T_{recRISE}$ (°C)	*F*	1.6±0.3$^{\#}$	1.4±0.3*	1.4±0.5	1.4±0.4
	M	1.0±0.3	1.1±0.3	1.3±0.4	1.2±0.5
Peak T_{sk} (°C)	*F*	37.1±0.8$^{\#}$	36.1±0.8*$^{\#}$	36.7±0.9	36.8±0.8
	M	36.0±1.2	35.3±0.7*	36.2±1.3	36.0±1.3
Peak HR (beats·min^{-1})	*F*	173±22$^{\#}$	163±18*$^{\#}$	168±11	166±18
	M	152±13	144±15*	153±12	152±10
Sweat Loss (%BM)	*F*	1.1±0.3$^{\#}$	1.3±0.3	1.3±0.4	1.0±0.3
	M	1.5±0.7	1.3±0.4	1.4±0.5	1.2±0.2
Forearm active sweat glands (per cm^2)	*F*	46±25	66±25*	44±13	40±20
	M	62±19	58±16	48±20	37±20
Upper back active sweat glands (per cm^2)	*F*	61±15	64±20	78±14	64±29*
	M	72±18	58±16*	61±13	42±4*
FBF (mL·dL tissue^{-1}·min^{-1})	*F*	2.77±1.61	3.11±1.80$^{\#}$	2.59±1.89$^{\#}$	2.49±0.92$^{\#}$
	M	3.83±2.32	8.30±4.63*	5.54±1.97	4.81±2.49

SAUNA, post-exercise sauna bathing intervention group; CON, control group; F, females; M, males; HR, heart rate; T_{rec}, rectal temperature; $T_{recRISE}$, rise in rectal temperature; T_{sk}, skin temperature; BM, body mass; FBF, forearm blood flow.
*Significantly different from Pre (p<0.05); $^{\#}$Significantly different from males at corresponding time-point (p<0.05)

7.4.5 Running heat tolerance test

7.4.5.1 Body temperatures

Body temperature absolute data and individual participant changes are presented in **Table 7.3** and **Figure 7.1**, respectively. SAUNA females had a higher peak T_{rec} and peak T_{sk} than SAUNA males across all HTTs (both *sex*: $p<0.01$). Both SAUNA females and males reduced peak T_{rec} and peak T_{sk} from Pre to Post (both *time*: $p<0.001$), with a trend for SAUNA females to experience a greater reduction in peak T_{rec} from Pre to Post (*time* × *sex*: $p=0.06$). Peak T_{sk} was reduced to a similar extent in both sexes (*time* × *sex:* $p=0.66$).

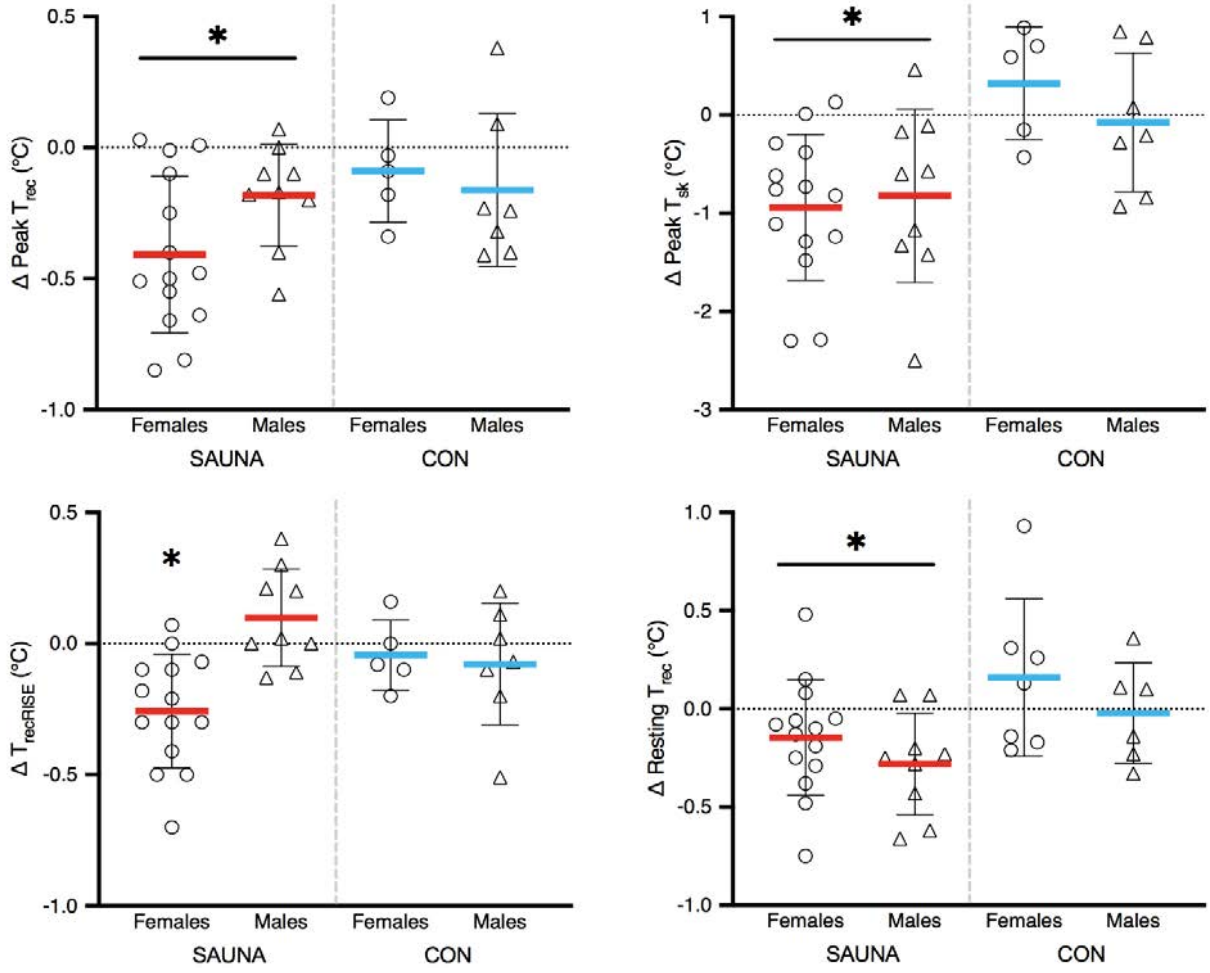


Figure 7.1 Changes (Δ) in body temperature responses during the running heat tolerance test (HTT; 40°C, 40%RH) from baseline (Pre) to following (Post) 3 weeks of post-exercise sauna bathing (SAUNA) or control (CON). Females are represented by circles and males by triangles. Horizontal black lines and error bars represent the group mean±SD. *Significant difference in Pre *vs* Post ($p<0.05$)
T_{rec}, rectal temperature; T_{sk}, skin temperature; $T_{recRISE}$, rise in rectal temperature

There was a *time* × *sex* interaction on $T_{recRISE}$ ($p=0.001$) and *post hoc* comparisons showed that only SAUNA females reduced $T_{recRISE}$ from Pre to Post (*time* × *sex:* $p<0.001$),

whilst $T_{recRISE}$ did not change in the SAUNA males (p=0.17). Therefore, in the SAUNA group, $T_{recRISE}$ was higher in females than the males at Pre (p<0.001), but not Post (p=0.10).

In the CON group, peak T_{rec}, $T_{recRISE}$ and peak T_{sk} were similar between sexes (*sex:* p=0.59-0.62) and did not change from Pre to Post (*time:* p=0.15-0.99; *time* × *sex:* p=0.29-0.71).

7.4.5.2 Heart rate

Heart rate absolute data are presented in **Table 7.3**. SAUNA females had a higher peak HR across all HTTs (*sex*: p=0.01). However, both SAUNA females and SAUNA males experienced a similar reduction in peak HR from Pre to Post (*time*: p=0.001; *time* × *sex*: p=0.63). CON Females tended to have a higher peak HR as compared to males (*sex*: p=0.06), though peak HR did not change from Pre to Post (*time*: p=0.43; *time* × *sex:* p=0.88).

7.4.5.3 Sweating

Absolute data and individual participant changes in sweating related outcomes are presented in **Table 7.3** and **Figure 7.2**, respectively. There was a *time* × *sex* interaction (p=0.03) on sweat loss in the SAUNA group. *Post hoc* comparisons showed sweat loss was greater in SAUNA males than in SAUNA females at Pre (p=0.05), but not Post (p=0.88). Sweat loss did not change in either SAUNA females nor SAUNA males from Pre to Post (p=0.14 and p=0.08, respectively). The CON group sweat loss was similar between sexes (p=0.14; *time* × *sex:* p=0.66) and did not change from Pre to Post (*time*: p=0.08).

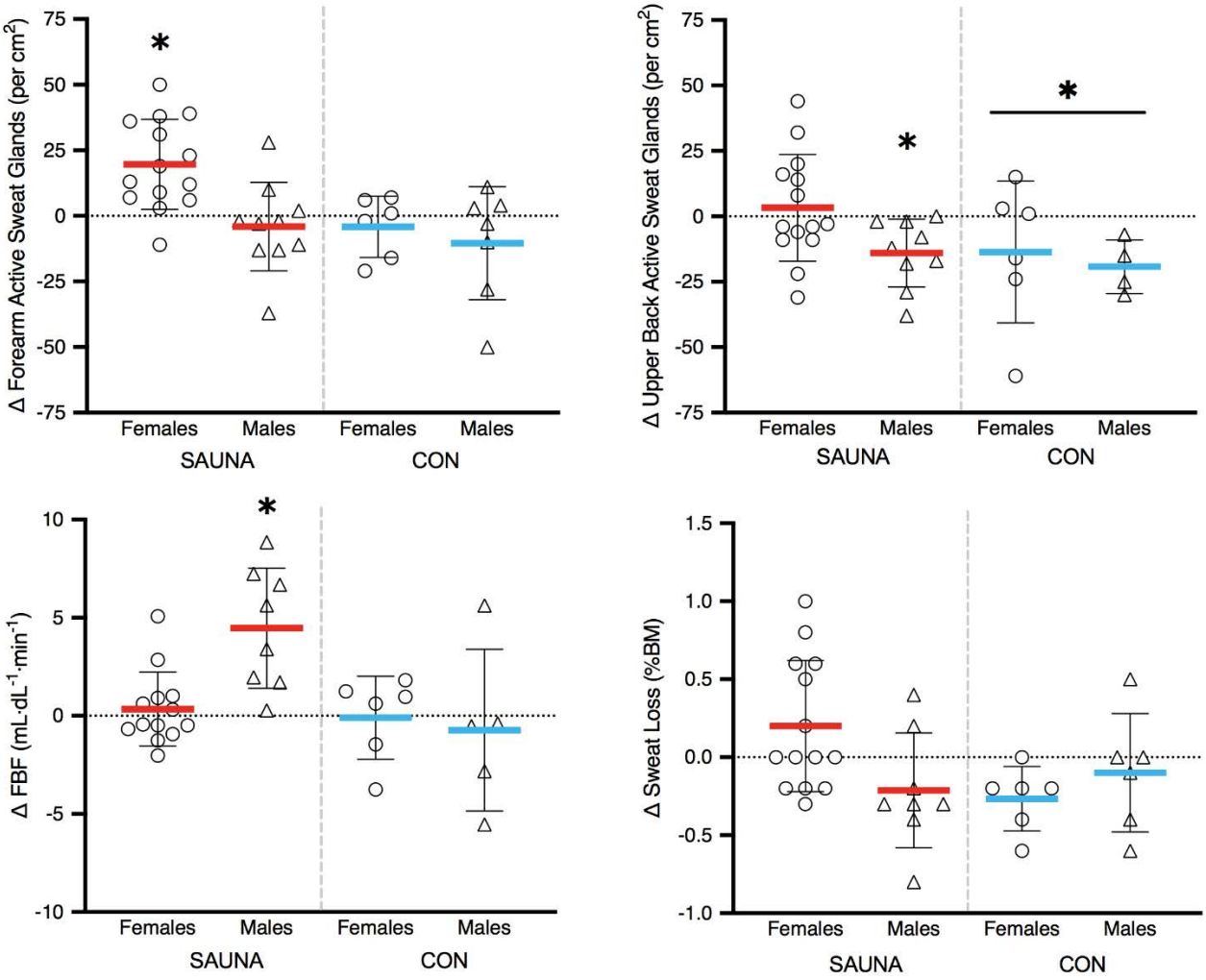


Figure 7.2 Changes (Δ) in thermoeffector responses during the running heat tolerance test (HTT; 40°C, 40%RH) from baseline (Pre) to following (Post) the 3-week post-exercise sauna bathing intervention (SAUNA) or control (CON). Females are represented by circles and males by triangles. Horizontal black lines and error bars represent the group mean±SD.
*Significant difference in Pre *vs* Post (p<0.05)
FBF, forearm blood flow

There were significant *time* × *sex* interactions on forearm (p<0.01) and upper back (p=0.03) sweat gland activity in the SAUNA group. *Post hoc* comparisons showed that SAUNA females increased sweat gland activity on the forearm by 57% from Pre to Post (p<0.001), but not on the upper back (p=0.51). In contrast, SAUNA males did not exhibit a change in sweat gland activity on the forearm from Pre to Post (p=0.47), and instead decreased sweat gland activity on the upper back by 18% (p=0.03). There were no differences between sexes in the SAUNA group in sweat gland activity at Pre or Post, at either site (p=0.12-0.42).

Forearm sweat gland activity in the CON group was similar between sexes (*sex*: p=0.96; *time* × *sex:* p=0.50) and did not change from Pre and Post (*time:* p=0.15). However,

both CON females and males experienced a similar, 22% reduction in sweat gland activity on the upper back from Pre to Post (*time*: $p<0.05$; *sex*: $p=0.08$; *time* × *sex*: $p=0.63$).

7.4.5.4 Forearm blood flow

FBF showed a *time* × *sex* interaction ($p=0.001$) in the SAUNA group (**Table 7.3**). *Post hoc* comparisons showed that SAUNA males increased FBF by 123% from Pre to Post ($p<0.001$; **Figure 7.2**), whilst FBF remained similar in SAUNA females ($p=0.61$). Consequently, FBF was similar between sexes at Pre ($p=0.23$), but was higher in SAUNA males than in SAUNA females at Post ($p<0.01$).

In the CON group, FBF did not change from Pre to Post (*time*: $p=0.68$; *time* × *sex:* $p=0.75$), though FBF was higher in CON males than in CON females overall (*sex*: $p<0.01$).

7.4.5.5 Perceptual

Overall, SAUNA females reported higher Thermal Sensation (*sex*: $p<0.01$) and Thermal Comfort (*sex*: $p=0.001$) ratings than SAUNA males (i.e. being hotter and less comfortable). SAUNA females and males reported similar reductions in peak Thermal Sensation (F: 11±1 [“very hot”] and 10±1 [“hot”]; M: 10±1 [“hot”] and 9±1 [“warm”], at Pre and Post, respectively; *time*: $p<0.001$; *time* × *sex*: $p=0.81$) and peak Thermal Comfort (F: 6±2 [between “uncomfortable” and “very uncomfortable”] and 5±2 [“uncomfortable”]; M: 4±1 [between “slightly uncomfortable” and “uncomfortable”] and 3±1 [“slightly uncomfortable”], at Pre and Post, respectively; *time*: $p=0.001$; *time* × *sex*: $p=0.08$).

