Effects of short-term heat acclimation on endurance cycling performance in the heat.

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Abstract

Heat training has been established as a tool to maximise physiological adaptations and enhance endurance performance in both hot and cool environmental conditions. However, recent research in the field of heat acclimation (HA) has uncovered physiological variances between sexes in their response to established heat protocols. The primary aim of this research was to determine the effects of short-term HA (5 days, 110 mins, 40°C and 40% relative humidity (RH)) on endurance cycling performance and physiological markers of HA - core temperature (°C), peak skin temperature (°C), heart rate (HR) (beats·min^{-1}), blood plasma volume (Δ%) and sweat rate (L·h^{-1}), in hot environments. Through the use of an emerging protocol, controlled heart rate, in tier two trained status females and males. The hypothesis tested was that five days of 110 mins of exercise at a controlled heart rate equivalent to heart rate at 65% of $\dot{V}O_{2max}$, in conditions of 40°C and 40% RH would significantly improve endurance cycling performance in the heat (20 km time trial at 33°C & 40% RH), and elicit significant improvements in physiological markers of HA as measured pre and post training using a heat stress test (40% of maximal aerobic power (MAP) for 30 mins in conditions of 40°C & 40% RH). Sixteen (eight females, eight males) participants who were undertaking at least three one hour plus sessions of cycling per week completed all HA sessions, resulting in improved time trial power (females: 4.9%, $p < 0.001$; males 6.7%, $p = 0.001$) and reduced duration (females: -2.0%, $p = 0.004$; males -2.6%, $p = 0.001$). Females saw significant improvement to markers of HA; peak HR reducing 15.9 ± 8.0 beats·min^{-1} (-9.6%, $p = 0.001$), peak core temperature reducing 0.33 ± 0.17°C (-0.9%, $p = 0.002$), thermal comfort reducing from slightly uncomfortable towards neutral ($p = 0.003$), thermal sensation reducing from hot to warm ($p = 0.049$) and RPE reducing from light towards very light ($p = 0.020$). While males saw significant improvement in peak core temperature reducing 0.30 ± 0.29°C (-5.5%, $p = 0.024$), thermal sensation reducing from hot to warm ($p = 0.003$) and tendency towards significance for peak HR reducing -8.0 ± 9.7 beats·min^{-1}, plasma volume expansion of 3.5% ± 10.9% and thermal comfort reducing from slightly uncomfortable towards neutral. These findings demonstrate the practical benefits for athletes in utilising a controlled heart-rate based HA protocol, to reduce performance decrement in the heat.

Declaration of Authenticity

"I, Damien Bovalino, declare that the Master of Research thesis entitled Effects of short-term heat acclimation on endurance cycling performance in the heat is no more than 50,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".

"I have conducted my research in alignment with the Australian Code for the Responsible Conduct of Research and Victoria University's Higher Degree by Research Policy and Procedures.

Signature:  Date: 7th February 2025

Ethics Declaration

"All research procedures reported in the thesis were approved by the Victoria University Human Research Ethics Committee HRE21-135."

Signature:  Date: 7th February 2025

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List of Abbreviations

Beats·min^{-1}	Beats per minute
ES	Effect size
FAM	Familiarisation session
GXT	Graded exercise test
HA	Heat acclimation
Hb	Haemoglobin
Hct	Haematocrit
HR	Heart rate
HST	Heat stress test
kg	Kilograms
kJ	Kilojoules
LTHA	Long-term HA
L·h^{-1}	Litres per hour
MAP	Maximal Aerobic Power
MTHA	Medium-term HA
NaCl	Sodium Chloride
OCP	Oral contraceptive pill
RH	Relative humidity
RPE	Rating of perceived exertion
STHA	Short-term HA
TT	Time trial
TTE	Time to exhaustion
USG	Urine specific gravity
$\dot{V}O_{2max}$	Maximum volume of oxygen consumption
W	Watts

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1. Introduction

With the exception of winter events, endurance sports are often held in warmer locations, in the summer months and outside at the mercy of the elements. Recent events where athletes endured difficult conditions include; the Tokyo Summer Olympics (34°C & 70% RH), Tour down under (>40°C), and 2019 Doha World Athletics Champs (32°C & 74% RH). Additionally climate change is expected to both increase maximum daytime temperatures and the frequency of days considered to be hot (Ridder et al., 2022). It's understood that exercising in hot environments, >25°C (Guy et al., 2014), impairs endurance performance by ~3% generally and specifically reduces time trial performance by 5% in conditions of 35°C & 60% RH and reduces running speed also by 5% in conditions of 33°C & 78% RH. Moreover, dangers exist when exercising in hot environments with exertional heat stroke the third most common cause of sudden death in athletes, rising to the primary cause of sudden death in summer. Therefore heat acclimation (HA) is vital for athletes wishing to compete in summer endurance sports.

To become heat adapted, requires athletes to follow a HA protocol, or experience heat acclimatisation, in order to gain the necessary stimulus to elicit physiological adaptations and for those adaptations to persist long enough to benefit the athlete in competition. Research in this area has shown that a short-term heat acclimation (STHA) protocol, considered to be < 7 exposures (Tyler et al., 2016), of 90 min HA sessions provides for improvements in males (Chalmers et al., 2014; Garrett et al., 2009, 2011, 2012, 2014; Mee et al., 2015; Poirier et al., 2015; Racinais et al., 2015b; Sunderland et al., 2008; Tyler et al., 2016). However, research on HA has largely been conducted with males, with numerous review papers revealing an under representation of female participation in HA research (Hutchins et al., 2021; Kelly et al., 2024; Wickham et al., 2021), resultingly, HA knowledge is largely based on male participants and evidence suggests protocols are not likely directly applicable to females. Females who are naturally menstruating or taking oral contraceptives (OCP), experience thermoregulator and fluid shifts in response to hormone levels (Aguree et al., 2020; Hutchins et al., 2021; Lei et al., 2019; Stachenfeld, 2008). Understanding the impact of female hormones on HA protocols is still in it's infancy. As such, there exists a sizeable gap in knowledge of HA as it relates to females.

Research that included female participants suggests a longer total duration is required to see similar significant physiological changes and performance improvement (Garrett et al., 2019; Kelly et al., 2023; Kirby et al., 2019; Mee et al., 2015, 2018; Wickham et al., 2021). More specifically, researchers found that post a STHA intervention, which was effective on males, females did not achieve the reduction in exercising core body temperature and heart rate that

males did (Mee et al., 2015) and did not see significant performance improvement in the heat (Kirby et al., 2019). However more recent research by Mee et al. (2018) found a pre-load of 20 min passive sauna did result in significant changes to the physiological markers of HA in females.

Mee et al. (2018) found that use of a sauna suit for 20 mins passively in conditions of 50°C and 30% RH prior to an active 90 min session in conditions of 40°C and 40% RH with controlled hyperthermia of 38.5°C, over five consecutive sessions, yielded desired outcomes. The protocol tested in the current study advances this research and consists of a STHA approach of five consecutive days of cycling, adopting the 110 minute heat exposure experienced by the sauna condition as session duration from Mee et al. (2018), with application of a controlled heart rate model (heart rate equivalent to heart rate at 65% of $\dot{V}O_{2max}$) and conducted in conditions of 40°C and 40% RH.