Peak $RPE_{6\text{-}20}$ showed a *time* × *sex* interaction ($p=0.01$) in the SAUNA group. *Post hoc* comparisons showed that SAUNA females reduced $RPE_{6\text{-}20}$ from Pre to Post (13±3 [“somewhat hard”] to 11±2 [“fairly light”], respectively; $p=0.001$). In contrast, SAUNA males reported a similar $RPE_{6\text{-}20}$ at Pre and Post (both 10±2 [between “fairly light” and “very light”]; $p=0.76$). Consequently, SAUNA females reported higher RPE than the SAUNA males at Pre ($p<0.01$), but not Post ($p=0.10$).

Overall, CON females reported lower Thermal Comfort ratings (i.e. being more comfortable) than CON males (F: 3±2 and 4±2; M: 5±1 and 6±2, at Pre and Post, respectively; *sex*: $p=0.04$). Both CON females and males reported higher Thermal Comfort ratings at Post compared to Pre (i.e. becoming less comfortable; *time*: $p=0.04$; *time* × *sex:* $p=0.92$). Thermal Sensation (F: 9±1 and 10±1; M: 10±1 and 10±1, at Pre and Post, respectively) was similar between sexes in CON (*sex:* $p=0.10$; *time* × *sex:* $p=0.35$) and did not change from Pre to Post (*time:* $p=0.80$). $RPE_{6\text{-}20}$ (F: 11±2 and 11±1; M: 11±3 and 11±2,

at Pre and Post, respectively) was similar between sexes in CON (*sex:* p=0.91; *time* × *sex:* p=0.98) and did not change from Pre to Post (*time:* p=0.75).

7.4.6 Temperate exercise tests

7.4.6.1 Lactate profile

In the SAUNA group, both sexes showed a similar increase in running speed at 4 mmol·L^{-1} [La^{-}] from Pre to Post (*time*: p=0.001; *time* × *sex*: p=0.58; **Table 7.4**), though SAUNA males reached 4 mmol·L^{-1} [La^{-}] at a faster running speed than SAUNA females (*sex*: p<0.001). The CON group did not exhibit any changes in running speed at 4 mmol·L^{-1} [La^{-}] from Pre to Post (*time*: p=0.69; *time* × *sex*: p=0.61), though CON males also reached 4 mmol·L^{-1} [La^{-}] at a faster running speed than CON females (*sex*: p<0.001; **Table 7.4**).

Table 7.4 Physiological responses to temperate exercise tests. Data are presented as mean±SD

		SAUNA		CON	
		Pre	*Post*	*Pre*	*Post*
$\dot{V}O_{2max}$ (L·min^{-1})	*F*	2.95±0.48$^{\#}$	3.10±0.33*$^{\#}$	2.83±0.20$^{\#}$	2.86±0.35$^{\#}$
	M	4.34±0.53	4.46±0.49*	4.06±0.37	4.03±0.19
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	*F*	52.6±6.9$^{\#}$	55.3±4.3*$^{\#}$	52.2±4.1$^{\#}$	52.6±2.3$^{\#}$
	M	64.6±2.4	66.6±3.7*	63.6±3.2	63.6±4.5
TTE (s)	*F*	412±69	450±68*	419±72	416±76
	M	428±52	448±73*	409±64	400±72
Speed at 4 mmol·L^{-1} [La^{-}] (km·h^{-1})	*F*	14.7±1.5$^{\#}$	15.0±1.4*$^{\#}$	15.1±0.9$^{\#}$	15.2±0.6$^{\#}$
	M	18.2±0.8	18.6±0.5*	17.7±1.0	17.7±1.0
Submaximal HR (beats·min^{-1})	*F*	187±11	184±8*	187±4	188±8
	M	187±11	186±13*	178±12	180±11
Submaximal RER	*F*	1.02±0.06	1.03±0.06	1.02±0.04	1.04±0.04
	M	1.05±0.03	1.03±0.04	1.04±0.03	1.05±0.04
Submaximal RE (mL·kg^{-1}·km^{-1})	*F*	190±15	192±13	178±22	187±18
	M	186±8	186±7	186±11	191±10

SAUNA, post-exercise sauna bathing intervention group; CON, control group; F, females; M, males; $\dot{V}O_{2max}$, maximal aerobic capacity; TTE, time to exhaustion; [La^{-}], blood lactate concentration; HR, heart rate; RER, respiratory exchange ratio; RE, running economy. *Significantly different from Pre (p<0.05); $^{\#}$Significantly different from males (p<0.05)

7.4.6.2 Submaximal gas exchange and heart rate

Submaximal temperate exercise data are presented in **Table 7.4**. Submaximal HR, running economy and RER at running speed at 4 mmol·L^{-1} [La^{-}] were similar between sexes

in the SAUNA group (*sex: p*=0.37-0.87). SAUNA females and males showed a similar reduction in submaximal HR from Pre to Post (*time*: *p*=0.02; *time* × *sex*: *p*=0.55). However, submaximal running economy and RER did not change from Pre to Post (*time*: *p*=0.42 and *p*=0.59; *time* × *sex*: *p*=0.98 and *p*=0.17; respectively).

Submaximal HR, running economy and RER at running speed at 4 mmol·L^{-1} [La^-] were similar between sexes in the CON group (*sex: p*=0.11-0.46) and did not change from Pre to Post (*time*: *p*=0.07-0.48; *time* × *sex*: *p*=0.29-0.65).

7.4.6.3 Heart rate recovery

There was a three-way interaction of *sex* × *time* × *interval* (*p*=0.04) on HR Recovery in the SAUNA group (**Figure 7.3**). *Post hoc* comparisons showed that SAUNA females experienced a reduction in HR at 1-min (*p*<0.01) and 3-min (*p*=0.01) from the cessation of exercise, but not significantly so at the immediate cessation (*p*=0.06) or at 2-min from the cessation of exercise (*p*=0.07) from Pre to Post. There were no differences at any time-point in SAUNA males from Pre to Post (*p*=0.61-0.75).

Overall, CON females exhibited a higher HR than CON males during HR Recovery (*sex: p*<0.01). However, the CON group did not show any changes from Pre to Post (*time: p*=0.75, *time* × *interval: p*=0.70; *sex* × *time* × *interval: p*=0.64) or other interaction effects related to sex (*sex* × *time p*=0.44; *sex* × *interval: p*=0.12) in HR recovery.

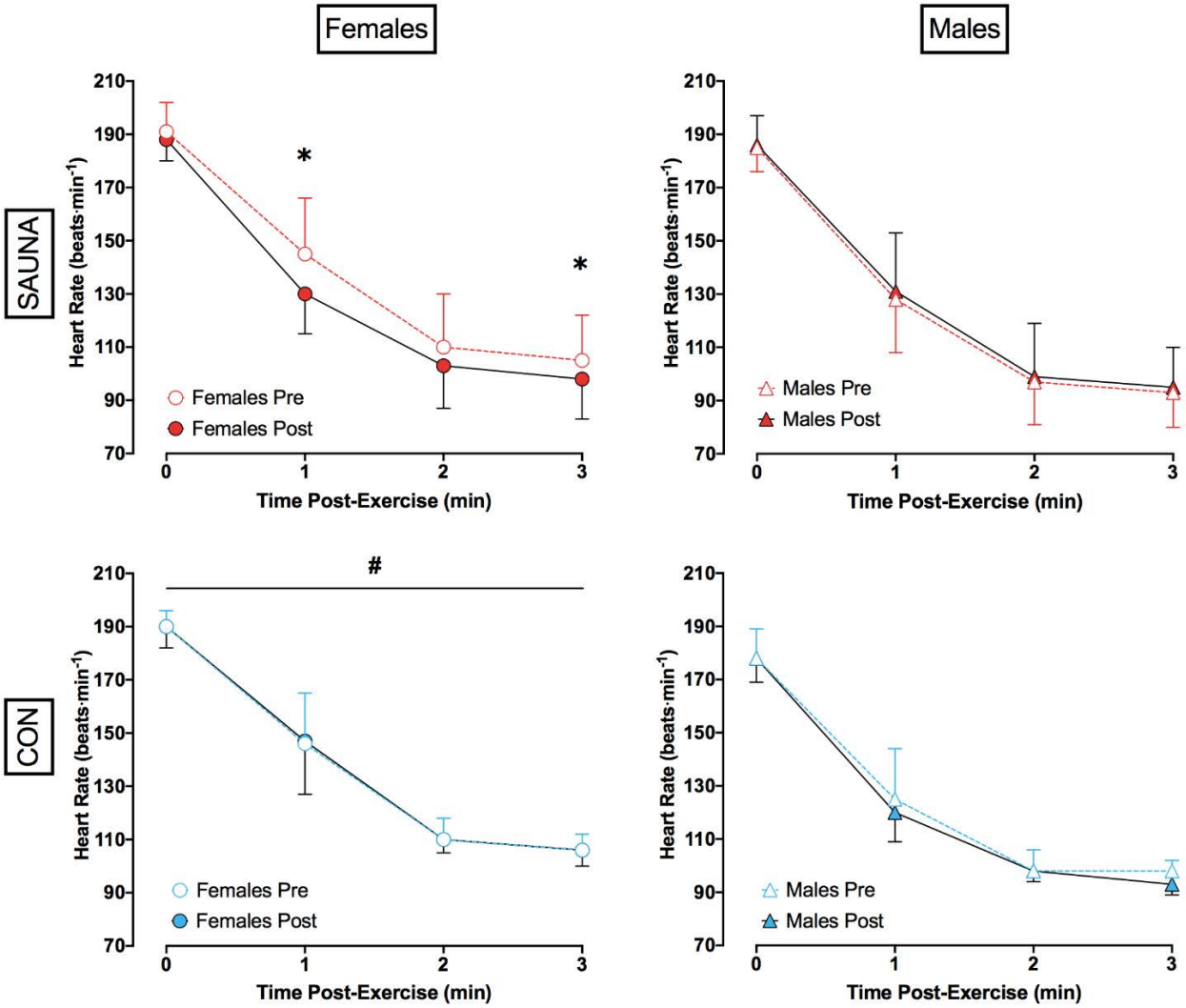


Figure 7.3 Heart rate recovery from submaximal exercise in temperate conditions (18°C) in females (*circles*) and males (*triangles*) at baseline (Pre; *open*) *vs* following (Post; *filled*) 3 weeks of post-exercise bathing (SAUNA; *top row*) or control (CON; *bottom row*). *Significant difference at Pre *vs* Post (p<0.05); #Significantly different from males (p<0.05).

7.4.7 Maximal aerobic capacity ($\dot{V}O_{2max}$) test

SAUNA females and males showed a similar increase in both absolute (*time:* p=0.03; *time* × *sex*: p=0.86) and relative (*time:* p=0.02; *time* × *sex*: p=0.73) $\dot{V}O_{2max}$ from Pre to Post (**Table 7.4**). However, overall absolute and relative $\dot{V}O_{2max}$ values were higher in SAUNA males than in SAUNA females (both *sex*: p<0.001). SAUNA females and males also showed similar increases (~8%) in TTE in the $\dot{V}O_{2max}$ test (*time*: p<0.001; *time* × *sex*: p=0.20) from Pre to Post (**Table 7.4**). TTE was not different between sexes in the SAUNA group (*sex*: p=0.79).

The CON group did not exhibit any changes in either absolute (*time:* p=0.99; *time* × *sex*: p=0.60) or relative $\dot{V}O_{2max}$ (*time:* p=0.86; *time* × *sex*: p=0.84), though CON males

exhibited higher absolute and relative $\dot{V}O_{2max}$ values than CON females (both *sex*: $p<0.001$; **Table 7.4**). TTE was similar between sexes in the CON group (*sex*: $p=0.73$) and did not change from Pre to Post (*time*: $p=0.66$; *time* × *sex*: $p=0.83$; **Table 7.4**).

7.4.8 Relationships between heat acclimation adaptations

Correlations between heat acclimation adaptations are presented in **Figure 7.4**. For all variables, adaptations were not related to training volume during the intervention period (all $p\geq0.17$). Baseline fitness (i.e. relative $\dot{V}O_{2max}$) showed a strong negative correlation with change in absolute $\dot{V}O_{2max}$ ($p<0.01$, $r=-0.63$), and tended to show a moderate positive correlation with change in submaximal HR during the Lactate Profile Test ($p=0.06$, $r=-0.41$). Other adaptations were not related to baseline fitness (all $p\geq0.19$).

Change in resting T_{rec} showed a strong positive correlation with change in peak T_{rec} ($p<0.01$, $r=0.62$) and showed a trend for a weak negative correlation with $T_{recRISE}$ ($p=0.08$, $r=-0.39$).

Change in peak HR showed a strong positive correlation with change in peak T_{rec} ($p<0.01$, $r=0.60$), a moderate positive correlation with change in $T_{recRISE}$ ($p=0.03$, $r=0.45$), a moderate positive correlation with change in submaximal HR during the Lactate Profile Test ($p=0.01$, $r=0.52$), a moderate negative correlation with change in speed at 4 $mmol \cdot L^{-1}$ $[La^-]$ ($p=0.02$, $r=-0.51$) and a moderate negative correlation with change in TTE ($p=0.04$, $r=-0.45$). Change in peak HR also showed a trend for a positive correlation with change in RPE_{6-20} ($p=0.10$), though this relationship was weak ($r=0.36$).

Change in sweat loss showed a moderate negative correlation with change in Thermal Comfort ($p<0.01$, $r=-0.57$), whereby those showing greater increases in sweat loss showed greater reductions in Thermal Comfort score (i.e. becoming more comfortable).

Change in peak T_{sk} showed a trend for a weak correlation with change in FBF ($p=0.10$, $r=-0.38$), whereby those showing greater reductions in peak T_{sk} also showed greater increases in FBF.

Perceptual adaptations during the HTT (RPE_{6-20}, Thermal Sensation and Thermal Comfort) were ($p<0.01$) or tended to ($p=0.07$) show moderate ($r=-0.39$-0.58) positive correlations with one another. All other heat acclimation adaptations were not related to one another ($p\geq0.16$).

Change in $\dot{V}O_{2max}$ showed a moderate positive correlation with TTE ($p=0.04$ $r=0.42$) and a moderate negative correlation with change in submaximal HR during the Lactate Profile Test ($p=0.02$, $r=-0.48$). Change in $\dot{V}O_{2max}$ also showed a trend for a positive correlation with change in speed at 4 $mmol \cdot L^{-1}$ $[La^-]$ ($p=0.06$), though this relationship was

weak (r=0.39). There was a moderate positive relationship between change in speed at 4 mmol·L^{-1} [La^{-}] and TTE (p<0.01 r=0.55).