The controlled heart rate HA model is novel to female only groups (Stephenson et al., 2019) and novel in its application to males in a STHA protocol. Research has examined the model via MTHA protocols consisting of 10 consecutive days of 90 minute HA sessions in conditions of 40°C and 40% RH to effect changes in performance and some markers of HA (Travers et al., 2020), and also with 50% of heart rate at VO_{2max} as determined during a hot test (Keiser et al., 2015).

Research questions are:
1. In tier two trained female and male cyclists, does STHA training for 110 mins at a heart rate equivalent to heart rate at 65% of $\dot{V}O_{2max}$ in ambient conditions of 40°C and 40% RH across five consecutive days significantly improve:

1) 20 km cycling endurance performance in the heat (33°C & 40% RH), and

2) physiological markers of HA; core temperature (°C), peak skin temperature (°C), heart rate (beats·min^{-1}), blood plasma volume (Δ%) and sweat rate (L·h^{-1}).

The current study builds on prior recent research conducted by Mee et al. (2018) and Travers et al. (2020). In regard to Mee et al. (2018), the current study looked to expand understanding of HA in females in three ways: 1) to test the application of an active model of HA which elicits the benefits of adequate, progressive cardiovascular strain and as yet had not been tested in females and compared to males (Périard et al., 2021b); 2) apply an applicable performance test in addition to understanding physiological and perceptual HA outcomes in females; 3) to test STHA across five sessions of 110 mins without the need to include 20 minutes wearing a sauna suit in a sauna, that is the full 110 mins as active HA in a heat chamber. Practical

benefits of these being expanding and understanding of a novel HA model that can be applied using the commonly used training measure of heart rate, along with an effective feasible STHA model for females without the need for sauna suit clothing and access to a sauna.

With regard to Travers et al. (2020), the current study looked to apply the same HA model, however, as a STHA protocol. In applying the HA model over five days as compared to 10 days, the duration of each session was 20 mins longer at 110 minutes compared to 90 minutes in Travers et al. (2020). The rationale for the longer sessions is to ensure an adequate heat dose over the shorter timeframe. Practically, application of this model using a STHA protocol provides an understanding of minimum duration and frequency for male athletes to obtain performance benefits from controlled intensity, utilising heart rate to optimise cardiovascular strain, and enabling less disruption to quality training, especially in the pre-competition tapering phase (Périard et al., 2015).

Overall, the current study adds to the understanding of female HA through inclusion of a performance test, expands knowledge in the application of controlled heart rate HA model, in a more practical for athletes STHA protocol, with comparison in it's application across males and females.

2. Literature Review

2.1. Effect of Heat on Performance

It is widely accepted that exercising in a hot environment, >25°C (Guy et al., 2014), reduces the capacity for human endurance performance. Exercising in a hot environment can result in all or some of; increase in core temperature, heart rate, discomfort, increased blood flow to the periphery for cooling, reduction in blood volume due to sweating, and impairment of skeletal muscle function (Nybo & Nielsen, 2001; Nybo et al., 2014; Racinais, & Oska., 2010; Racinais et al., 2015a). The effort of exercising in heat impairs performance through cardiovascular strain from increased body fluid loss and increased skin blood flow reducing stroke volume, and a shift towards anaerobic energy system contribution (Galloway & Maughan, 1997; González-Alonso & Calbet, 2003; Périard et al., 2011). Performance may additionally be impacted through heat impacting on a central critical temperature threshold, reducing neuromuscular drive or elevating the perception of effort, and through restriction in blood flow to systems and organs such as the gut and brain, with decremental flow on effects centrally and peripherally (Cheung & Sleivert, 2004). In total, these lead to performance decrements of greater than 13% for mean power and greater than 5% for time trial duration in

35°C and 60% RH, and greater than 5% for running speed in 33°C and 78% RH (Périard et al., 2011; Voltaire et al, 2002). Time to exhaustion tests in well trained males are impacted by up to 28% when core temperature is elevated by 1°C (Gonzalez-Alonso & Calbet, 2003). The decrease in performance is mostly evident in aerobic endurance sports such as race walking, distance running and time trial cycling compared to anaerobic based sports such as track sprinting. This is due to the prolonged nature of the effort from athletes in aerobic endurance sports requiring a higher dependence on aerobic metabolism (Racinais & Oksa., 2010; Guy et al., 2014).

2.2. Markers of Heat Acclimation

The physiological and performance decrements associated with exercise in the heat can be reduced via the application of HA protocols. HA involves deliberate exposure to a heat stimulus through training in a naturally hot environment, known as heat acclimatisation, or an artificially hot environment known as heat acclimation (Racinais et al., 2019). While other approaches, such as pre-exercise cooling and hydration, can assist with exercise in hot conditions. In isolation, HA is considered to be the superior approach to attenuate the rise in core temperature and extend heat storage capacity (Alhadad et al., 2019; Racinais et al., 2015a; Tyler et al., 2016). Once an athlete achieves HA, combination with pre-exercise cooling and hydration would likely yield on optimal approach (Alhadad et al., 2019). The physiological changes found to elicit HA manifest primarily in a lower heart rate, lower core temperature, higher sweat rate, and plasma volume expansion compared to an un-acclimatised athlete, which results in a reduced decrement in performance under similar exercise intensity in a hot environment (Sawka et al., 2011).

2.3. Methods for Heat Acclimation

With an adequate heat dose, different methods using active and/or passive approaches have successfully been employed across male and also female populations to enable HA (Kelly et al., 2023). Active methods include cycling, rowing or running in a heat chamber and simply training outside in a hot environment. The use of a heat chamber includes the benefits controlled temperature set points and allowing for active exercise, which elicits greater cardiovascular strain than passive sauna or hot water immersion and may lead to broader and more complete improvements in the markers of HA (Campbell et al., 2022). However, heat chamber facilities can also be impractical due to their cost, proximity to the location of the athlete or squad, scheduling complications and use by other athletes and researchers at undesired set points. Training outside is often more appealing to athletes, though has issues

with weather variability and ability to reach an appropriate heat stress stimulus safely in order to avoid potential heat illness due to the difficulties in monitoring core temperature while mobile. Passive methods include sauna and hot water immersion, which can provide an opportunity to be completed post training. This may allow athletes to complete quality event-specific “tune-up” training sessions in addition to passive HA in the lead up to competition rather than adding active HA on top of quality training sessions, resulting in fatigue. Or lowering intensity and duration to simultaneously complete active HA and event-specific training, resulting in under preparing for competition in elite athletes (Casadio et al., 2016). For some participants, sauna in hot, humid conditions (55°C 54% RH) was found to not be as well tolerated for the planned duration as other methods, and its passive mode may not see the same extent of HA in athletes (Campbell et al., 2022). While each method has appealing aspects and limitations, in order to be effective the HA method and protocol needs to produce a heat stress impulse that is great enough to disturb homeostasis. This in turn reduces the physiological strain of future exposure to heat with a series of sufficient sessions required for this to occur optimally (Taylor, 2014).

2.4. Model of Heat Acclimation

Active HA model options include controlled hyperthermia, controlled work rate, controlled heart rate, and self-regulated with controlled work rate being the most commonly used (Tyler et al., 2016). Across studies including female participants, the controlled work rate model had a greater impact on performance improvement in the heat across mean power and time to exhaustion, with the effect size for controlled work rate 1.53 (change of 58% ± 32%) compared to 0.31 (change of 7% ± 3%) for controlled hyperthermia (Kelly et al., 2023). However, the controlled work rate approach has limitations in that as participants acclimatise, the heat strain can reduce and restrict further HA. In contrast, the controlled hyperthermia model can maintain adequate heat strain through progressive overload (Taylor, 2014), though the high cost of core temperature pills, or use of invasive rectal probes to accurately measure core temperature can be a limitation for the controlled hyperthermia model. The controlled heart rate model, however, avoids these limitations through utilising the commonly used measure of heart rate, and presents as an alternative model that also results in progressive overload (Périard et al., 2015; Tyler et al., 2016). Further, it may be less impacted where core temperature is elevated due to specific hormonal phases in females (Wickham et al., 2021).

The use of the controlled heart rate model has been successful in improving performance and inducing markers of HA in tier 2 trained status males after ten 90 min sessions in conditions of 40°C and 40% RH. Core temperature was maintained at ~38.4°C for the final 75 mins of

the sessions and increased sweat rate by 0.19 ± 0.18 $L \cdot h^{-1}$, decreased mean skin temperature by 0.6°C ± 0.5°C, and increased time trial performance by 19 ± 16 W (Travers et al., 2020). Using this method, the exercise work rate is adjusted in order to maintain the required heart rate throughout the session and across the duration of intervention sessions, thus providing a progressive, effective and time efficient model (Périard et al., 2021b).

2.5. Heat Acclimation Protocols

Exposure to heat provides a stimulus for adaptation and this can be achieved to greater or lesser extents through the length of intervention and a trade-off between intensity and session duration (Kelly et al., 2023; Périard et al., 2015; Tyler et al., 2016). Common groupings of intervention length are STHA < 7 exposures, medium-term HA (MTHA) 8-14 exposures, and long-term HA (LTHA) 15+ exposures (Tyler et al., 2016). Physiological changes occur most rapidly in the first week (Flouris et al., 2014; Poirier et al., 2015; Racinais et al., 2015a) and trained female athletes can see changes in as little as four sessions using active heat acclimatisation (Sunderland et al., 2008). The rate of adaptation to HA reduces with greater exposure and requires a complex set of regulated and key physiological interactions take place over the longer term for complete HA which may be seen in active inhabitants of hot environments (Taylor, 2014). Given athletes seeking HA are likely unable to feasibly engage in long-term exposure, a STHA protocol provides an attractive option for athletes to schedule around other training and avoid compromising training (Chalmers et al., 2014; Galloway & Maughan., 1997) while obtaining important cardiovascular adaptations (Garrett et al., 2011).

Research has established in moderately trained males that a STHA protocol of five consecutive 90 min sessions in 40°C and 30-60% RH commencing with 60-65% $\dot{V}O_{2max}$ to obtain controlled hyperthermia of ~38.5°C elicits changes in the markers of HA or performance improvement during a short-term acclimation phase (Garrett et al., 2009; Gibson et al., 2015; James et al., 2017; Mee et al., 2015; Patterson et al., 2004b). However, the aforementioned controlled heart rate model has not been tested in STHA using a performance test and heat stress test (HST) to determine changes in the markers of HA. There therefore exists a need to expand the understanding of the application of the controlled heart rate model to STHA protocol, so that males can confidently choose that approach and avoid the limitations described previously of the controlled hyperthermia model.

2.6. Performance Improvement from Heat Acclimation

Improvement to performance post HA, has been found through a number of methods and models utilising a STHA protocol. In a meta-analysis of predominately male participants, Tyler et al. (2016) found that across short to long-term duration HA provided for a mean time trial performance improvement of 7% ± 7% and mean time to exhaustion (TTE) improvement of 23% ± 29%, both in hot conditions. In a meta-analysis of female participants, Kelly et al. (2023) found across time trial and TTE tests a mean performance improvement in hot conditions of 30% ± 25% for short-term HA interventions and 55% ± 44% for long-term HA interventions. While these meta-analysis show substantial improvements in performance, given relatively large standard deviations reported, it is worth noting that there is inter-individual variance in response to HA with regarding to performance.

2.7. Physiological adaptations

Various HA studies, predominantly using male participants, have found a reduction in resting and exercise heart rate post-intervention period (Périard et al., 2015; Tyler et al., 2016). Resting heart rate was reported to have reduced by 10 beats·min^{-1}, after 16 heat sessions of 90 mins in 40°C and 60% RH using controlled hyperthermia with males (Patterson et al., 2004a), while Mee et al. (2018) reported a 12 beats·min^{-1} reduction in resting heart rate for females after five heat sessions of 110 mins in 40°C and 40% RH.

The majority of core temperature improvements generally occur by seven days and are limited beyond ten days (Périard et al., 2015). Given this, STHA may not reduce resting core temperature significantly, however, it can reduce exercising core temperature (Garrett et al., 2009, 2012). In LTHA, resting core temperature can be reduced up to 0.32°C in well trained male participants (Neal et al., 2015), with a reduction of 0.2°C considered likely to improve performance across predominately male participants (Tyler et al., 2016).

Sudomotor function changes in response to HA include a reduced onset temperature for sweating, increased sensitivity to changes in body temperature and increased sweat rate capacity leading to greater cutaneous blood flow (Sawka et al., 2011; Tyler et al., 2016). These changes to sensitivity have been found to be central through a reduced required magnitude of change in core temperature (Patterson et al., 2004), and peripheral through an endothelium dependent vasodilator (Lorenzo et al., 2010). While longer HA interventions, 14+ exposures, are required to achieve complete sweat response adaptations, these beneficial sudomotor adaptations begin to occur within the first week of HA, allowing for improved sub-maximal performance due to their cooling effects (Périard et al., 2015; Tyler et al., 2016).

In addition to evaporative cooling from increased sweat rate, cooling can also occur through convection of core heat to the periphery for loss into the environment. A core to skin temperature gradient is required to promote heat exchange in this manner, and has been found to be a critical factor in performance in the heat (Cuddy et al., 2014; Ely et al., 2009; Sawka et al., 2011). HA can improve the ability to remove heat through an increase to skin blood flow occurring earlier and with a reduction in skin temp of 0.5°C ± 0.4°C during exercise (Garrett et al., 2019). Despite the presence of a core to skin temperature gradient, where the ambient temperature exceeds skin temperature this method for cooling is lost and greater dependency on evaporative cooling ensues (Sawka et al., 2011)

During exercise in the heat, the cardiovascular demands for exercise and peripheral blood flow for cooling, lead to reduced cardiac output despite rises in heart rate due to reduced venous pressure and resulting lower stroke volume (Sawka et al., 2011). An expansion of blood plasma volume achieved through HA via overall extracellular fluid enlargement as a result of electrolyte retention and increased plasma protein content such as albumin, can stabilise cardiac output in hot conditions (Patterson et al, 2004b). Examples in the literature where HA has led to plasma volume expansion include Lorenzo et al. (2010) who reported that for a mixed-sex cohort of well-trained participants, ten active 90 min exposures to heat training in 40°C at 50% $\dot{V}O_{2max}$ increased plasma volume by 6.5%. Also, in tier two trained status females according to the framework established by McKay et al. (2022) with reference to hours trained, Mee et al. (2018) found a plasma volume expansion of 9% following five 20 min passive plus 90 min active exposures to 40°C and 40% RH suggesting that a STHA approach can yield desired blood plasma volume expansion.