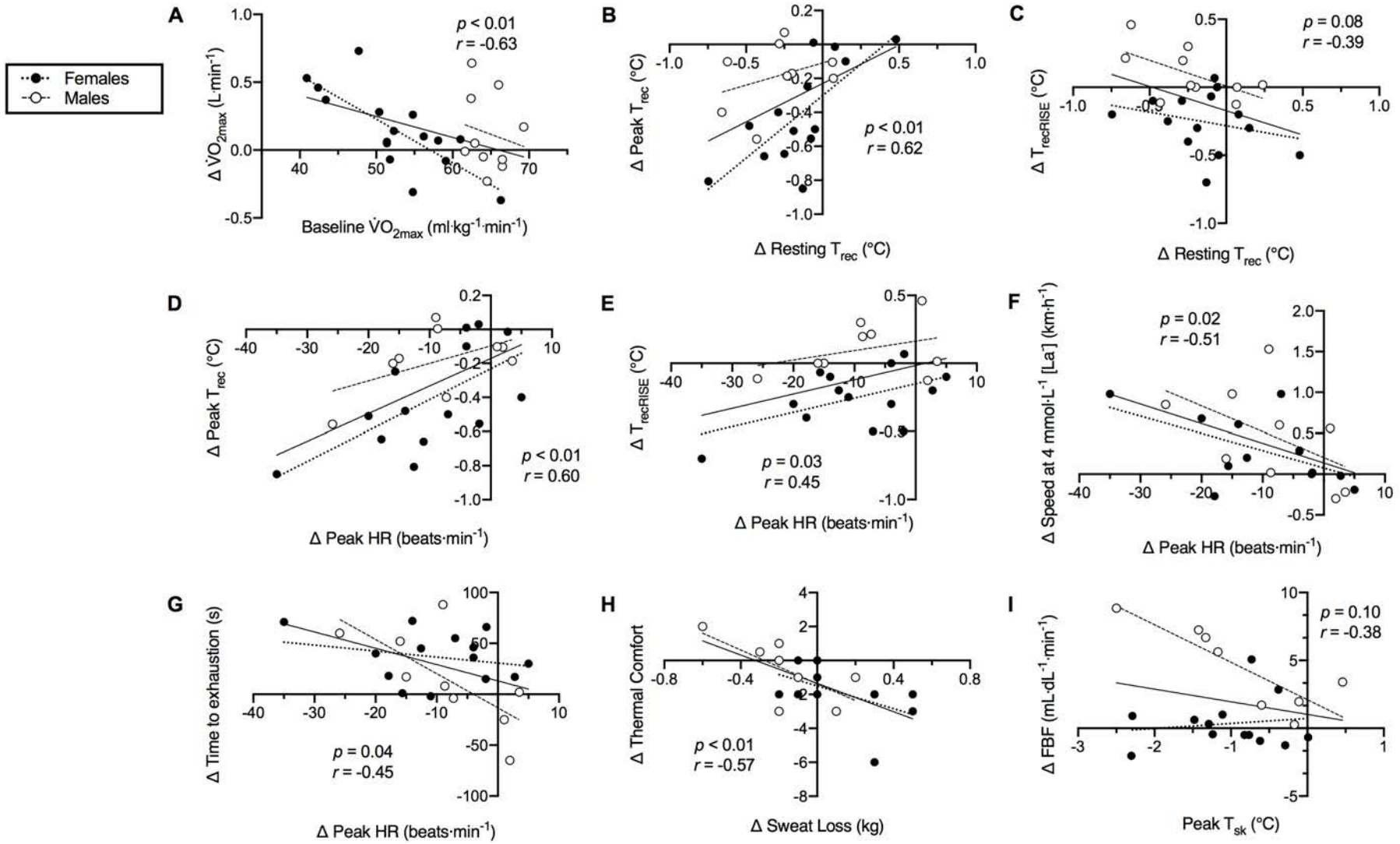


Figure 7.4 Correlations between heat acclimation adaptations following 3 weeks of intermittent post-exercise sauna bathing. Adaptations are represented by change scores from pre- to post-acclimation. Female data and regression lines are denoted by filled symbols and dotted lines, respectively. Male data and regression lines are denoted by open symbols and dashed lines, respectively. The solid regression lines, *p*-values and correlation coefficients relate to pooled female and male data.
$\dot{V}O_{2max}$, maximal aerobic capacity; T_{rec}, rectal temperature; $T_{recRISE}$, rise in rectal temperature; HR, heart rate; [La^-], blood lactate concentration; FBF, forearm blood flow; T_{sk}, skin temperature

7.5 Discussion

The main findings of this study were that, contrary to the first hypothesis, both female and male endurance athletes exhibited similar physiological strain during an acute bout of post-exercise sauna bathing. Furthermore, contrary to the second hypothesis, 3 weeks of intermittent post-exercise sauna bathing was *not* more effective in males. In fact, post-exercise sauna bathing was arguably more effective in female athletes, as both sexes showed similar reductions in thermal and cardiovascular strain while only females showed an attenuated *rise* in rectal temperature during exercise-heat stress at post-intervention. Moreover, there were novel sex differences thermoeffector adaptations. Males exhibited marked increases in forearm blood flow in response to exercise-heat stress, and reduced sweat gland activity on the back but not the forearm post-intervention. Conversely, females exhibited increased sweat gland activity on the forearm and not the back, and did not experience any changes in forearm blood flow.

Temperate performance results also contradict our second hypothesis, as both sexes in the SAUNA group showed similar improvements in markers of temperate exercise performance (i.e. $\dot{V}O_{2max}$, running speed at 4 mmol·L^{-1} [La^{-}], TTE and submaximal HR) following 3 weeks of intermittent post-exercise sauna bathing. HR recovery from submaximal temperate exercise was faster at post- as compared to pre-intervention in SAUNA females only. Importantly, female and male athletes in the CON group did not show any favourable improvements following three weeks of normal training, indicating that findings in the SAUNA group resulted from the post-exercise sauna intervention, in line with the findings from **Chapter 6**.

7.5.1 Intervention and training

Overall, the post-exercise sauna bathing intervention appeared to invoke a similar physiological stimulus in the subset of females and males sampled at the first sauna session (i.e. similar T_{rec}, HR, sweat loss and thirst, as indexed by similar *ad libitum* fluid consumption), as well as in those observed during the course of the intervention (i.e. similar HR). These similarities were somewhat unexpected, considering females likely experienced greater dry heat gain in the hot-dry conditions of the sauna because of their greater surface-area-to-mass ratio (Shapiro et al., 1980). Furthermore, both sexes entered the sauna at T_{rec} ~37.6°C, despite female participants running at a higher mean HR than male participants in the training session immediately before the first post-exercise sauna bathing session. Peak T_{rec} in the sauna was also similar between sexes at the first post-exercise sauna session,

reaching ~38.5°C, the suggested core body temperature threshold for inducing thermoregulatory adaptations (Taylor & Cotter, 2006). It should be noted that the first post-exercise sauna session, and most of those thereafter, occurred in the evenings (19:00-20:00), when circadian rhythms would have aided in reaching this T_{rec} threshold (Waterhouse et al., 2005; Heathcote et al., 2019).

Although participants reached a peak T_{rec} of ~38.5°C in the first sauna session, it is likely that the thermal impulse (i.e. elevated T_{rec}) diminished as the protocol progressed (Taylor, 2014). Indeed, Tyka et al. (2008) observed that males' peak T_{rec} during a sauna bath (independent of exercise) was ~38.5°C at the first session, but was reduced to, and subsequently plateaued at ~37.8°C by the fifth session. It is also possible that T_{rec} was lower upon entering the sauna after exercise by the end of the protocol, since Zurawlew et al. (2016) showed that 6-days post-exercise hot water immersion reduced peak T_{rec} during exercise in 18°C. Nonetheless, heat acclimation adaptations were observed despite thermal impulse likely waning by the latter sauna exposures.

Lastly, despite different overall running distances in training, there were no differences in weekly type, frequency and perceived exertion of sessions between sexes. This indicates a similar relative training stimulus, especially as participants were from the same running club. Furthermore, training volume was not correlated with any adaptations observed during either the HTT or the temperate exercise tests.

7.5.2 Exercise-heat tolerance

Both females and males in the SAUNA group showed similar reductions in peak and resting T_{rec} post-intervention, with mean reductions reaching or exceeding the 0.2°C threshold identified as physiologically meaningful (Tyler et al., 2016). Both sexes in the SAUNA group also reduced peak T_{sk} and peak HR to a similar extent, with the rate of rise in T_{rec} (i.e. $T_{recRISE}$) attenuated post-intervention in the SAUNA females only. That females showed similar thermoregulatory and cardiovascular adaptations, or sometimes superior to males, was contrary to the hypothesis, which was informed by findings in **Chapter 4** and by the robust sex-comparison study by Mee and colleagues (2015b). To achieve these adaptations, female and male participants in the current study received ~275 min of exogenous, passive heat exposure via post-exercise sauna bathing in addition to their normal training over a three-week period. In contrast, Mee et al. (2015b) used a traditional isothermic heat acclimation protocol and found that 5 consecutive days of 90-min active heat exposures (i.e. a total 450 min of heat exposure) resulted in body temperature and cardiovascular adaptations in males but not in females. Mee and colleagues showed that

females required ten days (and thus 900 min) of isothermic heat exposure (T_{rec} ≥38.5°C) to achieve the same level of adaption as their male counterparts (and a similar degree of adaptation as demonstrated in the current study). Importantly, both the current study and Mee et al. (2015b) used the same heat tolerance test (Mee et al., 2015a) to examine heat adaptation, allowing us to compare the magnitude of adaptation. We observed a ~0.4°C reduction in peak T_{rec}, ~1.0°C reduction in T_{sk} and ~10 beats·min^{-1} reduction in HR in our female cohort, similar to the ~0.5°C, ~1.1°C and ~10 beats·min^{-1} reductions, respectively, reported in females by Mee et al. (2015b) following 10 days of isothermic heat acclimation. These similarities were in spite of ~70% less total exogenous heat exposure time in the current study as compared to the 10-consecutive day heat acclimation protocol by Mee et al. (2015b), albeit the 10 exposures in the current study were spread across three weeks. Whether sex differences similar to that observed by Mee et al. (2015b) would have been apparent after 5 post-exercise sauna bathing sessions (i.e. ~1.5 weeks) is unknown. However, the higher fitness level of female participants in the current study as compared to that by Mee et al. (2015b) (~53 *vs* 45 mL·kg^{-1}·min^{-1}) may have facilitated a faster rate of adaptation (Pandolf et al., 1977).

It appears that the reduction in peak T_{rec} in males was achieved through a lower resting T_{rec}. However, females achieved a reduction in peak T_{rec} through both a lowered resting T_{rec} and a reduced $T_{recRISE}$ during the HTT, culminating in the trend for a greater reduction in peak T_{rec} in females. However, the attenuated $T_{recRISE}$ in the SAUNA females and the lack of change in $T_{recRISE}$ in males may have been influenced by the tendency for participants with “worse” baseline values (i.e. females) to show a greater improvement. The fixed workload of the HTT was not specific to sex, fitness or anthropometry. Males in the SAUNA group were therefore working at a lower intensity relative to their fitness level and at a lower metabolic heat production relative to their larger body size than females (Jay & Cramer, 2015). Consequently, males in the SAUNA group consistently achieved lower $T_{recRISE}$ values at baseline than females, and would need to show a greater relative improvement in heat dissipation and/or reduction in heat production to exhibit the same absolute improvement in $T_{recRISE}$ as females. These differences in metabolic heat production as well as relative intensity make it somewhat difficult to compare the magnitude of the change in core body temperatures between sexes.

Only females in the SAUNA group increased sweat gland activity on the forearm post-intervention. **Chapters 4** also showed an increase in forearm sweat gland activity in a female cohort following consecutive-day heat acclimation. However, in the current study, which is consistent with others (Inoue et al., 1999; Lee et al., 2010; Poirier et al., 2016), males showed no change in forearm sweat gland activation following repeated heat exposures. Thus, the increase in forearm sweat gland activity in **Chapter 6** was likely driven

by the predominantly female cohort (9F, 3M). Considering females exhibit relatively lower sudomotor thermosensitivity and maximal sweat output per gland (Gagnon & Kenny, 2011), the increase in sweat gland activity observed in the current study may be due to peripheral changes to the cholinergic sensitivity of the forearm sweat glands or size of the sweat glands in response to heat acclimation, allowing more active sweat glands to be identified (Sato & Sato, 1983; Buono et al., 2009a). Though mechanisms are yet to be delineated, adaptations that increase the number of active sweat glands appear to be unique to female cohorts undergoing heat acclimation. Curiously, this 43% increase in forearm sweat gland activation in females was observed without an increase in overall total-body sweat loss. This may indicate a more efficient distribution of sweating, whereby the relative contribution to whole-body sweat loss is shifted away from the back and towards the upper limbs following acclimation, as observed by Smith and Havenith (2019). Moreover, it is possible that there was no change in whole-body sweat loss in the current study because the post-exercise sauna bathing intervention probably did not maintain thermal impulse throughout (i.e. T_{rec} ~38.5°C; Taylor, 2014) , as discussed above. Maintaining thermal impulse through an isothermic heat acclimation protocol has previously been suggested to induce preferential sudomotor adaptations (Patterson et al., 2004b; Gibson et al., 2015c).

Only males in the SAUNA group increased FBF post-intervention, which is consistent with what others have shown in male cohorts following active heat acclimation (Roberts et al., 1977; Armstrong & Kenney, 1993; Fujii et al., 2012). This increase in FBF is likely a result of improved cutaneous vascular function (Lorenzo & Minson, 2010a), which is largely nitric oxide-dependent (Brunt et al., 2016a). Females are less reliant on nitric oxide-dependent dilation to increase blood flow to the skin during local heating, as oestrogen can stimulate vasodilation in a nitric oxide-independent manner (Orshal & Khalil, 2004; Stanhewicz et al., 2014), which might limit these functional adaptations. Of note, whilst females and males are known to exhibit similar skin blood flow responses to passive heating at the forearm, females show markedly greater skin blood flow at the thigh (Inoue et al., 2005). Thus, the single-site measurement in the current study may not be representative of whole-body changes in skin blood flow. Nevertheless, it should be stipulated that although females did not increase FBF following the post-exercise sauna intervention, they achieved a similar FBF at a ~0.4°C lower T_{rec}, indicating greater vasomotor thermosensitivity. Overall, the dichotomous profiles of thermoeffector adaptations observed between sexes is both novel and interesting, considering post-exercise sauna bathing incited a similar thermal burden in females and males in the initial session, and probably throughout (given similar HR responses).

Although trained athletes have previously been observed to heat acclimate more rapidly (Williams et al., 1967; Pandolf et al., 1977), fitness may not predict the *magnitude* of

these adaptations. In line with data from Corbett et al. (2018), baseline fitness and training volume were not correlated with heat acclimation adaptations during the HTT in the current study. However, in contrast to data from Corbett et al. (2018), some indices of heat acclimation were related to one another. Namely, T_{rec} (resting, peak and rise) and HR adaptations showed positive relationships, whereby "high-responders" showed similar responses across T_{rec} and HR adaptations. One exception to this was the trend for a negative relationship between resting T_{rec} and $T_{recRISE}$. Thus, participants that had lesser reductions in resting T_{rec} may have compensated by either reducing heat production or increasing heat loss to achieve a lower $T_{recRISE}$. Furthermore, the relationship between sweat loss and Thermal Comfort adaptations indicates that those showing greater sweat loss adaptations also reported being more comfortable. This could have implications for self-paced exercise. Notably, none of the measures of body temperature adaptations (i.e. peak T_{rec}, resting T_{rec}, $T_{recRISE}$ and T_{sk}) were related to any perceptual adaptations.

7.5.3 Temperate exercise performance

Both females and males in the SAUNA group improved absolute and relative $\dot{V}O_{2max}$, running speed at 4 $mmol \cdot L^{-1}$ $[La^-]$ and TTE to a similar extent. Data presented in **Chapter 6** showed that 3 weeks of intermittent post-exercise sauna bathing is effective for improving markers of submaximal and maximal temperate exercise performance in a mixed-sex cohort. With the addition of more participants of both sexes, this study further supports the literature evidencing the ergogenic effects of heat acclimation, and importantly, it supports the ergogenic benefits of heat acclimation in both sexes.

Quantification (and debate) of improvements in exercise performance and $\dot{V}O_{2max}$ in relatively cooler conditions following heat acclimation have been mainly limited to male data. Only two studies to date have investigated changes in $\dot{V}O_{2max}$ following heat acclimation in a female cohort (Fortney & Senay, 1979; Bailey et al., 2016). Fortney and Senay (1979) employed 90-min cycling at 30%$\dot{V}O_{2max}$ in 45°C, 30%RH daily for two weeks, then every other day for an additional two weeks, and did not observe any changes in $\dot{V}O_{2max}$. Conversely, Bailey et al. (2016) employed 30-min hot-water immersion (42°C water to the chest) thrice weekly for eight weeks, and observed a 5.6% improvement in $\dot{V}O_{2max}$. This is similar to the ~5% improvement in females in the current study. However, participants in both previous studies were sedentary or untrained. Thus, the current study provides the first evidence of improved $\dot{V}O_{2max}$ following heat acclimation in a trained female cohort. Moreover, the current study shows that this improvement is similar to that experienced in a male cohort training within the same running club. Thus, contrary to the study's hypothesis,

the female cohort in the current study did not show a reduced trainability of $\dot{V}O_{2max}$, contrasting with previous findings from exercise interventions in females (Diaz-Canestro & Montero, 2019).

Females and males in the current study, given a similar relative training stimulus and a similar thermal stimulus in the sauna, showed similar improvements in speed at 4 $mmol \cdot L^{-1}$ [La^-]. This is supported by previous research, showing a similar reduction in submaximal exercise metabolism in males and females exercising in cool conditions following heat acclimation (Sawka et al., 1983a). Furthermore, Hafen et al. (2018) reported increases in mitochondrial biogenesis and function (the mechanisms expected to largely underpin improvements in speed at 4 $mmol \cdot L^{-1}$ [La^-]; Holloszy & Coyle, 1984) following six days of 2-h localised thigh heating in a mixed-sex cohort (10 F, 10 M). However, the current study is the first to specifically investigate the effects of sex on metabolic thresholds in temperate conditions following heat acclimation. Neither males nor females in the control group experienced improvements in these performance outcomes, which indicates that 3 weeks of normal training alone was not sufficient to improve $\dot{V}O_{2max}$, running speed at 4 $mmol \cdot L^{-1}$ [La^-] or TTE in this well-trained cohort. Such adaptations have been shown to strongly correlate with time trial improvements (Lorenzo et al., 2011).

Baseline $\dot{V}O_{2max}$ was related to changes in absolute $\dot{V}O_{2max}$. It is possible that those with a higher $\dot{V}O_{2max}$ at baseline may have a lesser capacity to further increase $\dot{V}O_{2max}$. Furthermore, whilst these responses were correlated, individual data in the current study shows that a high $\dot{V}O_{2max}$ did not necessarily prevent adaptations in those with the highest baseline scores, as a number of participants with baseline $\dot{V}O_{2max}$ >60 $mL \cdot kg^{-1} \cdot min^{-1}$ experienced absolute $\dot{V}O_{2max}$ increases of ≥0.4 $L \cdot min^{-1}$.

Improvements in markers of temperate exercise performance (i.e. $\dot{V}O_{2max}$, speed at 4 $mmol \cdot L^{-1}$ [La^-], TTE and submaximal HR) appeared to be related. Thus, indices of temperate exercise performance appear to increase holistically with post-exercise sauna bathing. Furthermore, change in peak HR during the HTT was related to changes in speed at 4 $mmol \cdot L^{-1}$ [La^-], TTE and submaximal HR (though not to changes in $\dot{V}O_{2max}$). Therefore, the magnitude of improvement in peak HR during a standardised HTT may be useful to predict an athlete's performance improvements in temperate conditions.

Only females in the SAUNA group had a faster HR recovery in temperate conditions post-intervention. Pre- to post-intervention differences were especially pronounced at 60 s post-exercise (i.e. the "fast phase" of HR recovery), whereby vagal reactivation is the predominant neural factor controlling HR recovery post-exercise (Dupuy et al., 2012). Increased autonomic, and specifically vagal, control of the heart characterises the initial transient phase of the biphasic pattern of heat acclimation (Horowitz & Meiri, 1993; Horowitz et al., 1996). Indeed, a faster HR recovery 60 s post-submaximal temperate exercise was

previously observed in males following a 10-consecutive-day post-exercise sauna bathing protocol (Stanley et al. 2015). However, in the current study, males in the SAUNA group did not show changes in HR recovery post-intervention, possibly because the sauna exposures were spread over 3 weeks. A pattern of initial increase in autonomic excitability, followed by autonomic withdrawal has previously been shown when using ~3 weeks of heat acclimatisation in males (Stacey et al., 2018). Thus, perhaps in the male cohort in the current study, the 3-week, intermittent nature of the intervention allowed time for more stable cardiovascular adaptations to underpin changes in HR (i.e. enhanced physiological efficiency and decreased excitability, in line with characteristics of the second, stabilisation phase of heat acclimation; Horowitz et al., 1996), whilst females, who have been shown to take longer to develop the initial cardiovascular adaptations in response to heat acclimation (Mee et al., 2015b), remained in the initial transient phase of adaptation at post-intervention. Sex differences in autonomic control of the cardiovascular system in response to various stressors (e.g. exercise, hypoxia, head-up tilt) are well-documented (Dart et al., 2002) and may affect the biphasic response to heat acclimation.

There were no changes in plasma volume measures in either group. However, it was somewhat unlikely that a heat-mediated shift in relative plasma volume (observed as quickly as after the first two heat exposures; Harrison et al., 1981) would persist for the duration of the three-week intervention. As detailed in the investigation by Stanley and colleagues (2015), relative plasma volume shifts were no longer significantly different from baseline by the sixth consecutive day of post-exercise sauna bathing, and mean values returned to baseline by the ninth day. Similarly, whilst Scoon and colleagues (2007) observed increases in *absolute* plasma volume following 3 weeks of intermittent post-exercise sauna bathing, haemoglobin concentration and haematocrit (the measures from which our plasma volume data were derived), were the same at pre- and post-intervention. Considering similarities with the current protocol and that of Scoon et al. (2007), it is likely that participants experienced absolute increases in plasma volume and that plasma volume expansion underpinned performance improvements to some extent (Lorenzo et al., 2010).

7.5.4 Perspectives and considerations

Given current recommendations for athletes to utilise post-exercise sauna bathing to prepare for athletic events in the heat (Racinais et al., 2019; World Athletics, 2019) plus the increased interest in using heat as an ergogenic aid (Minson & Cotter, 2016; Nybo & Lundby, 2016), these data are important because they confirm that post-exercise sauna bathing is an appropriate and effective training intervention for both sexes *and* in a trained

population (despite some apparent sex differences in mechanisms). The accessible and pragmatic nature of post-exercise sauna bathing makes these data especially meaningful for female athletes, who may not otherwise have access to traditional methods of heat acclimation involving climatic chambers or to other costly ergogenic aids, given the comparatively limited funding at present in some female sporting environments (Emmonds et al., 2019). That being said, the contributions of skin blood flow and sweating to heat dissipation differ depending on the ambient temperature (above or below skin temperatures) and humidity of exercise heat stress (Sawka et al., 2011). Thus, observed sex differences in forearm blood flow and sweat gland activity adaptations following a post-exercise sauna bathing intervention may impact heat tolerance and/or performance under different environmental conditions, or during longer/higher-intensity exercise. Notably, this novel observation of sex dimorphism in thermoeffector adaptations in response to post-exercise sauna bathing has not previously been described in response to any other heat acclimation protocol.

The HTT employed in the current study was chosen because: i) it has been validated for use in both sexes (Mee et al., 2015a), ii) it has previously been observed to detect hallmark heat acclimation adaptations in both females and males (Mee et al., 2015b), iii) it is specific to the modality most meaningful to the cohort of middle distance/cross-country runners in the current study, and iv) it allowed for a direct comparison of outcomes with a key study that informed the hypothesis of the current study. However, the main limitation of this test was that the fixed external workload (i.e. 9 $km \cdot h^{-1}$, 2% grade) likely resulted in different rates of metabolic heat production between the female and male cohorts, given they were significantly different physical sizes (i.e. body mass and body surface area; Jay & Cramer, 2015). This makes it somewhat difficult to attribute sex differences in thermoeffector outcomes to the intervention alone. It is possible that the different heat loads experienced during the HTT confounded observations of forearm blood flow and sweat gland activity. This possible adaptive sex difference should be further investigated using a heat tolerance test matching metabolic heat production.

The random sampling of the female cohort across menstrual cycle phases and hormonal contraceptive use provides greater confidence in the ecological validity of these findings for female athletes because females were not tested in just one menstrual cycle phase (Stanhewicz & Wong, 2020). The so-called “flipside” of the enhanced ecological validity is that there may be greater associated variability. Additionally, this meant we did not have the opportunity to gain insight into the underlying effects (if any) of endogenous or exogenous female sex hormones on our results (Wenner & Stachenfeld, 2020). However, it was deemed *most* important to structure the protocol around the club’s training schedule to ensure a similar exercise training stimulus between groups and sexes.

It is possible that female participants may have experienced a greater thermal stimulus when undergoing post-exercise sauna bathing during their luteal phase. However, as body temperatures were not measured after the first post-exercise sauna bathing session, we cannot ascertain this. Nevertheless, overall peak HR in the sauna (somewhat indicative of thermal strain) was not different between sexes across the duration of the intervention.

Two female SAUNA group participants completed their HTTs in different menstrual phases. This likely caused very minor, if any, variation in the results of the total 14 SAUNA females who completed both HTTs. With regards to temperate exercise tests, a recent and thorough investigation of submaximal and maximal exercise responses in oral contraceptive users and non-users showed no effects of either menstrual cycle phase or oral contraceptive pill phase (i.e. active or inactive) on $\dot{V}O_{2max}$, TTE, [La^-] nor submaximal gas exchange or HR (Mattu et al., 2020). Therefore, differences in menstrual cycle phase from Pre to Post in 3 out of 16 female participants were unlikely to have affected temperate exercise findings.

Finally, due to the nature of the running HTT, FBF was assessed post-exercise. The ~5 min period immediately post-exercise is associated with dynamic changes in heart rate and blood pressure, amongst other haemodynamic variables (Carter et al., 2002); although cardiovascular responses during recovery from exercise-heat stress are similar between males and females (Gagnon et al., 2008). Thus, whilst every effort was made to replicate the FBF measurement conditions (i.e. only ~26 s difference between Pre and Post FBF measurement timing), it is possible that the adaptive sex differences observed in these data may not necessary reflect FBF changes *during* exercise.

7.6 Conclusions

The findings from the current study demonstrate that, in an ecologically valid setting, females and males demonstrated similar cardiovascular, thermoregulatory, sweat and *ad libitum* fluid consumption responses during an acute session of post-exercise sauna bathing. Chronically, 3 weeks of post-exercise sauna bathing resulted in similar HR and peak skin and core body temperature reductions during exercise-heat stress in both female and male endurance athletes, indicating a similar level of improvement in these main markers of heat adaptation. Although there appeared to be sex differences in thermoeffector adaptations (i.e. FBF and sweat gland activity), as well as in the rise in T_{rec} during exercise-heat stress, it is unclear how differences in metabolic heat production due to the fixed work-load of the HTT contributed to these differences. There were also sex differences in HR recovery from submaximal temperate exercise, possibly indicating differences in the temporal patterning of autonomic heat acclimation adaptions. Lastly, post-exercise sauna bathing induced similar

meaningful improvements in temperate exercise performance in female and male athletes, as evidenced by both sexes showing similar changes in $\dot{V}O_{2max}$, running speed at 4 $mmol \cdot L^{-1}$ [La^-] and TTE following the post-exercise sauna intervention. Taken together, the findings of this study highlight that post-exercise sauna bathing is an effective method of heat acclimation and ergogenic aid for both sexes.

8. HEAT ACCLIMATION IS PARTIALLY RETAINED FOR TWO WEEKS FOLLOWING THREE WEEKS OF INTERMITTENT POST-EXERCISE SAUNA BATHING

8.1 Abstract

Background: Intermittent post-exercise sauna bathing is a pragmatic method of inducing heat acclimation. However, the rate of acclimation decay using this method is unknown. Therefore, this study investigated the decay of heat adaptations accrued using three-weeks intermittent post-exercise sauna bathing following two weeks without exposure to hot ambient conditions.

Methods: Eight trained middle-distance runners (3 female; mean±SD, age 19±1 years, $\dot{V}O_{2max}$ 60.5±5.9 mL·kg^{-1}·min^{-1}) performed a running heat tolerance test (30-min, 9 km·h^{-1}/2% gradient, 40°C/40%RH; HTT) before (Pre), following three-weeks normal training with 30 min post-exercise sauna bathing 3±1 times per week (Post) and subsequently following two-weeks without exposure to hot ambient conditions (Decay).

Results: Post-exercise sauna bathing reduced resting rectal temperature (T_{rec}) by ~0.5°C, peak T_{rec} by ~0.4°C, peak skin temperature (T_{sk}) by ~1.1°C and peak heart rate (HR) by ~10 beats·min^{-1} during the HTT from Pre to Post (all p<0.05). Sweat loss during the HTT was similar (p=0.51), though at lower body temperatures at Post and Decay. Following two-weeks decay, peak T_{rec} did not change from Post (p=0.20) and remained ~0.3°C lower than at Pre (p=0.03). Resting T_{rec}, and peak T_{sk} and HR also did not change from Post to Decay (all p≥0.31), but did not remain lower at Decay than at Pre (all p≥0.12). Decay of resting T_{rec} and peak HR were correlated (p=0.01, r=0.82), and decay of peak HR and sweat loss tended to be correlated (p=0.06, r=-0.74), but decay of other variables was not (all p≥0.16).

Conclusions: Adaptations induced using three weeks of post-exercise sauna bathing (i.e. reduced HR, T_{rec} and T_{sk}) are partially retained following two weeks without exposure to hot ambient conditions.

8.2 Introduction

The rate of induction of heat acclimation adaptations has been well documented and reviewed (Periard et al., 2015), however the literature surrounding retention of heat acclimation adaptations is comparatively limited (Garrett et al., 2011; Daanen et al., 2018). Nevertheless, understanding the time course of heat acclimation decay has important practical implications for athletes using heat acclimation to prepare for competition in the heat. For example, athletes travelling to hot climates would benefit from retaining adaptations accrued during a specifically-designed and controlled heat acclimation protocol rather than relying on natural acclimatisation alone, since conditions for natural acclimatisation are not always specific or guaranteed (Gerrett et al., 2019).

Adaptations accrued through heat acclimation are transient and subject to decay at a rate of ~2.5% per day without exogenous heat exposure (Daanen et al., 2018). By this calculation, participants typically retain a considerable percentage of the initial adaptations for at least two weeks. However, this consensus is largely based off data from protocols employing active heat acclimation. This is problematic because passive heat acclimation may be preferred by athletes for practicality and accessibility reasons (Heathcote et al., 2018). To address this knowledge gap, Zurawlew et al. (2019) recently showed that, following 6 days of post-exercise hot-water immersion, males retained the accrued reductions in body temperatures, heart rate, core body temperature threshold for sweating and perceptual ratings for two-weeks post-intervention. These data indicate that heat acclimation induced via relatively short bouts (30-40 min) of post-exercise passive heating is as stable as that using traditional active heat acclimation, and is encouraging for adaptations accrued using post-exercise sauna bathing. Moreover, post-exercise sauna bathing involves dry (~10%RH) heat exposure, which may contribute to favourable retention of heat acclimation mediated adaptations (Pandolf et al., 1977; Daanen et al., 2018).