2.8. Differences in Heat Acclimation Between Sexes

HA research with female participants, where results have been reported by sex, has found the application of the same protocol across sexes has not provided similar outcomes (Kirby et al., 2019; Mee et al., 2015; Morton., et al., 2008; Wickham et al., 2021). Mee et al. (2015) found that after five 90 min sessions at 40°C and 40% RH with cycling at 65% of $\dot{V}O_{2max}$ before adjusting to controlled hyperthermia of 38.5°C, that females did not achieve the reduction in exercising core body temperature and heart rate that males did with female participants requiring an additional 5 days to see significant changes and only increasing sweat rate post five sessions compared to males. At five days, females resting core temperature decreased 0.02°C ± 0.08°C (males -0.24°C ± 0.16°C), peak core temperature decreased 0.07°C ± 0.18°C (males -0.39°C ± 0.36°C), resting HR reduced 7 beats·min^{-1} (males -5 beats·min^{-1}), and peak HR reduced 5 ± 3 beats·min^{-1} (males -14 ± 12 beats·min^{-1}).

In a study by Kirby et al. (2019), which tested at four and nine days during the HA protocol, nine days were required, and not four, to see significant changes in moderately trained females in 90 min sessions in conditions of 40°C 30% RH with controlled hyperthermia of 38.5°C. During a self-paced 15 min performance test in conditions of 33°C and 33% RH the participants had a significant increase of 8.1% to mean power at nine days (3% at four days). Physiological adaptions of a reduced mean (0.2°C) and peak (0.1°C) core temperature, reduced peak skin temperature (0.5°C) and decreased mean heart rate of 5 beats·min^{-1} during exercise, were only observed between days five to nine of the HA protocol (Kirby et al., 2019).

Research by Garret et al. (2019) did result in some acclimation in moderately trained females using a STHA protocol of five 90 min sessions at 39.5°C and 60% RH, no fluid intake with cycling commencing at 60 W before controlled hyperthermia of 38.5°C. HSTs were conducted during the luteal phase with a nine x five min high intensity intermittent performance test in conditions of 31°C and 50% RH. The authors found skin temperature decreased 0.5°C ± 0.4°C, and plasma volume expanded 7% ± 7.5% (large effect size 1.27). A core temperature decrease of 0.2°C ± 0.1°C was found to not be significant, nor was the change in mean power output between the intermittent tests, which increased 56 ± 82 W. The large variance in these findings suggests that inter individual responses to the HA sessions varied. Given control for phase of menstrual cycle, variance driven by fluid shifts should have been limited (Aguree et al., 2020). In a systematic review of studies with female participants, Kelly et al. (2023) found that STHA led to improvements in exercise core and skin temperature, and heart rate. However, MTHA was required to see significant changes in resting core temperature and sweat rate.

Both studies by Mee et al. (2015) (2018) included a HST but no performance test, while the study by Garrett et al. (2019) did not examine the effect on endurance performance in the heat, that is a key parameter for athletic populations. Thus, there is a gap in the research to test a STHA protocol which will achieve an effective outcome for females and improve endurance performance in hot environments.

Research by Mee et al. (2018) has shown promise in a STHA protocol for females that elicits improvements to markers of HA as found with males. This cross-over designed study involved a treatment protocol where participants first rested in a 100% vinyl sauna suit for 20 mins in conditions of 50°C and 30% RH prior to undertaking a 90 min training session of exercise at 65% of maximal aerobic power in ambient conditions of 40°C and 40% RH, across five consecutive days and using controlled hyperthermia of 38.5°C. The other condition involved

resting for 20 mins in a sports bra and shorts in conditions of 20°C and 40% RH, before undertaking the same training session. During the 20 min period, rectal core temperature was not significantly different between the two conditions, however, average heart rate, sweat rate and temperature sensation were significantly higher for the sauna condition while resting in the hot environment. During the 90 min training session, rectal core temperature, average heart rate, relative exercise intensity (% of $\dot{V}O_{2max}$), and total work were not significantly different with only sweat NaCl significantly lower in the sauna condition. Post intervention the authors found significant differences between the sauna and non-sauna condition regarding plasma volume expansion (9% vs 1%), increase to sweat rate (81% vs 58%), reduced sweat NaCl (-16 vs -5 $mMol \cdot L^{-1}$), reduced onset temperature of sweating (-0.29°C vs -0.08°C), reduced resting heart rate (-10 beats.min-1 vs -4 beats.min-1), reduced peak heart rate (-12 $beats \cdot min^{-1}$ vs -3 $beats \cdot min^{-1}$), reduced rectal core temperature at rest (-0.28°C vs -0.07°C), reduced rectal peak core temperature (-0.42°C vs -0.05°C), reduction in peak skin temperature (-0.89°C vs ± 0.03°C), reduced temperature sensation (-1 vs 0), and reduced RPE (-2 vs -1). The authors concluded that the additional 100 mins of heat exposure and subsequent elevated heart rate, sweat rate and temperature sensation during the 20 min sauna exposure resulted in an altered autonomic control of heat balance. Additionally, the authors suggest that plasma volume expansion, which provided cardiac stability and lowered heart rate, was driven by sweating in the sauna increasing arginine vasopressin and aldosterone secretion to increase fluid balance via vasoconstriction and electrolyte reabsorption (Hew-Butler, 2012). It is therefore apparent that either longer HA sessions or greater total HA protocol duration may be required for females compared to males.

2.9. Physiological Impacts from Female Hormones

As related to HA, the hormones estrogen and progesterone impact thermoregulation and fluid shifts in females as levels rise. The high level of these hormones, and subsequent effects, can be driven by natural fluctuations across the menstrual cycle and also by use of contraceptive pills and implants (Lei et al., 2019; Stachenfeld, 2008).

Fluid shifts are driven by a number of factors, with a subsequent impact on plasma volume. Estrogen alters the renin-angiotensin-aldosterone system which can increase sodium reabsorption (Kuroski de Bold, 1999) and in turn extracellular fluid and plasma volume. Progesterone based implants or estrogen patches, however, do not have the same effect as oral estradiol, as OCPs lead to estradiol being metabolised in the liver where angiotensinogen is synthesized, and thus an oral route is required (Stachenfeld, 2008). Additionally, thirst and renal fluid elimination is altered through arginine vasopressin when progesterone and

estrogen are taken via OCP (Calzone et al., 2001). In naturally menstruating females, estrogen and progesterone have been found to have small impacts on renal sodium reabsorption, expanding extracellular fluid and in turn plasma volume. Further, estrogen and progesterone reduce albumin capillary leakage, resulting in plasma volume expansion despite evidence estrogen can also contract extracellular fluid (Stachenfeld, 2008). Clearly there are numerous interactions driven by variation in hormone levels throughout the menstrual cycle, with mean plasma volume highest during the early follicular phase and lowest at mid-luteal (Aguree et al., 2020).