Notably, an *intermittent* post-exercise sauna bathing protocol, though pragmatic (i.e. flexibility around training), does not reflect the current recommendations to use a consecutive-day protocol to maximise heat acclimation retention (Daanen et al., 2018). Since Gill and Sleivert (2001) reported suboptimal heat acclimation *induction* with an intermittent active heat acclimation protocol as compared to a traditional consecutive-day protocol, Daanen et al. (2018) suggested that intermittent protocols may also lead to suboptimal adaptation retention. However, Duvnjak-Zaknich et al. (2019) is the only study to directly compare heat acclimation decay from a consecutive-day protocol (8 consecutive heat exposures) to that from an intermittent protocol (8 heat exposures over 15 days). The authors reported that both groups improved repeated-sprint performance to a similar extent, and that neither group experienced any significant decay in repeated-sprint performance in

the heat at 7- and 14-days post-intervention. Thus, an intermittent heat acclimation protocol may not lead to suboptimal retention, as previously suggested (Daanen et al., 2018). However, the protocol used by Duvnjak-Zaknich et al. (2019) included heat exposures every other day for 15 days, whereas the 3-week protocol described in **Chapters 6** and **7** spreads heat exposures over a longer intervention period. Thus, whether the intermittent nature of the current protocol would modify the expected ~2.5% per day rate of heat acclimation decay is unknown.

8.2.1 Aim

The aim of the present study was to determine whether a 3-week post-exercise sauna bathing intervention would induce heat acclimation adaptations that would be retained for 2 weeks without exogenous heat exposure.

8.2.2 Hypotheses

It was hypothesised that 3 weeks of post-exercise sauna bathing would induce heat acclimation adaptations that would be partially lost (i.e. decay at a rate of ~2.5% per day) after 2 weeks without post-exercise sauna bathing, equating to ~35% decay, in line with current literature on heat acclimation decay.

8.3 Methods

8.3.1 Participants

Thirty-nine trained middle-distance and cross-country runners were recruited from the university's athletics club to participate in this study. However, due to various reasons (injury, scheduling, etc.) only fourteen athletes (SAUNA, *n*=8; CON, *n*=6) completed all experimental trials detailed herein. Data from these participants were also included in the analyses in **Chapter 7**. One additional participant completed the SAUNA intervention but was excluded from these analyses as they demonstrated physiological signs of illness at post-intervention testing (i.e. resting rectal temperature elevated >2 SD from group mean and urine osmolality of 940 $mOsm \cdot kg^{-1}$, indicating hypohydration). SAUNA participant characteristics are summarised in **Table 8.1**. The CON group was treated as a time-control. As **Chapter 6** showed the 3-week post-exercise sauna intervention to be superior to normal endurance training (CON) for all variables presented herein (i.e. resting and peak T_{rec} and

HR, peak T_{sk}, sweat loss), all CON participant characteristics and data for the intervention described in the current chapter are reported in **Appendix 3**.

Table 8.1 SAUNA participants' characteristics. Data are presented as mean±SD

Participants (female/male)	3/5
Age (years)	19±1
Height (cm)	171±9
Body mass (kg)	64±10
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	60.5±5.9

SAUNA, post-exercise sauna bathing intervention group; $\dot{V}O_{2max}$, maximal aerobic capacity

Female participants in the SAUNA group were eumenorrheic (regular menstrual cycles lasting between 28-30 days; *n*=2) or using a contraceptive coil (*n*=1). Eumenorrheic participants completed Pre and Post tests in the luteal phase, and Decay tests in the follicular phase.

Participants complied with all criteria and instructions for participation (**Section 3.3** General participant instructions, **Section 3.6** Menstrual cycle questionnaire and self-reported log and **Section 3.9.1** Instructions for normal hydration). Throughout the protocol, participants were asked to keep a training diary (including session type and running distance).

8.3.2 General overview and design

Participants completed a running heat tolerance test (HTT) at baseline (Pre), following 3 weeks (Post) of either normal endurance training (CON) or normal endurance training with the post-exercise sauna bathing intervention (SAUNA; **Section 3.7.3**), and again following 2 weeks without post-exercise sauna bathing (Decay). The SAUNA and CON interventions, and experimental tests at Pre and Post described in the current study matched those described in **Chapter 7**. Participants' $\dot{V}O_{2max}$ was determined on a treadmill as described in **Section 3.7.6**.

Experimental trials at Pre and Post were arranged ~28 days apart to test female participants during the same menstrual cycle phase, as per a typical menstrual cycle. Participants took daily iron tablets from 2 weeks prior to the first experimental trial until the completion of the protocol (**Section 3.3.1**). This experiment was conducted in the United

Kingdom between the months of October and December to minimise any natural heat acclimatisation.

8.3.3 Experimental procedures

8.3.3.1 Post-exercise sauna bathing

Participants entered the sauna (101-108°C, 5-10%RH at ~1.8 m; **Section 3.4.3**) within ~5 min of cessation of outdoor exercise and were asked to remain in the sauna for 30 min or until they could no longer tolerate the discomfort (**Section 3.7.3)**. Sauna bathing typically followed scheduled low-intensity, continuous exercise training sessions (i.e. 'easy runs' or 'long runs'). Peak heart rate (HR; **Section 3.9.4**) was recorded during the final minute of exposure.

8.3.3.2 Running heat tolerance test

The HTT was performed in hot conditions (40°C, 40%RH; **Section 3.4.7**). Participants wore socks, shoes, and either shorts (males) or shorts and a sports bra (females). Three CON males opted to wear a t-shirt for all tests. Resting T_{rec} was measured in the anti-chamber (i.e. room temperature). Rectal temperature (T_{rec}), skin temperature (T_{sk}), and heart rate (HR) were measured continuously (**Sections 3.9.3.1**, **3.9.3.2** and **3.9.4**, respectively). Sweat loss was measured via changes in towel-dried, nude body mass (**Section 3.9.6**).

8.3.4 Data analyses

Peak T_{rec}, $T_{recRISE}$, and weighted T_{sk} were calculated as described in **Section 3.9.3**. Peak HR was calculated as described in **Section 3.9.4**. Decay in heat acclimation adaptations was calculated according to Pandolf et al. (1977), as described in **Section 3.11**. Briefly, %Decay is defined as the difference between scores at Post and Decay, divided by the difference between scores at Pre and Post (i.e. (Post–Decay)/(Post–Pre)). %Decay was only calculated when there was a significant change in a dependent variable from Pre to Post.

8.3.5 Statistical approach

Physiological variables were compared across *time* (i.e. Pre, Post, Decay) within each of the SAUNA and CON groups separately using a repeated-measures one-way

analysis of variance (ANOVA). **Chapter 7** showed that females and males exhibit similar responses to the post-exercise sauna bathing intervention for the above variables, and thus sex was not included as a covariate for these analyses.

Average weekly training distance and frequency between the intervention period (i.e. three weeks between Pre and Post) and the decay period (i.e. two weeks between Post and Decay) were compared within each of the SAUNA and CON groups separately using a repeated-measures one-way ANOVA. **Chapter 7** showed that male participants from this running club ran greater weekly distances, but did not complete more sessions than their female counterparts. Thus, sex was included as a covariate when assessing weekly running distance only.

Normality of the data was assessed using the Shapiro-Wilk Test (not violated on any occasion). Sphericity was assessed using Mauchly's Test of Sphericity, and Greenhouse–Geisser corrections were applied where assumptions of sphericity were violated. When a significant main effect was found, a Dunn-Bonferroni-corrected *post hoc* analysis was used to compare pairwise differences. Significance was set at $p \leq 0.05$. Absolute data are expressed as mean ± standard deviation (SD).

A power analysis was conducted *posteriori* (G*Power version 3.1, Germany) to examine whether the analyses were sufficiently powered to detect an effect. This analysis used an accepted parameter of power ($\beta \geq 0.80$) at an α level of 0.05. Results of the power analysis are reported in **Appendix 4**.

Pearson's correlations were performed *posteriori* to examine the relationships between variable decay (i.e. change scores from Post to Decay) and: i) other physiological variables, ii) their respective changes from Pre to Post, iii) training volume, and iv) baseline fitness.

8.4 Results

All data reported in this section are from the SAUNA group only. Data from the CON group can be found in **Appendix 3.**

8.4.1 Training and post-exercise sauna bathing intervention

Participants completed a similar ($p=0.77$) number of training sessions per week between Pre and Post (6±1), and between Post and Decay (6±1), consisting of 2±1 easy runs, 1±0 tempo run, 1±0 long run, 1±1 high-intensity running session and 1±1 cross-training session (all $p \geq 0.16$). Running distance was also similar, with participants reporting running

57±19 km per week between Pre and Post, and 58±24 km per week between Post and Decay (p=0.85).

Participants attended sauna sessions 3±1 times per week, accumulating 9±1 sauna sessions before completing the HTT at Post. Participants remained in the sauna for 30±0 min each session, totalling 275±32 min of sauna exposure. Across all sessions, peak HR reached 120±7 beats·min^{-1} whilst in the sauna.

8.4.2 Running heat tolerance test

Body temperature data for the SAUNA group are presented in **Figure 8.1**. There was a main effect of *time* on resting T_{rec} (p<0.01). *Post hoc* analysis showed that resting T_{rec} was reduced from Pre to Post (p<0.01). However, resting T_{rec} at Decay was not different from either Pre (p=0.12) or Post (p=0.31). From Post to Decay, there was a 33% decay in the mean resting T_{rec} adaptations observed from Pre to Post.

There was a main effect of *time* on peak T_{rec} (p<0.01). *Post hoc* analysis showed that peak T_{rec} was reduced from Pre to Post (p=0.02). Peak T_{rec} did not change from Post to Decay (p=0.20) and remained lower at Decay than at Pre (p=0.03). From Post to Decay, there was a 25% decay in the mean peak T_{rec} adaptations observed from Pre to Post.

There was a main effect of *time* on peak T_{sk} (p<0.01). *Post hoc* analysis showed that peak T_{sk} was reduced from Pre to Post (p<0.01). However, peak T_{sk} at Decay was not different from either Pre (p=0.19) or Post (p=0.53). From Post to Decay, there was a 33% decay in the mean peak T_{sk} adaptations observed from Pre to Post.

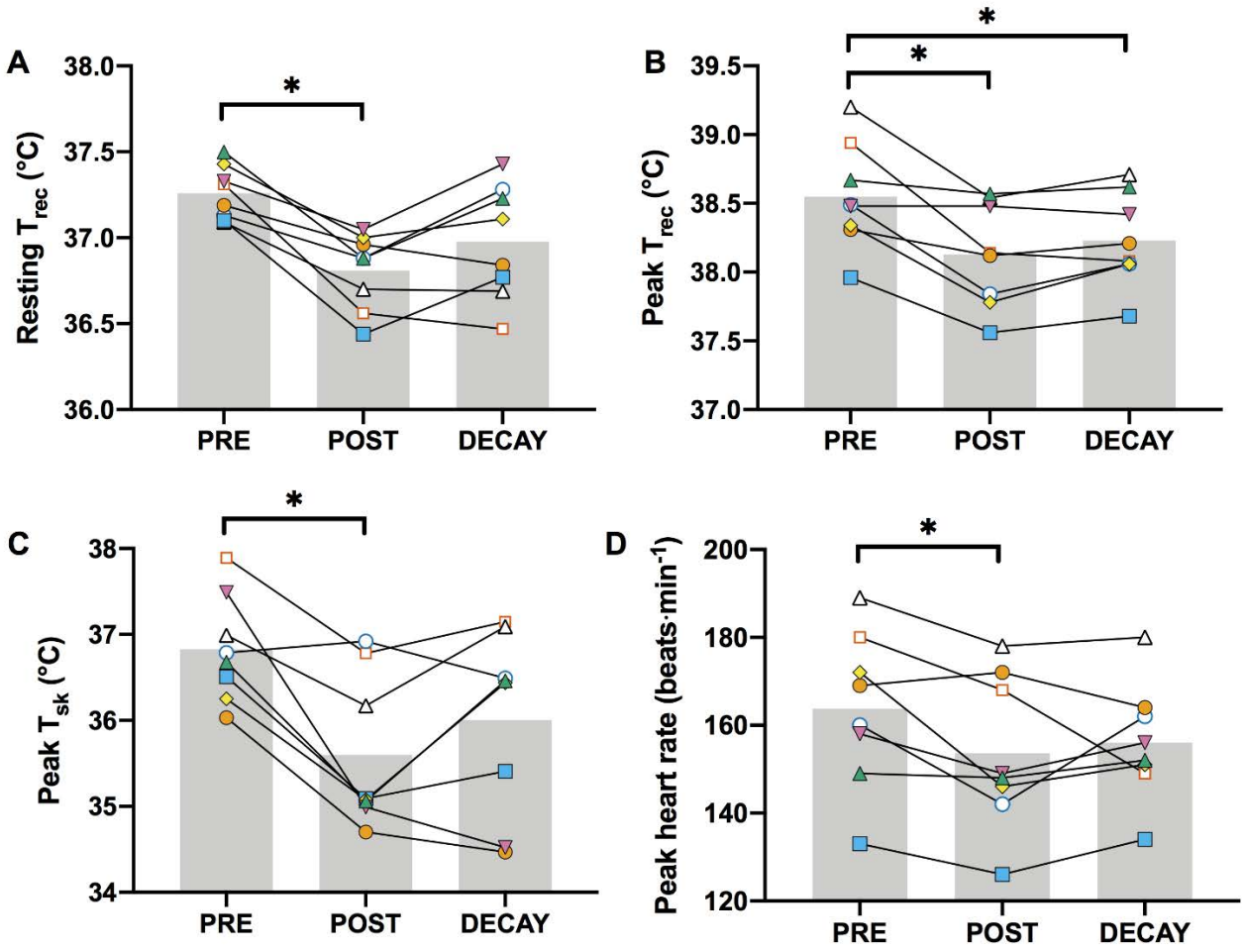


Figure 8.1 Resting rectal temperature (T_{rec}; *A*), peak T_{rec} (*B*), peak skin temperature (T_{sk}; *C*) and peak heart rate (*D*) pre- (Pre), post- (Post) and two-weeks after (Decay) a three-week post-exercise sauna bathing intervention. Individual participant data are marked with their respective symbols. Open symbols indicate female participants. The female participant using a contraceptive coil is indicated by an *open square* and the two eumenorrheic female participants are indicated by an *open circle* and *open triangle*. Bars indicate group mean. *Significant difference between indicated time-points (p<0.05)

Peak HR data are presented in **Figure 8.1**. There was a main effect of *time* on peak HR (p=0.05). *Post hoc* analysis showed that peak HR was reduced from Pre to Post (p<0.05). However, peak HR at Decay was not different from either Pre (p=0.35) or Post (p=1.00). From Post to Decay, there was a 25% decay in the mean peak HR adaptations observed from Pre to Post.

Sweat loss was not affected by the SAUNA intervention (Pre: 1.3±0.9 %BM, Post: 1.2±0.5 %BM, Decay: 1.2±0.5 %BM; p=0.51; n=7).

8.4.3 Relationships between heat acclimation decay and other variables

There was a strong positive correlation between decay of resting T_{rec} and decay of peak HR (p=0.01, r=0.82), whereby those showing greater decay of resting T_{rec} also showed greater decay of peak HR adaptations (**Figure 8.2**). There was also a trend (p=0.06) for a strong negative correlation (r=-0.74) between decay of peak HR and decay of sweat loss, whereby those showing greater decay of peak HR also showed greater attenuation of sweat loss. Changes from Post to Decay were not correlated between other variables (all p≥0.16). Additionally, for any given outcome variable, changes from Post to Decay were not related to their respective changes from Pre to Post (all p≥0.36).

For all variables, changes from Post to Decay were not related to training volume during the intervention period (all p≥0.29), during the decay period (all p≥0.36) or during the decay period when normalised as a percentage of the intervention-period training volume (all p≥0.15). Changes from Post to Decay were not related to baseline fitness (all p≥0.16).

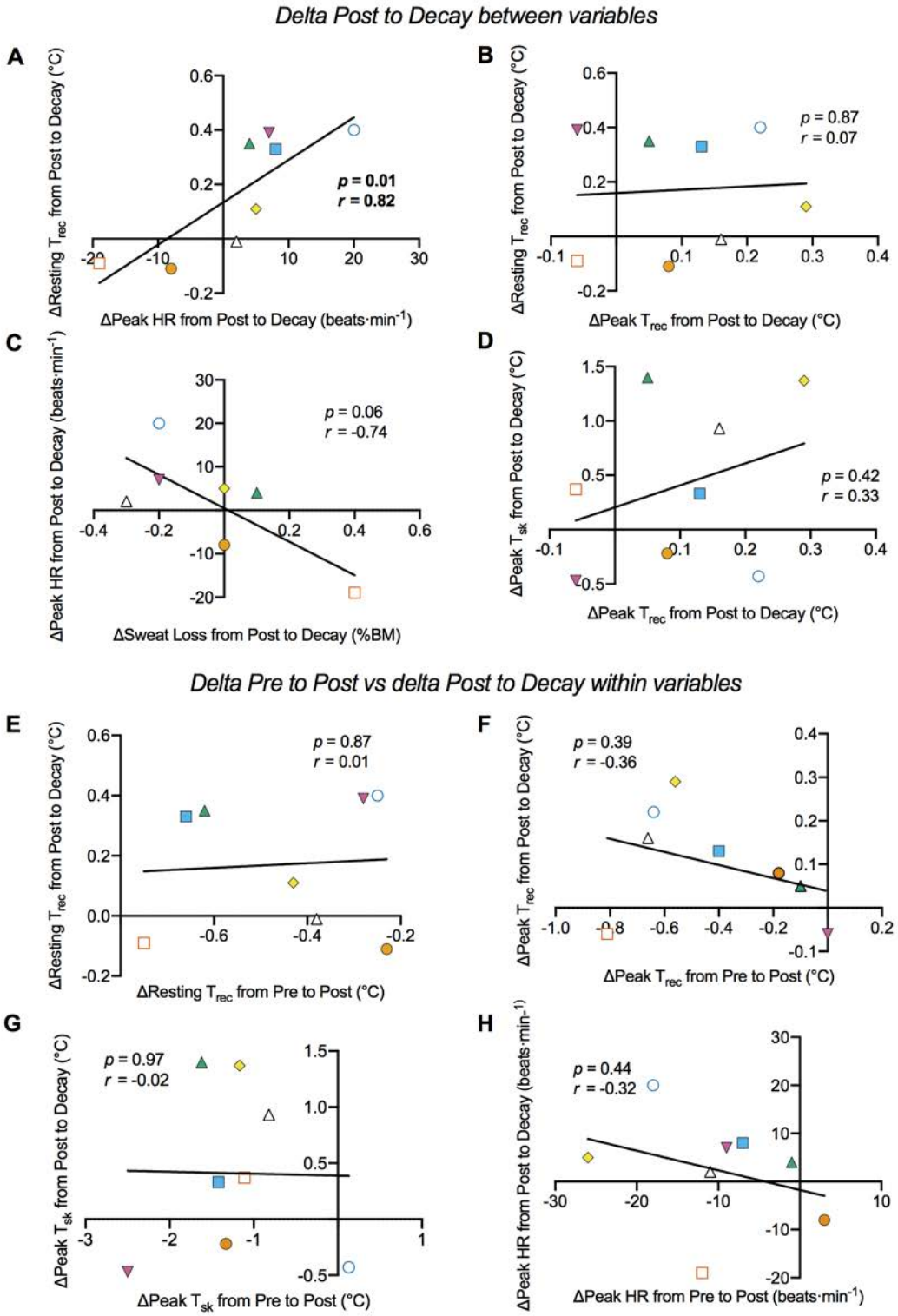


Figure 8.2 Relationships between the change from Post (following the 3-week intermittent post-exercise sauna bathing intervention) to Decay (2 weeks after Post, without sauna bathing), between variables (**A-D**) and relationships between changes from Pre (baseline) to Post and Post to Decay, within variables (**E-F**). Individual participant data are marked with their respective symbols, with open symbols indicating female participants. The female participant using a contraceptive coil is indicated by an *open square* and the two eumenorrheic female participants are indicated by an *open circle* and *open triangle*. BM, body mass; HR, heart rate; T_{rec}, rectal temperature; T_{sk}, mean weighted skin temperature

8.5 Discussion

The main findings of this study are that adaptations induced using 3 weeks of post-exercise sauna bathing (i.e. reduced HR, T_{rec} and T_{sk}) are partially retained following 2 weeks without exposure to hot ambient conditions, in line with our hypothesis. Specifically, adaptations decayed by 25-33%, which was consistent with the ~35% hypothesised. Adaptations associated with a reduced peak T_{rec} during exercise-heat stress were especially well retained, given that peak T_{rec} at Decay was still ~0.3°C lower than at Pre. Participants' decay of resting T_{rec} was associated with the decay of their peak HR adaptations. However, the magnitude of decay for other variables were independent of one another.

8.5.1 Heat acclimation induction and decay

Heat acclimation induction in the current study was in line with data in the previous chapters. From Pre to Post, resting T_{rec} was reduced by ~0.5°C, peak T_{rec} by ~0.4°C, peak T_{sk} by ~1.1°C and peak HR by ~10 beats·min^{-1}. Furthermore, sweat loss during the running heat tolerance test was similar at Pre and Post despite reductions in body temperatures, indicating improved sweat sensitivity (i.e. same sweat rate as at Pre but at a lower body temperature). These data indicate that heat acclimation was successfully induced using 3 weeks of intermittent post-exercise sauna bathing and that changes were unlikely to have been induced by normal training alone, given the lack of improvements in the control groups in previous chapters and in the current protocol (**Appendix 3**).

Following 2 weeks without exogenous heat exposure, peak T_{rec} during exercise-heat stress remained lower than at pre-intervention. Whilst peak T_{rec} did not significantly change from Post to Decay, mean values were 0.1°C higher at Decay than at Post, representing a ~25% decay of the 0.4°C reduction accrued post-intervention. This magnitude of decay is well within the wide range of decay reported in the literature (Daanen et al., 2018). For example, Poirier et al. (2015) observed a ~43% decay in peak T_{rec} following 2 weeks without heat exposure, whilst Weller et al. (2007) observed that peak T_{rec} adaptations only decayed by ~5% after 26 days without heat exposure. Notably, sweat rate still did not change at Decay as compared to either Pre or Post. This, coupled with the reduced T_{rec} at Decay from Pre, indicates that participants retained a greater sweat sensitivity following 2 weeks without exogenous heat exposure.

Adaptations related to resting T_{rec}, and peak T_{sk} and HR were not as well maintained as peak T_{rec} adaptations following 2 weeks without post-exercise sauna bathing. Although these variables did not significantly change from Post to Decay, they also showed no

significant difference from Pre. The individual data shows that some participants fully retained adaptations, whilst others displayed either a partial or complete loss of resting T_{rec}, and peak T_{sk} and HR adaptations. Thus, the SAUNA group as a whole did not demonstrate a uniform response at Decay, resulting in data that were not significantly different from either Pre or Post. Given that the decay of heat acclimation adaptations appears to be more variable between individuals than their induction, the low sample size probably compromised the statistical power in the current study. Indeed, a *posteriori* power analysis (**Appendix 4**) indicated that resting T_{rec}, and peak T_{sk} and HR analyses were not adequately powered beyond the 0.80 threshold (Cohen, 1969) to detect a significant effect (power [1− β]=0.78, 0.45 and 0.34, respectively). Thus, a larger sample size would have provided greater statistical power for the current study, as was initially recruited (i.e. 16 SAUNA participants). Unfortunately, due to high rates of injury unrelated to the intervention (van Gent et al., 2007) and high dropout rates as described previously, the current study's results are somewhat unclear. Still, despite being statistically underpowered, the 25-33% decay observed for the group on average is in agreement with previous active, consecutive-day heat acclimation literature describing ~2.5% decay per day without exogenous heat exposure (Daanen et al., 2018). Therefore, it appears that adaptations related to resting T_{rec}, and peak T_{sk} and HR were likely partially retained for 2 weeks following the post-exercise sauna bathing intervention, though the response appears to be highly individual.

The *partial* retention of heat acclimation adaptations reported in the current study is in contrast to what was reported in the only other investigation of heat acclimation decay following post-exercise passive heating (Zurawlew et al., 2019). Zurawlew et al. (2019) reported that body temperature and heart rate adaptations accrued following 6 days of post-exercise hot-water immersion were *fully* maintained following 2 weeks without heat exposure (i.e. variables remained either strikingly similar to post-intervention values or were even slightly improved upon following 2 weeks "decay", and were significantly lower than pre-intervention values). This is an interesting difference, given that Zurawlew et al. (2019) only employed a 6-day protocol and reported a similar degree of initial adaptation at post-intervention tests as in the current study (e.g. ~0.3°C reduction in peak exercising T_{rec} and ~13 beats·min^{-1} reduction in peak exercising HR). Thus, is appears unlikely that the magnitude of heat acclimation induction is the reason for differences in the retention of adaptations. However, Zurawlew et al. (2019) employed an exercise-heat stress test (40-min running at 65%$\dot{V}O_{2max}$ in 33°C, 40%RH) to quantify decay at both 1- and 2-weeks post-intervention. This additional heat exposure incurred at 1 week post-intervention may have facilitated better retention of adaptations at 2 weeks post-intervention, as Pryor et al. (2019b) demonstrated that a single exercise-heat stress exposure every 5 days was sufficient to preserve heat acclimation status for at least 25 days. Nevertheless, it is possible that the

intermittent nature of the heat acclimation protocol in the current study was not as “stable” as a consecutive-day protocol, as indicated by data from Gill and Sleivert (2001) comparing induction of consecutive-day and intermittent active heat acclimation responses.

Given the highly individual responses at Decay, an exploratory analysis of the relationships between the magnitude of decay and other variables was conducted to investigate potential factors involved in this individual response. Decay in resting T_{rec} and peak HR were well-correlated, and there was a trend for a relationship between peak HR and sweat loss. These relationships were somewhat unexpected, since Corbett et al. (2018a) observed that the magnitude of induction of thermal, sudomotor and cardiovascular adaptations were not related following active heat acclimation. Whilst these variables are known to decay at similar rates (Daanen et al., 2018), decay of thermal, cardiovascular and sudomotor adaptations have not previously been reported to demonstrate a relationship to one another within the individual, *per se*.

Interestingly, decay in resting T_{rec} and peak T_{rec} were not related. This contrasts with findings by Zurawlew et al. (2019), who reported that resting and exercising T_{rec} did show a relationship, and thus that the retention of a lower exercising T_{rec} in their study was driven by the retention of a lower resting T_{rec} at the start of exercise. Yet in the current dataset, this was not the case. Instead, peak T_{rec} appears to have been largely maintained by adaptations related to whole-body heat loss and/or heat production *independent* of the shift in thermal “set-point” (Hessemer et al., 1986) for resting T_{rec} post-intervention. Finally, there were no relationships between the magnitude of induction and the respective magnitude of decay in any of the outcome variables measured. Therefore, a large initial response (i.e. being a “high responder”) to the intervention did not appear to facilitate better retention 2 weeks later in this cohort. Nonetheless, any inferences drawn from these correlation analyses should be taken with some caution given the small sample size in the current study.

Athletes in the current study were well-trained, which has been observed to aid in the retention of adaptations (Williams et al., 1967; Pandolf et al., 1977), although baseline fitness and training volume were not correlated with heat acclimation decay within this cohort. Possibly opposing the benefits of their high training status, participants trained mainly outdoors (during both the intervention and decay periods) in November and December in Birmingham, UK (daily temperatures ~10°C). Saat et al. (2005) previously showed that exercising in the cold (~18°C) during a 14-day heat acclimation decay period resulted in faster decay of adaptations than passive exposure to cooler temperatures. Likewise, Williams et al. (1967) observed that adaptations accrued during heat acclimatisation were lost at a greater rate in the winter as compared to the summer. Thus, outdoor training during the decay period in the current study may have facilitated the rate of decay of heat

acclimation adaptations. It is unclear whether athletes training in more temperate conditions (i.e. indoors) during the decay period would experience similar rates of decay.

8.5.2 Perspectives and considerations

The 25-33% decay of adaptations aligns closely with the rate of decay reported in the literature, but was induced using an intermittent heat acclimation intervention that is more practical and accessible for athletes. This provides more evidence, in addition to that by Duvnjak-Zaknich et al. (2019), that heat acclimation adaptations attained using an intermittent protocol are retained to a similar magnitude as when using a consecutive-day protocol. Moreover, strategies used to maintain heat acclimation adaptations following a consecutive-day protocol, such as weekly “top-up” heat exposures (Casadio et al., 2016; Pryor et al., 2019b) or implementing a rapid re-acclimation protocol (Weller et al., 2007; Racinais & Periard, 2020), are probably viable options to better maintain heat acclimation adaptations following an intermittent post-exercise sauna bathing intervention.

In an applied setting, the withdrawal from heat exposure would presumably be combined with a training taper immediately prior to a competition. In the current study, athletes continued their normal training volume and intensity during the decay period. It is unknown whether the reduced training volume (and thus reduced time at an elevated body temperature) during a taper would result in a similar or more rapid decay in heat acclimation-mediated adaptations, or if the higher fitness levels typically observed following a taper (Mujika et al., 1998) would attenuate the decay in adaptations.

Every effort was made to recruit a suitable number of female and male athletes to allow for a between-sex comparison of heat acclimation decay. Unfortunately, due to a similar drop-out rate as reported in previous chapters, this was not possible in the current analysis. To date, only one study has investigated heat acclimation decay in a female cohort (Stephens & Hoag, 1981), and this decay was amongst the fastest reported in the literature (see **Section 2.6**). Thus, it is possible that including a mixed-sex cohort contributed to the variability in resting T_{rec}, peak T_{sk} and peak HR responses at Decay; albeit the scarcity of data investigating heat acclimation decay in females makes these suggestions mainly speculative. Future studies should aim to elucidate this response.

Two SAUNA participants completed Pre and Post trials in the luteal phase and the Decay trial in the follicular phase. This may have affected changes in their resting T_{rec} at Decay, as a meta-analysis by Giersch et al. (2020) showed that resting internal temperatures are typically elevated by ~0.2°C during the luteal phase. Data from Giersch et al. (2020) also indicated that end-exercise T_{rec} was minimally elevated (~0.05°C) during the

luteal phase as compared to the follicular phase, whilst heart rate and sweat rate were not clearly affected by the menstrual cycle (Giersch et al., 2020). Thus, it appears unlikely that the menstrual cycle had any meaningful effects on other variables in the current study.

8.6 Conclusions

This study was the first to investigate decay of heat acclimation adaptations following post-exercise sauna bathing. In the current study, heat acclimation adaptations were partially retained, as indicated by a 25-33% decay on average following 2 weeks without exogenous heat exposure. This rate of decay is similar to previous literature from active, consecutive-day protocols, but was observed using a more pragmatic, intermittent heat acclimation intervention. Peak T_{rec} during exercise-heat stress remained ~0.3°C lower than pre-intervention following 2-week decay, whilst retention of adaptations associated with resting T_{rec}, and peak T_{sk} and HR was highly individual, though somewhat related. Thus, athletes competing in the heat 2 weeks after an intermittent post-exercise sauna bathing intervention will retain some thermoregulatory adaptations.