Estrogen and progesterone impact thermoregulation through core temperature and sudomotor activity, with estrogen attenuating the effect of progesterone on increases to core temperature Stachenfeld et al., 2000). Across the menstrual cycle naturally menstruating women can see a rise in core temperature of between 0.3°C to 0.7°C at mid-luteal compared to the follicular phase (Baker et al., 2020). During exposure to heat, core temperature has also been found to be up to 0.2°C higher during the follicular phase, with the threshold for onset of sweating 0.29°C higher (Inoue et al., 2005). There have are also findings, that despite monophasic OCP usage there is an increase in core temperature at rest in the quasi-luteal phase of 0.15°C and an increase to the temperature onset of sweat rate (Charkoudian & Johnson, 1997; Sunderland & Nevill, 2003; Lei et al., 2019). While performance outcomes are equivocal between quasi-phases, recent research by Lei et al. (2019) regarding the active OCP period suggests that quasi-phase differences disappear after 12 mins of fixed intensity exercise in warm to hot conditions and behavioural thermoregulation is unaffected. These physiological impacts of hormones related to female participants requires careful analysis of results with reference to contraceptive use and phase of menstrual cycle.

3. Methodology

3.1. Experimental Design

The research was conducted across winter and spring to minimise any influence from heat acclimatisation across warmer months. Across this period local monthly mean maximum temperature ranged from 13.4°C to 23.9°C and monthly mean 9 am temperature 8.9°C to 17.8°C, with the latter being the typical time a local cyclist would be finished training given work and parental commitments (where relevant). Sixteen participants completed a graded exercise test to determine training intensity relative to $\dot{V}O_{2max}$ while cycling on a calibrated fixed Lode cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Participants then completed a 20 km time-trial familiarisation session in the same conditions as the performance test (33°C and 40% RH) to understand the process and reduce variability through

improved pacing across performance tests. These conditions are in line with those used in prior research; Garrett et al. (2019) used 31°C and 50% RH in an intermittent interval HST with females, Kirby et al. (2019) used 33°C and 33% RH in a 15 min capacity test with females, and Wingfield et al. (2016) used 33°C and 60% RH in a 20 km performance test with recreational males. Further, the conditions and distance are typical of those faced by endurance athletes competing in summer. For baseline measurements, the participants attended the laboratory on a separate day, after at least one day's rest, where hydration status of participants was confirmed before blood being taken. Participants then completed a 30 min HST in conditions of 40°C and 40% RH and at 40% of maximal aerobic power. A duration with conditions expected to induce adequate sweating for measurement (Schmit et al., 2017). Following a 60 minute rest period, completed a 20 km time trial in conditions of 33°C and 40% RH. Both were conducted by cycling on an electronically-braked cycle ergometer (Velotron, RacerMate Inc., Seattle, USA). Post one day's rest, participants completed five days of training for 110 minutes each session in conditions of 40°C and 40% RH, with the first 15 minutes at 65% of their power at $\dot{V}O_{2max}$ and the remainder at a heart rate equivalent corresponding to their heart rate at 65% of $\dot{V}O_{2max}$.

After one day's rest post training sessions, participants completed a post-intervention HST and time trial performance test. The day's rest allowed for participants to recover from training sessions with decay to physiological changes expected to be minimal from the day's rest given the view that for every two days spent without working in the heat, one day of acclimatisation is lost (Périard et al., 2015). Figure 1 displays a timeline of the experimental design and measures taken at each timepoint.

Day	-4	-2	1-5	+2
Session / **Measures**	GXT & Fam.	HST_1 & TT_1	Training Sessions (controlled heart rate)	HST_2 & TT_2
MAP, $\dot{V}O_{2max}$	✓			
Core & Skin Temp		✓	✓ (tympanic temp. only)	✓
Blood Plasma & Hormones		✓		✓
RPE, TS & TC		✓		✓
Sweat Rate		✓	✓	✓
Mean Power & Duration		✓	✓	✓
HR, Mean HR	✓	✓	✓	✓

Figure 1. Timeline of experimental design

3.2. Participants.

The 16 participants comprised of 8 female and 8 male cyclists aged 17 to 49 who regularly complete three or more cycle training sessions per week, for a minimum of 60 mins per session. This level of training classifies the cyclists as tier-two: trained/developmental as per established criteria (McKay et al., 2022). Trained/developmental cyclists having been chosen in order for the participants to be able to manage the demands of the stationary bike training, testing, familiarity with the performance test like efforts. Further, this selection of participants assists in the application of findings to athletic populations seeking better performance in hot environments. While the selection of trained/developmental cyclists may limit applicability to higher-level athletes, accessibility to higher-level athletes was not possible during the study period in the numbers required for appropriate statistical power. Participants were able to commence their participation provided they had not had heat exposure for a minimum of four weeks prior and at any time during their participation. Baseline participant characteristics are presented in Table 1. G*power 3.1.9.7 software was used to determine the required sample size to detect a statistically significant difference in 20 km cycling time trial performance post-intervention, assuming a 4.1% worthwhile performance improvement (Borg et al., 2018). Calculations resulted in a total of fourteen participants being required to achieve a statistical power of 95% and a significance level of 0.05 using two-way mixed model ANOVA.

Table 1. Baseline participant characteristics. Data expressed as mean ± SD.

	Females (n = 8)	Males (n = 8)
Age (years)	33.00 ± 6.65	30.00 ± 11.05
Height (cm)	167.75 ± 4.15	182.75 ± 4.99
Body mass (kg)	62.83 ± 4.47	73.89 ± 9.32
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	45.89 ± 6.20	61.13 ± 9.24
MAP (W)	260.00 ± 44.50	383.18 ± 47.51

Abbreviations: $\dot{V}O_{2max}$ – maximal oxygen consumption, MAP – maximal aerobic power, W = watts.

Research by Inoue et al. (2005) and Stachenfeld et al. (2000) suggests that the menstrual cycle may play a significant role in thermoregulation during exercise in the heat, with menstrual cycle having been controlled in previous HA studies (Garret et al., 2019; Mee et al., 2018). However, for this research, an approach was taken that competitive and amateur female athletes do not have the luxury of necessarily scheduling events around their menstrual cycle, and therefore, sessions and testing were not scheduled for a particular menstrual cycle phase. Instead, information on recent menstrual cycles, length of cycle, contraception type and formulation, and post hoc hormone analysis of blood to determine menstrual cycle phase was

undertaken to inform analysis of results. These steps taken result in a silver classification standard for methodological control as per the framework developed by Smith et al. (2022). Upon expressing interest to participate in the study, participants were asked to complete a menstrual cycle and contraceptive questionnaire (See Appendix A) with summary presented in Table 2. In addition to information in the questionnaire, further discussion during testing was also held with relevant female participants to identify phase of menstrual cycle. This information was combined with hormone analysis from blood samples for determination of menstrual cycle phase as described in Section 3.4. Those using copper or hormonal intrauterine devices, or a contraceptive implant were not excluded from participating. Participants were also screened via a medical questionnaire with any listed medications reviewed for safety and potential impact to thermoregulatory response.