9. GENERAL DISCUSSION

9.1 Revisiting the problem and objectives

Historically, there has been a comparative lack of research related to and including female participants' physiological and performance responses to exercise (Costello et al., 2014). This scarcity of research in females is particularly apparent in the areas of thermoregulation and heat acclimation (Tyler et al., 2016; Benjamin et al., 2019; Morris et al., 2020). This is concerning, given the growing number of females working and competing in hot environments. Thus, this thesis aimed to close the gap in the knowledge of performance outcomes from training interventions for female athletes, specifically when responding and adapting to a repeated heat stimulus, better known as heat acclimation. Heat acclimation research has recently expanded to include alternative and more pragmatic methods, such as post-exercise heating. Namely, this thesis aimed to investigate post-exercise sauna bathing as one such emerging, pragmatic heat acclimation method. Finally, this thesis aimed to investigate the use of heat acclimation as an ergogenic aid, given the ongoing debate of its efficacy (Minson & Cotter, 2016; Nybo & Lundby, 2016).

This thesis addressed the above aims by firstly gathering and critically examining findings in the literature in **Chapter 2.** The current literature surrounding thermoregulation and heat acclimation was reviewed, with a particular focus on female physiology. **Chapter 3** then described the use of and rationale for specific methodological techniques, as well as the holistic, and when possible, gold-standard tests appropriate to address the hypotheses. The data chapters are written in chronological order of conception and execution. **Chapters 4** and **5** describe this thesis' initial investigation into the efficacy of short-term *vs* traditional active heat acclimation for endurance performance in a female cohort. The findings and experiences gained from **Chapters 4** and **5** were integral in the shift to a method of heat acclimation (i.e. post-exercise sauna bathing) that could be more easily integrated into athletes' training. Furthermore, post-exercise sauna bathing is more accessible, making it especially useful to the population of interest (i.e. female athletes), given existing inequalities in funding of high-level female sport (Emmonds et al., 2019). Therefore, the objectives in **Chapters 6-8** centred on investigating the efficacy of an intermittent post-exercise sauna bathing protocol for inducing heat acclimation. Specific aims of these chapters included evaluating heat acclimation markers during exercise-heat stress, assessing the required duration of the intervention to induce meaningful adaptations, examining sex differences in the acute and adaptive response, and assessing heat acclimation decay. These chapters also investigated the use of post-exercise sauna bathing for improving objective markers of

exercise performance in temperate conditions. Importantly, the investigations detailed in in **Chapters 6-8** were methodologically designed to include and consider both sexes in order to challenge the current status quo in heat acclimation research: that novel hypotheses and ideas are tested on a male cohort, and pending positive results, are (sometimes) later repeated in a female cohort. This practice has likely contributed to the comparative paucity of data in female cohorts. Instead, this thesis serves as an example of including both sexes when investigating emerging methods and advancing understanding in the field.

9.2 Summary of findings

The first study of the current thesis (**Chapter 4**) addressed the hypothesis that, despite numerous successful short-term heat acclimation (STHA) protocols in male cohorts recorded in the literature, 4 days of heat acclimation would not be sufficient to improve self-paced exercise performance in the heat in a female cohort. This hypothesis was correct, as there were no significant improvements in any physiological or performance measures following 4 days of a high-intensity exercise, isothermic heat acclimation protocol with permissive dehydration. However, there was some individual variation in the response following 4 days acclimation. Following 9 days heat acclimation there was a meaningful and significant performance improvement (e.g. ~8% greater mean power output than at baseline) in a 15-min cycling time trial in 35°C, 30%RH. This affirmed previous findings by Mee et al. (2015), which demonstrated that STHA consisting of daily 90-min exposures was not sufficient for female athletes, even using an isothermic method. **Chapter 4** was also the first study to document the magnitude of improvement for female athletes during self-paced exercise in the heat following a more traditional, medium-term heat acclimation protocol (i.e. 9 days), which was similar to what has been reported in male cohorts in the literature.

The same female cohort was tested in **Chapter 5**. This study tested the hypotheses that females i) would *not* improve self-paced exercise performance in cool conditions (15°C) following 5 days of heat acclimation, given the lack of physiological adaptations believed to underpin performance improvements in cool conditions following STHA; but ii) *would* improve performance following 10 days of heat acclimation. These hypotheses were correct. As in the previous chapter, no performance improvements were observed in cool conditions following 5 days of heat acclimation. However, power output (+5%) and time trial distance (+2.5%) were improved in cool conditions following 10 days of heat acclimation. Notably, there was a negative correlation between distance cycled at baseline and the improvement in distance cycled, whereby the participants that performed best at baseline showed the least improvement. Thus, this protocol was *less* of an ergogenic aid for the best performing

participants in the study. Additionally, most participants (6 of 8) did not surpass the smallest worthwhile change associated with a ~15 min cycling time trial. These considerations, as well as anecdotal reports from participants that the protocol was overly strenuous, unpleasant and fatiguing, prompted reconsideration of the protocols used in **Chapters 4** and **5** when designing the remaining studies.

The remaining studies investigated a more accessible and practical approach to heat acclimation; namely, post-exercise sauna bathing. The study detailed in **Chapter 6** tested the hypotheses that intermittent post-exercise sauna bathing would: i) elicit hallmark heat acclimation adaptations and improve thermoregulation during exercise-heat stress more than endurance training alone, and ii) improve physiological markers of exercise performance in temperate conditions to a greater extent than endurance training alone. Consistent with these hypotheses, 3 weeks of intermittent post-exercise sauna bathing (~10 sessions) induced heat acclimation adaptations (i.e. reduced heart rate and body temperatures and increased sweat gland activity), and improved markers of exercise performance in temperate conditions (i.e. maximal aerobic capacity, running speed at 4 $mmol \cdot L^{-1}$ blood lactate concentration and time-to-exhaustion), to a greater extent than endurance training alone. However, extending the post-exercise sauna bathing intervention to seven weeks only resulted in further improvement in peak rectal temperature (additional -0.1°C reduction) during exercise-heat stress. This study demonstrated that 3 weeks of post-exercise sauna bathing is sufficient to be an effective and pragmatic method of heat acclimation and an effective ergogenic aid. An additional finding of this study was that >50% of athletes experienced a training-related injury since beginning the study that either prevented them from training normally at some point during the study or from completing the experimental tests. This highlights the difficulty of implementing a long-term intervention in a highly trained endurance cohort.

The study detailed in **Chapter 7** expanded on the findings in **Chapter 6** by increasing the size of the cohort in order to formally compare female and male responses. Therefore, this study was designed to investigate the effect of sex on the efficacy of intermittent post-exercise sauna bathing to induce heat acclimation and improve markers of temperate exercise performance in trained athletes. This chapter addressed the hypotheses that: i) an acute bout of post-exercise sauna bathing would be more physiologically challenging in females, however despite a greater hypothesised acute thermoregulatory strain, ii) 3 weeks of intermittent post-exercise sauna bathing would be more effective in males for inducing desirable adaptations during both exercise-heat stress and temperate exercise. Results contradicted the hypotheses. Firstly, both female and male endurance athletes exhibited similar physiological strain (as indicated by similar rectal temperature, heart rate and sweat loss) during an acute bout of post-exercise sauna bathing. Second, 3 weeks of post-exercise

sauna bathing was not more effective in males. In fact, post-exercise sauna bathing was arguably more effective in female athletes, as both sexes showed similar reductions in thermal and cardiovascular strain, whilst only females showed an attenuated *rise* in rectal temperature during exercise-heat stress at post-intervention. There was also a sex dimorphism in changes in thermoeffector outcome measures following the post-exercise sauna bathing intervention. Only males exhibited marked increases in forearm blood flow from pre- to post-intervention, whereas only females exhibited increased sweat gland activity on the forearm. However, due to the fixed external workload employed in the running heat tolerance test, it is unclear how these differences may have been affected by differences in metabolic heat production due to differences in physical size. These findings warrant further investigation of a possible adaptive sex difference. Temperate performance results also contradicted our hypotheses, as both sexes showed similar improvements in all markers of temperate exercise performance following 3 weeks of intermittent post-exercise sauna bathing.

Finally, **Chapter 8** tested the hypothesis that 3 weeks of intermittent post-exercise sauna bathing would induce heat acclimation adaptations that would be partially lost (i.e. decay at a rate of ~2.5% per day) after 2 weeks without post-exercise sauna bathing. Specifically, it was hypothesised that this would equate to ~35% decay, in line with current literature describing the decay of heat acclimation (Daanen et al., 2018). Consistent with this hypothesis, heart rate and body temperature adaptations decayed by 25-33% on average. Rectal temperature during exercise-heat stress remained significantly reduced from baseline following 2 weeks without exogenous heat exposure. However, other adaptations were not different from pre-intervention following 2-week decay, and retention appeared to have a large individual variance. Some indices of decay were or tended to be related, namely decay of peak heart rate reduction with decay of resting core body (rectal) temperature and with decay of sweat loss. However, decay of resting core body temperature and peak core body temperature were not related, which implicates other mechanisms besides a reduced resting temperature in maintaining a reduction in peak core temperature following 2 weeks decay.

Collectively, these studies have established a number of important findings. Short-term, active heat acclimation is not sufficient to improve exercise performance in either hot or cool conditions in female athletes. More traditional, active heat acclimation protocols can be considered an ergogenic aid for females. However, active heat acclimation may not be as effective in well-trained female athletes and carries considerable barriers related to accessibility, time and cost. Instead, intermittent post-exercise sauna bathing offers a pragmatic and accessible alternative method for heat acclimation and importantly, appears to be an effective ergogenic aid for both sexes, as evidenced using gold-standard and objective tests. It is also an important and novel finding that post-exercise sauna bathing

induces similar thermal and cardiovascular adaptations in both sexes. However, these similarities in hallmark adaptations were observed amid potential sex differences in thermoeffector adaptations following post-exercise sauna bathing. Finally, hallmark adaptations to post-exercise sauna bathing are somewhat retained for up to 2 weeks, though more so in some individuals than in others.

9.3 Considerations and assumptions

There are some considerations when interpreting the results of the studies in this thesis. Firstly, with respect to study design. The studies in **Chapters 4** and **5** had no male comparison group, so it was assumed but not confirmed that the short-term protocol that was ineffective in females would have indeed been effective in males. In the remaining chapters, the rigor of findings would have been strengthened had the study been of a randomised-control, cross-over design. Since groups were not randomised, results may have been subject to some selection bias. The control group also did not receive a placebo intervention, and thus were not completely blinded to the expected outcome of the study. However, the ethos of the running club, the similar self-reported training intensity between groups and the objective nature of the experimental tests in **Chapters 6-8** suggest that motivation was not a contributing factor; although it remains a possibility.

Food intake following post-exercise sauna sessions was not recorded. Given that heat stress has been observed to increase appetite-suppressing hormones (Shorten et al., 2009), post-exercise sauna bathing could reduce the likelihood that athletes consume enough food to adequately match the caloric expenditure of the preceding exercise bout. Furthermore, glycogen resynthesis following endurance exercise is optimised by ingesting carbohydrates in the early phases of post-exercise recovery (Ivy et al., 1988; Jeukendrup, 2011; Stellingwerff et al., 2011). Post-exercise sauna bathing sub-optimally prolongs the timing of post-exercise carbohydrate intake, which should be considered when implementing this protocol, especially given that the addition of heat stress could also reduce appetite.

Recent data from Waldron et al. (2019) have highlighted the importance of the timing of post-intervention testing following a heat acclimation intervention, whereby aerobic capacity in temperate conditions appears to be suppressed in the days immediately following a heat acclimation intervention and subsequently rebounds above pre-intervention values at four-days post-intervention. Unfortunately, the investigations detailed in this thesis had commenced prior to the publication of the data by Waldron et al. (2019), and so their findings were not incorporated into the study designs herein. The post-heat acclimation tests in the current thesis were conducted 1-2 days post-intervention, which may have affected exercise performance outcomes.

The final delimitation imposed on the study designs was the recruitment of young, able-bodied and moderately to well-trained participants. Therefore, these findings cannot be readily applied to other populations.

The outcomes of these studies were largely descriptive of responses but not mechanisms. The exact mechanisms driving adaptations and performance improvements following heat acclimation, specifically as it pertains to cool or temperate conditions, remains largely speculative. The investigation of blood markers in **Chapter 6** were hypothesised to inform on possible haematological or vascular adaptation pathways, however many of the samples fell below detection limits. A resting blood sample was collected for participants that completed the interventions in **Chapters 7** and **8**, however, laboratory access became limited due to the COVID-19 pandemic and these samples remain unanalysed at the time of writing.

Some consideration is also given to the tests and equipment used. For example, rectal temperature was used as an index for core body temperature. The concept of “core body temperature” in-and-of itself is subject to interpretation. Considering the variations reported between brain, heart, muscle and organ temperatures (Dunleavy, 2010), it is debateable whether a single measurement site is truly representative of the body’s “core”. Moreover, rectal temperature is not as dynamic a measure as other methods of core body temperature measurement, such as gastro-intestinal (via telemetric pills) or oesophageal temperatures. Additionally, the tests, such as the running heat tolerance test and the hot cycling time trial, were relatively short (15-30 min) as compared to other endurance events. The magnitude of adaption observed in the studies in this thesis may not necessarily be the same in the context of a longer athletic event.

In a point/counter-point style debate, Wenner and Stachenfeld (2020) and Stanhewicz and Wong (2020), present two ideologies of how research in female populations should be conducted. Both parties agree that more physiology research needs to include females; however, Wenner and Stachenfeld (2020) argue that controlling for menstrual cycle is crucial. Specifically, studies should recruit multiple female cohorts (or test the same participants in different menstrual phases) to accurately represent the responses of female participants. Such research would help to determine the influence of sex hormones, given the wide-ranging effects of variations in hormonal status on physiological responses. The authors argue that one cannot be certain that hormonal fluctuations do not affect the end-result without testing the respective null-hypothesis. This approach certainly represents a limitation for the current thesis. Whilst this thesis had a central focus on female athletes and sex differences, the possible effects of the menstrual cycle and hormonal contraceptives were not investigated. Therefore, this thesis offers little understanding as to how sex hormones independently affected the results. This was especially relevant for **Chapters 4**, **5**

and **8,** where the length of the intervention or timing of tests did not always allow for participants to be tested and re-tested in the same menstrual phase or week of pill-taking. Furthermore, there was no way of discerning whether the menstrual cycle/hormonal phase during which the intervention was administered affected the intervention's efficacy. For example, the total thermal load of an isothermic heat acclimation protocol could differ if conducted during the luteal phase as compared to the follicular phase, since lower resting temperatures in the latter (~0.2°C; Giersch et al., 2020) require a greater increase in core body temperature to reach the 38.5°C threshold, considered one of the most important drivers of adaptation to chronic heat stress (Taylor & Cotter, 2006). Whether this, amongst other hormonal effects, impacts the efficacy of a heat acclimation intervention is unknown (Wickham et al., 2020). However, this point was less relevant for outcomes in **Chapters 6** and **7**, where the length of the intervention typically encapsulated a regular menstrual cycle (at least for the eumenhorreic participants) and most participants were tested and re-tested in the same phase. Thus, the menstrual cycle could not have affected the efficacy of the intervention itself, *per se*. This does not account for any possible differences between the eumenhorreic participants as compared to the 7 of 16 female participants in the intervention group in **Chapter 7** who were either using hormonal contraceptives or not normally menstruating (i.e. oligomenhorreic or amenorrheic). The capacity of hormonal contraceptive users to heat-acclimate as compared to normally menstruating females is unknown. Curiously, Garrett et al. (2019) and Perrotta et al. (2018), two studies that observed otherwise elusive heat acclimation adaptations following short-term heat acclimation in females, only tested hormonal contraceptive users. This is interesting, given that i) endogenous oestrogen levels are suppressed in oral contraceptive users (Lei et al., 2019), and ii) oestrogen has been suggested to dampen the heat shock protein response to heat acclimation (Paroo et al., 2002b; Gillum et al., 2013), which is necessary for adaptation (Kuennen et al., 2011).

In opposition to the points made by Wenner and Stachenfeld (2020), Stanhewicz and Wong (2020) contend that such stringent restraints are not necessary, especially considering that variations amongst males' sex hormones (i.e. testosterone) are not typically controlled for, despite the important physiological influence exerted by testosterone (e.g. its anabolic qualities; Hooper et al., 2017). Setting a standard of imposing strict control of female sex hormones in all physiological studies creates an additional barrier to including an already under-represented group and may even further limit data to females in a single menstrual phase (commonly, the "low-hormone" early-follicular phase). Instead, they suggest including a female cohort regardless of endogenous or exogenous hormonal state, because it is most representative of the female population as a whole (unless of course the research questions are specifically aimed at investigating the effects of sex hormones or the

menstrual cycle). This approach promotes ecological validity and accepts that responses with some hormone-related variability are the norm – because they reflect the variability that females normally experience. In the case of the current thesis, the female cohorts reported herein were composed of a random sample of female athletes and their responses were assumed to be the "normal" female response. Notably, there was no obvious variability in the female cohort as compared to the male cohort for main sex-comparison outcome measures in **Chapter 7**. Taken together, although the specific effects of female sex hormones were not examined within this thesis, the added ecological validity of the approach taken in the studies determines that the observed results of this thesis are applicable to the population that they represent.

9.4 Perspectives and implications

These data inform heat acclimation strategies for future events in hot conditions, including the summer Olympic Games in Tokyo, set to be the hottest in modern history (Gerrett et al., 2019). In the context of the ongoing climate crisis, increases in average temperatures and the frequency of extreme heat events (Lehner et al., 2018; Power & Delage, 2019) mean that heat acclimation will be of even greater importance for sport and athletic competition. If heat acclimation strategies become a standard for elite athletes, personalising these strategies can be expected to also become paramount. This thesis demonstrates that sex should be considered as a modifying factor, especially when considering active heat acclimation programme *duration* for female athletes. Furthermore, post-exercise passive heating, and specifically sauna bathing, offers a pragmatic alternative to active heat acclimation and offers the unique opportunity for athletes to heat acclimate without altering normal training. Whilst post-exercise sauna bathing has been recommended to athletes as a form of heat acclimation (World Athletics, 2019), the data in this thesis present the first conclusive evidence demonstrating hallmark thermal, cardiovascular and sudomotor adaptations during exercise-heat stress. The most optimal avenue for *performance* improvements in the heat is probably a mix of active and passive heat acclimation (Ruddock et al., 2016; Stephenson et al., 2019), since athletes minimise training disruption, but have the perceptual experience of exercising in the heat (though these additive effects remain speculative; Pryor et al., 2019). Whilst post-exercise sauna bathing did improve perceptual responses of athletes, this implies but does not necessarily guarantee an improvement in self-paced exercise performance.

These findings also represent an important body of evidence towards the ongoing debate on the efficacy of using heat acclimation to improve performance in cooler conditions (Minson & Cotter, 2016; Nybo & Lundby, 2016). Firstly, conclusions from **Chapter 5** highlight

that a high-intensity (i.e. incorporating HIIT, permissive dehydration and hot conditions), consecutive-day active heat acclimation protocol may not reliably produce meaningful performance improvements in all (female) athletes. Notably, this debate has largely surrounded medium-term, consecutive-day heat acclimation protocols similar to that employed in **Chapter 5.** However, these protocols may not be the optimal way to integrate heat as an ergogenic aid. Active heat acclimation sessions may compromise the integrity of a training session planned for cooler conditions or be overly fatiguing (Reeve et al., 2019), especially considering that this stress is applied on consecutive days. Instead, post-exercise passive heating allows training sessions targeting specific training adaptations to continue undisturbed, whilst providing a thermal stimulus for heat adaptation (Pryor et al., 2019a). Moreover, post-exercise sauna bathing adds heat stress without adding mechanical load, which could be an important factor in endurance populations with a high rate of injury, such as the cohort of middle-distance runners studied in this thesis. Finally, post-exercise sauna bathing provokes a similar acute stimulus in both male and female athletes in an ecologically valid setting, and is subsequently equally effective in driving thermal and cardiovascular adaptations when applied chronically. Overall, heat stress appears to be ergogenic for both sexes when it is correctly integrated as a supplement to normal training, specifically in the context of post-exercise sauna bathing.

9.5 Future directions

Considering the comparative lack of information surrounding heat acclimation in females, there is a wide scope for future research. Sex differences in the temporal patterning of adaptations should be examined closely by observing hallmark thermal, cardiovascular and sudomotor measures at a fixed intensity during each day of acclimation. In the current thesis and in the literature (Mee et al., 2015b), females have been assessed at 4-5 and 9-10 day cut-offs, but heat acclimation may be attained following, for example, a 6-day heat acclimation intervention (still considered “short-term”; Garrett et al., 2011). Moreover, the mechanisms underpinning sex differences observed in the efficacy of short-term heat acclimation are yet to be elucidated. It is possible that sex differences identified thus far are a product of females being typically smaller and thus producing a comparatively lower thermal load (i.e. the combination of metabolic heat production, environmental heat gain and heat storage; Wickham et al., 2020) and lower thermoeffector drive (Notley et al., 2017).

The possible sex dimorphism in thermoeffector adaptations in response to post-exercise sauna bathing also warrants further investigation. Firstly, mechanisms facilitating sweating and skin blood flow adaptations should be examined in both sexes to identify if there is a difference in thermoeffector adaptation to chronic heat stress. Second, the careful

measurement of heat production and heat dissipation (in dry and humid heat) is required to better understand exactly how these possible adaptive differences contribute to whole-body heat loss in various conditions. The implications of better understanding thermoeffector differences include enhanced individualisation of heat acclimation programs through a targeted, sex-specific approach.

To even better optimise heat acclimation for female athletes, future investigations should compare the efficacy of implementing a heat acclimation intervention at various points in the menstrual cycle and/or as compared to in hormonal contraceptive users. This paradigm of optimising adaptations by capitalising on fluctuations in females' menstrual cycle is not necessarily new to exercise sciences. For example, the 'anabolic' hormone milieu in the follicular phase favours strength-trainability and muscle mass gain (Chidi-Ogbolu & Baar, 2018). Thus, improvements are optimised when a classic resistance training program is modified to load resistance-training sessions in the follicular phase (Reis et al., 1995; Sung et al., 2014). Considering the direct effects that female sex hormones exert on body temperatures (Stephenson & Kolka, 1999; Kim et al., 2008) and the vasculature (Orshal & Khalil, 2004), further investigation of a menstrual cycle-based optimisation approach is warranted.

Akin to the vast opportunities for further investigation in females, research in the area of post-exercise heating is just beginning and is rightfully gaining increased attention (Heathcote et al., 2018). Details yet to be ascertained include the minimal required dose for meaningful adaptations, in the context of session duration and frequency, protocol duration and intensity of ambient conditions. This also includes optimising the integration of a post-exercise heating protocol before competition and retaining adaptations during a taper (Casadio et al., 2016). As evidenced in **Chapter 8**, by 2 weeks post-intervention, adaptations had waned in some individuals, so some form of "top-up" or re-acclimation is likely required during a taper that lasts ~2 weeks in order to maximise heat acclimation benefits (Racinais & Periard, 2020).

Investigations related to post-exercise sauna bathing should be repeated in other populations for which it would presumably be applied. For example, the athletes in the current thesis were well-trained (training ~6 times per week), but the 1.5 cumulative hours of post-exercise sauna bathing added a significant cardiovascular (heart rate ~132 beats·min^{-1}) and thermal stimulus (rectal temperatures ~38.5°C) to their weekly training. However, many elite-level athletes have a much greater training volume (e.g. often training twice daily) and thus whether these post-exercise sauna bathing sessions would be equally ergogenic is unknown. Furthermore, better understanding of the efficacy of post-exercise heating for inducing thermal tolerance to prevent heat injury would be particularly pertinent for military

personnel, especially with the greater incidences of exertional heat injury in females than in males in the military (Kazman et al., 2015).

As alluded to throughout this chapter, many of the outcome measures in this thesis act as quantifiable “markers” of heat acclimation, but do not necessarily elucidate the mechanisms of adaptation. For instance, this thesis consistently reported a reduction in exercising heart rate following the post-exercise sauna bathing interventions (**Chapters 6-8**). In **Chapter 2,** a number of possible mechanisms contributing to the reduced heart rate are presented (including plasma and blood volume expansion, improved myocardial efficiency, increased autonomic excitability and parasympathetic control, and a reduced thermal stimulus resulting from a lower core body temperature, amongst others). However, the relative contribution of the plausible mechanistic pathway(s) is not well-understood. Thus, **Chapter 8** offers little explanation as to *why* the decay of some variables were related within individuals whilst others were not. Better understanding of the independent and integrative mechanisms of heat acclimation would aide in the understanding of both its induction and decay; and may help to optimise and personalise heat acclimation by allowing for pursuit of a more targeted response. This is a merited avenue for future research, as variation in individual responses has been highlighted throughout this thesis (e.g. **Chapter 4** and **Chapter 8**) and in the literature (Corbett et al., 2018a).

9.6 Closing comments

This thesis contributed new knowledge to the area of heat acclimation, with specific investigations of the responses of female athletes, sex differences, post-exercise sauna bathing and repeated heat stress as an ergogenic aid. Short-term active heat acclimation does not appear to be effective in a female cohort, although more traditional medium-term heat acclimation enhances self-paced performance in the heat similar to what has been documented in males. Post-exercise sauna bathing provides a practical alternative for heat acclimation for both sexes without disrupting training and without the cost of accessing specialised facilities such as an environmental chamber. There may be a sex difference in the thermoeffector adaptations that facilitate the reduction in body temperatures following heat acclimation. This challenges previous assumptions that males and females achieve improved heat loss during exercise-heat stress following heat acclimation through similar mechanisms (Sawka et al., 2011) and provides evidence that warrants further investigation.

Heat acclimation can be ergogenic; however, it is important that chronic heat stress is applied in a manner that does not compromise normal training, such as using post-exercise sauna bathing, and does not cause fatigue to a degree that becomes detrimental to performance. As heat acclimation becomes a more common training element for athletes

aiming to improve performance in both hot and cool conditions, it is imperative that female and male athletes are equally represented in research that pushes the boundaries of our knowledge and understanding.

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APPENDIX 1: MENSTRUAL CYCLE QUESTIONNAIRE

Participant ID Code (to be completed by the researcher): _____

1.	When was the start date of your last period?		
2.	Would you consider yourself to have a regular cycle?	YES	NO
3.	How many days is your average cycle?		
4.	Do you normally experience any significant side effects during your period that could affect exercise or performance? If so, please describe:		
5.	Have you missed a period in the last 6 months?	YES	NO
	If so, please describe how many and when:		
6.	Are you on oral contraception?	YES	NO
	If so, what brand?		
7.	Are you on any other type of hormonal contraception (i.e., coil, injection, implant, patch, other...)	YES	NO
	If so, which one?		
8.	How long have you been taking oral or other methods of hormonal contraception?		

I have completed these questionnaires to the best of my knowledge and any questions I had have been answered to my full satisfaction ☐

Date: .....

APPENDIX 2: SENSITIVITY ANALYSIS

A sensitivity analysis was performed on main outcome measures from **Chapter 6** by removing the *n*=2 "cross-over" participants' (i.e., participants who completed both SAUNA and CON interventions, with a ~5-weeks washout in-between) second intervention data set from the ANCOVA analyses (i.e., participant's data from the intervention they completed first was *only* included).

After removing *n*=2 participants, the SAUNA group showed trends for greater reductions in peak HR (p=0.07, d=1.07) and peak T_{rec} (p=0.06, d=0.59) than the CON group. The CON group still showed an attenuated sweat loss (p=0.03, d=0.85) as compared to the SAUNA group. Consistent with our original analysis, the SAUNA group exhibited a greater improvement in running speed at 4 mmol·L^{-1} [La^{-}] (p=0.05, d=0.94), $\dot{V}O_{2max}$ (p=0.02, d=0.96) and TTE (p<0.01, d=1.54) than the CON group.

APPENDIX 3: CONTROL GROUP DATA FROM CHAPTER 8

The control (CON) group's data from the time-points detailed in Chapter 8 (investigating heat acclimation decay) are detailed below. Participant characteristics are summarised in **Table A3.1**.

Table A3.1 CON participants' characteristics. Data are presented as mean±SD

Participants (female/male)	2/4
Age (years)	19±1
Height (cm)	174±13
Body mass (kg)	58±7
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	59.3±6.6

CON, control group; $\dot{V}O_{2max}$, maximal aerobic capacity

Female participants in the CON group were oligomenorrheic (menstrual cycle >40 days; *n*=1) or using a contraceptive implant (*n*=1).

Data from the CON participants during HTTs are detailed in **Table A3.2**. There were no main effects of time in the CON group for resting T_{rec} (p=0.27), peak T_{rec} (p=0.52), peak T_{sk} (p=0.18), peak HR (p=0.49) or sweat loss (p=0.73).

Table A3.2 Physiological responses of the control group during the running Heat Tolerance Test pre- (Pre), post- (Post) and 2-weeks post-intervention (Decay). Data are presented as mean±SD

	Pre	Post	Decay
Resting T_{rec} (°C)	37.3±0.2	37.4±0.5	37.1±0.3
Peak T_{rec} (°C)	38.4±0.3	38.5±0.5	38.3±0.3
Peak T_{sk} (°C)	36.4±1.2	36.0±1.2	36.5±0.8
Peak HR ($beats \cdot min^{-1}$)	163±12	160±11	162±15
Sweat loss (%BM)	1.3±0.5	1.2±0.3	1.2±0.3

T_{rec}, rectal temperature; T_{sk}, mean weighted skin temperature; HR, heart rate; BM, body mass

APPENDIX 4: POWER ANALYSIS FOR CHAPTER 8

A power analysis was conducted *posteriori* to examine whether the analyses conducted in **Chapter 8** were sufficiently powered to detect an effect. Effect size (as defined by partial eta-squared [η_p^2]), Greenhouse-Geisser nonsphericity correction (ε), error probability (α=0.5), sample size and number of time-points was entered into G*Power (G*Power version 3.1, Germany) for each main outcome variable. Results of the power analysis are reported in **Table A4.1**.

Table A4.1 Statistical power of analyses to detect a change in physiological responses of the SAUNA intervention group at pre- (Pre), post- (Post) and 2-weeks post-intervention (Decay).

	Power (1− β)
Resting T_{rec}	0.78
Peak T_{rec}	0.83
Peak T_{sk}	0.45
Peak HR	0.34
Sweat loss	0.08

T_{rec}, rectal temperature; T_{sk}, mean weighted skin temperature; HR, heart rate; BM, body mass