Table 2. Female participant menstrual cycle summary.

Part.	Age	MC or Contr.	Length of MC (days)	HST_1			HST_2		
				Estradiol (pg/ml)	Proges. (ng/ml)	Phase	Estradiol (pg/ml)	Proges. (ng/ml)	Phase
1	29	Implant[1]	N/A	26.56	5.22	N/A	12.21	4.59	N/A
2	37	Nat. M	26	19.02	3.79	Early Fol.	3.17	20.98	Mid Lut.
3	28	Nat. M	31	5.42	5.16	Early Fol.	80.70	4.31	Mid Fol.
4	25	Nat. M	34	194.93	5.03	Late Fol.	91.62	6.11	Early Fol.
5	26	No. MC	N/A	27.81	5.78	N/A	3.34	6.52	N/A
6	39	OCP[2]	28	2.22	2.46	Late Lut.	68.82	3.74	Mid Fol.
7	35	Nat. M	26	9.39	4.59	Early Fol.	11.84	5.71	Late Lut.
8	45	Nat. M	27	57.31	8.08	Late Fol.	2.30	4.77	Late Lut.

Abbreviations: Part. – participant, MC – menstrual cycle, Contr. – contraceptive, Nat. M – naturally menstruating, OCP – oral contraceptive pill, Implant – contraceptive implant, No. MC – neither using contraceptive or menstruating, Proges. – progesterone, Fol – follicular, Lut – luteal.

1 Brand: Implanon

2 Brand: Monofeme (monophasic).

3.3. Graded Exercise Test

A GXT was conducted on an electronically-braked cycle ergometer (Lode Excalibur Sport, v2.0, Groningen, The Netherlands) at ~19°C to determine each participant's $\dot{V}O_{2max}$ and maximal aerobic power. With $\dot{V}O_{2max}$ defined as the highest recorded reading over a 30 sec period. Maximal aerobic power was defined as the power output of the last completed stage, or if stage not completed calculated according to the equation described by Kuipers et al. (1985) as (power of completed stage + (time completed of final stage reached / length of final stage reached X step stage change in power).

3.4. Plasma volume and hormone level analysis

Ten minutes prior to venepuncture, the participants were placed into a supine position to rest prior to taking blood. A 10 ml venous blood sample was taken, with 1 ml used immediately to assess the changes in plasma volume. The remainder was sampled into an EDTA blood collection tube, centrifuged for 10 minutes at 4000 revs·min-1 and 4°C and the separated plasma frozen at – 80°C until analysed. Changes in plasma volume pre- and post-intervention were calculated as per Dill & Costill (1974) using haematocrit (Hct) and haemoglobin (Hb) on the assumption that haemoglobin cannot leave the blood. Plasma volume pre-intervention was calculated as 1 - haematocrit (Hct), while plasma volume post-intervention was calculated as (Hb pre / Hb post x (1 - Hct post)). Change in plasma volume was then determined as ((plasma volume post-intervention - plasma volume pre-intervention) / plasma volume pre-intervention) expressed as a percentage. Remaining blood was centrifuged, with plasma separated and frozen for subsequent analysis of estrogen and progesterone levels for female participants. Plasma levels of estrogen were tested using an Invitrogen Human Estradiol E2 ELISA assay kit (ThermoFisher, Waltham, USA) and progesterone using an Invitrogen Human Progesterone Rapid ELISA assay kit (ThermoFisher, Waltham, USA), both with inter-assay and intra-assay coefficient of variation of <10%, according to manufacturer's instructions in the Victoria University Exercise Biochemistry Laboratory. With marked differences in the levels of estrogen and progesterone between the luteal and follicular phases of the menstrual cycle (Stachenfeld & Taylor, 2014), allowing for the determination of the menstrual cycle phase for each female participant at the time of HST blood collection. A combined approach of self-reported date onset of menses for up to three prior cycles, contraceptive use, and blood hormone analysis of estradiol and progesterone levels was used to make a determination of menstrual cycle phase for each female participant across each testing session (Allen et al., 2016).

3.5. Heat Stress Test

One day prior to the HST, the participants were provided with a packaged core temperature pill (CorTemp, HQInc, Palmetto, USA) and instructed to swallow the pill at least four hours before reporting to the laboratory. For each participant, testing was performed at the same time of the day to avoid possible diurnal effects (Reilly et al., 1984). Participants refrained from intensive exercise longer than one hour for 72 hours prior to testing (Rose Chrismas et al., 2017). Nutritional intake was recorded by each participant for 24 hours prior to pre-intervention testing, with the same nutritional intake repeated in the 24 hours prior to post-intervention testing. Exercise undertaken and nutritional intake was verbally reviewed prior to sessions for compliance, with no exceptions noted. To ensure euhydration on the day of the testing, the

participants were advised to drink 500 ml of water 2 h before arriving at the laboratory. Upon arriving at the laboratory, urine specific gravity (USG) was measured from a provided sample using a handheld refractometer (URC-NE, Atago, Japan) and the hypohydration threshold set at USG ≤ 1.020 (Sawka et al. 2007). Two participants arrived at the laboratory hypohydrated, and were provided with 600 ml of water with hydration status reassessed after 40 min, as recommended by Logan-Sprenger & Spriet (2013). Participants were then instructed to lie on a bed in a supine position prior to venepuncture, as described in the section above. Post venepuncture, participants were given privacy to measure body mass wearing only cycling bib-shorts on a calibrated scale to the nearest 10 g and to then place a heart rate monitor (FT1 model, Polar Electro OY, Kempele, Finland) across their chest. Thereafter, skin temperature sensors (Thermodata, Brisbane, Australia) were attached using film patches (Tegaderm, 3M, Saint Paul, Minnesota, USA) to the muscle mid-belly of their gastrocnemius, rectus femoris, brachioradialis and also equidistant between the xyphoid process and manubrium of the sternum. The participants then moved into the climatic chamber where after 5 minutes of rest, baseline physiological and perceptual measurements, (excluding RPE) were taken. Perceptual scales used were RPE (Borg, 1982), thermal sensation (Toner et al., 1986) and thermal comfort (Zhang, 2003). The HST was conducted in conditions of 40°C and 40% RH with participants cycling on an electronically-braked cycle ergometer (Velotron, RacerMate Inc., Seattle, USA) for 30 minutes at 40% of their maximal aerobic power as determined during the GXT. No fluid was allowed to be taken during the HST, as prior research has found that ingestion of fluid may influence core temperature readings (Wilkinson et al., 2008). Physiological and perceptual measures of RPE, thermal sensation and thermal comfort were recorded every 5 minutes during the test and at the end of the test. Skin temperature was recorded at a frequency of every five minutes across the four muscle sites, with an overall skin temperature calculated for each five minute interval using a weighted mean approach as per Ramanathan (1964). Core temperature was also recorded at a frequency of every five minutes. Immediately at the conclusion of the heat stress test the participant exited the heat chamber and skin temperature sensors and heart rate monitor were promptly removed. The participant was then given privacy to measure body mass wearing only cycling bib-shorts after having thoroughly towel dried any excess sweat from their person. Sweat rate ($L \cdot h^{-1}$) was determined by the difference in body weight measured before and after the test, but not for respiratory fluid loss and weight loss due to fuel oxidation, which would be negligible (Baker et al., 2009; Maughan et al., 2007). Subsequently, the participants were given one hour to passively relax at room temperature (~20°C) and monitored to ensure compliance. This recovery time has been demonstrated to be adequate to allow body core temperature to return to resting levels after cycling for one hour at 60% of $\dot{V}O_{2max}$ at 32°C (Naperalsky et al., 2010) and as applied in research by Zurawlew et al. (2016). During the recovery period, the

participants were asked to drink an amount of water equal to 150% of their body mass loss during the heat stress test, as recommended by the current hydration guidelines (McDermott et al., 2008).

3.6. Time Trial Test

The 20 km time trial test was completed in a climatic chamber and preceded by a warm-up protocol consisting of cycling for 10 minutes on an electronically-braked cycle ergometer (Velotron, RacerMate Inc., Seattle, USA). The warm-up protocol involved an initial intensity corresponding to 40% of maximal aerobic power, as determined during the GXT, with 4 x 20 second accelerations at 60% maximal aerobic power, at the beginning of the 4th, 5th, 6th and 7th minutes (Ihsan et al., 2010). The participants were then instructed to ride the 20 km time trial as fast as possible in conditions of 33°C and 40% RH with no encouragement provided. These thermal conditions are in line with those used in prior research; Garrett et al. (2019) used 31°C and 50% RH in an intermittent interval heat stress test with females, Kirby et al. (2019) used 33°C and 33% RH in a 15 min capacity test with females, and Wingfield et al. (2016) used 33°C and 60% RH in a 20 km performance test with recreational males. In line with the research methodology of previous HA studies in cyclists (Keiser et al., 2015; Racinais et al., 2015b), during the time trial the participants had access to power output, speed, heart rate and distance data. Participants were able to adjust the power output throughout the test by changing gears as well as modifying cadence. Upon reaching 20 kms, the time to complete the test (s) and mean power (W) were recorded.

3.7. Training Sessions

HA sessions were completed in a climatic chamber at 40°C and 40% RH cycling on an electronically-braked cycle ergometer (Velotron, RacerMate Inc., Seattle, USA). During the sessions, exercise intensity was first set at a power corresponding to the participant's power at 65% of their $\dot{V}O_{2max}$ for 15 minutes, followed by 95 minutes using the controlled heart rate method. This involved adjusting power to maintain a heart rate equivalent to the participant's heart rate at 65% of their $\dot{V}O_{2max}$, as calculated using linear regression from the heart rate to $\dot{V}O_2$ relationship determined during their GXT (Périard et al., 2021; Travers., 2020). Since hypohydration may act as an independent stimulus for HA by enhancing plasma volume expansion (Garrett et al. 2014), the same steps as before the heat stress test were taken to ensure that the participants commence each HA session in a euhydrated state. To remain euhydrated during the sessions, the participants were instructed to drink 150 ml every 15 min throughout the sessions. This drinking pattern has been shown to maintain euhydration during HA sessions (Garrett et al., 2014).

Prior to and post training sessions, participants had their body mass measured to determine required post training session fluid intake as 150% of change in body mass. Further, participants were advised of carbohydrate intake requirements utilising work (kJ) as calculated from power data obtained during the training session and in line with current sports nutrition guidelines to consume 5-8 g/kg/day for the given session and testing workload (Kerksick et al., 2018).

3.8. Statistical Analysis

Statistical analyses were conducted using R Studio and R base (versions 2024.12.0 and 4.4.2 respectively). Initially, the data were analysed for outliers using box and whisker plots before testing for normality of distribution using the Shapiro-Wilk test and Q-Q plots. Data were further tested for equality of variance using Levene's test. For performance and physiological dependent variables where residuals were normally distributed two-way mixed model ANOVA tests were applied using the rstatix package. Where residuals were non-parametric or the dependent variable was ordinal, robust mixed method tests were applied using the WRS2 package while training sessions were analysed using paired t-tests. Where a result of significant main effects or interactions occurred, pairwise Bonferroni-corrected post-hoc analysis was applied. The statistical significance level was set at $p < 0.05$. In addition, within-group effect sizes were calculated and reported for dependent variables to determine the magnitude of observed effects, with paired t-tests calculated according to Dankel and Loenneke. (2021). Effect size having been interpreted using the following thresholds trivial (<0.2), small (0.2-0.59), moderate (0.6-1.19), large (1.2-1.99), and very large (>2.0) (Batterham & Hopkins, 2006). Independent two-sample t-tests were applied to examine percentage performance improvement between sexes. Smallest worthwhile change for noted variables are; reduction in resting core temperature (−0.18 °C), reduction in peak core temperature (−0.31 °C), reduction in peak skin temperature (−0.57 °C), reduced resting HR (−6 beats·min^{-1}), reduced peak HR (−12 beats·min^{-1}), plasma volume expansion (4.3%), increase in sweat rate (38%), reduction in thermal sensation (−0.9), and reduction in rate of perceived effort (−1) (Tyler et al., 2016). Unless otherwise stated, descriptive data are presented as mean ± standard deviation and 95% confidence intervals. Exact p values are reported unless $p < 0.001$.

4. Results

4.1. 20 km time trial performance test

Table 3 presents measures pre- and post-intervention during the 20km time trial performance tests. Figure 2 displays individual and mean responses for power and duration during pre- and post-intervention 20 km time trial performance tests

Performance measures within groups

Analysis of mean power output during the 20 km time trial test at 33°C and 40% R.H. revealed a significant main effect for sex ($F_{(1, 14)} = 21.956$, $p < 0.001$), time ($F_{(1, 14)} = 60.910$, $p < 0.001$), and also for sex X time interaction ($F_{(1, 14)} = 8.481$, $p = 0.011$). Post-hoc analysis revealed a statistically significant increase in power of 4.9% ± 7.1% pre- to post-intervention for the female group ($p < 0.001$) with trivial within-group effect size (0.197), and also a significant increase in power of 6.7% ± 10.0% for the male group ($p < 0.001$) with small within-group effect size (0.387).

Analysis of duration during the 20 km time trial test at 33°C and 40% R.H. revealed a significant main effect for sex ($F_{(1, 14)} = 12.379$, $p = 0.003$), and also for time ($F_{(1, 14)} = 45.325$, $p < 0.001$), however not for sex X time interaction ($F_{(1, 14)} = 0.082$, $p = 0.778$). Post-hoc analysis revealed a statistically significant reduction in duration of 2.0% ± 3.3% pre- to post-intervention for the female group ($p = 0.004$) with trivial within-group effect size (0.151), and also a significant reduction in duration of 2.6% ± 4.0% for the male group ($p = 0.001$) with small within-group effect size (0.423).

Performance changes between groups

Analysis of the mean change in power pre- to post-intervention during the 20 km time trial test at 33°C and 40% R.H. between groups did not reveal a significant difference between sexes ($t_{(8)} = 0.828$, $p = 0.422$), with small effect size (0.418) in favour of males. Also, analysis of the mean change in duration pre- to post-intervention during the 20 km time trial test at 33°C and 40% R.H. between groups did not reveal a significant difference between sexes ($t_{(8)} = -1.148$, $p = 0.270$), with small effect size (0.569) in favour of males.

Table 3. Performance measures pre- and post-intervention during the 20km time trial performance tests. Data expressed as mean ± SD (95% CI), effect size (95% CI).

	Females				Males				ANOVA *p* values		
	Pre-Intervention	Post-Intervention	Change	Within-group ES	Pre-Intervention	Post-Intervention	Change	Within-group ES	Sex	Time	S x T
Power (W)	161.4 ± 41.9	169.2 ± 40.4	7.9 ± 3.6* (4.9, 10.9)	0.197 (-0.79, 1.17)	257.6 ± 45.2	274.9 ± 45.1	17.3 ± 8.4* (10.3, 24.2)	0.387 (-0.61, 1.37)	<0.001	<0.001	0.011
Duration (s)	2,419 ± 338	2,371 ± 314	-47.8 ± 31.9* (-74.4, -21.2)	0.151 (-0.83, 1.13)	1,987 ± 128	1,935 ± 120	-52.0 ± 27.2* (-74.8, -29.2)	0.423 (-0.57, 1.41)	0.003	<0.001	0.778

Abbreviations: W = watts, s = seconds, ES = effect size. * Significant within group difference p ≤ 0.05.

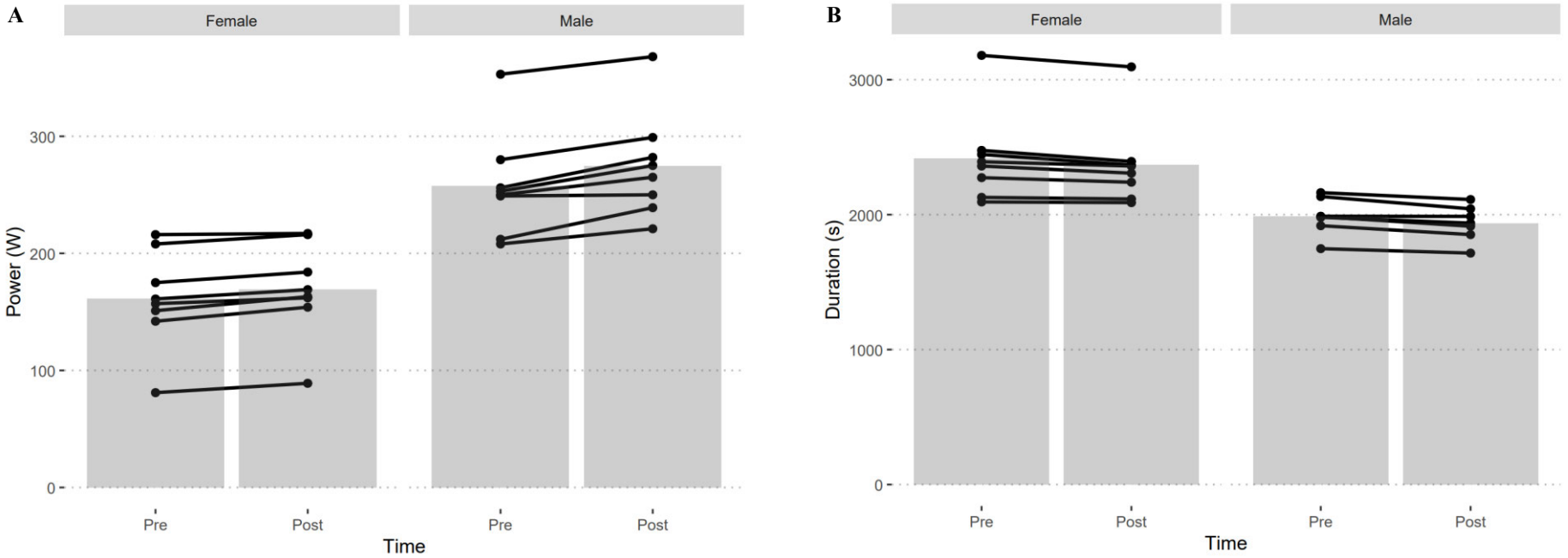

Figure 2. 20 km time trial test individual data points (black lines) and mean group values (grey bars) pre- and post-intervention; **A**: mean power (W) output, and **B**: mean duration (s) to complete.

4.2. 30-minute continuous heat stress test

Physiological measures

Table 4 presents a summary of the physiological and perceptual measures pre- and post-intervention during the 30 minute continuous heat stress test in conditions of 40°C and 40% R.H. by each group, with Figure 3 displaying group responses across pre- and post-intervention heat stress tests, and Figure 4 displaying individual comparisons between pre- and post-intervention heat stress tests.

Resting HR showed a significant main effect for sex ($F_{(1, 14)}$ = 4.726, p = 0.049), with no significant main effect for time ($F_{(1, 14)}$ = 0.413, p = 0.531) or sex X time interaction ($F_{(1, 14)}$ = 0.060, p = 0.810). Post-hoc analysis; however, revealed no statistically significant difference between groups pre- (p = 0.094) or post-intervention (p = 0.083), though a small effect size was determined for males (0.248).

Peak HR showed a significant main effect for sex ($F_{(1, 14)}$ = 7.751, p = 0.015) and also time ($F_{(1, 14)}$ = 28.683, p < 0.001), however, not for sex X time interaction ($F_{(1, 14)}$ = 3.121, p = 0.099). Post-hoc analysis revealed a statistically significant reduction in pre- to post-intervention peak HR for the female group (p = 0.001) with moderate within-group effect size (1.020) and greater than the smallest worthwhile change (-15.9 beats·min^{-1} vs −12 beats·min^{-1}), with males showing a tendency towards significant improvement (p = 0.053) and moderate within-group effect size (0.662).

Resting core temperature showed a significant main effect for sex ($F_{(1, 14)}$ = 5.002, p = 0.042), with no significant main effect for time ($F_{(1, 14)}$ = 0.303, p = 0.591) or sex X time interaction ($F_{(1, 14)}$ = 0.032, p = 0.861). Post-hoc analysis; however, revealed no statistically significant difference between groups pre- (p = 0.092) or post-intervention (p = 0.068), though a small effect size was found for females (0.290).

Peak core temperature showed a significant main effect for sex ($F_{(1, 14)}$ = 9.223, p = 0.010) and also time ($F_{(1, 14)}$ = 25.065, p < 0.000), however, not for sex X time interaction ($F_{(1, 14)}$ = 0.073, p = 0.791). Post-hoc analysis revealed a statistically significant reduction in pre- to post-intervention peak core temperature for the female group (p = 0.002) with moderate within-group effect size (1.120) and greater than the smallest worthwhile change (-0.33°C vs -0.31°C), and also for the male group (p = 0.024) with moderate within-group effect size (0.865) which was close to the smallest worthwhile change (-0.30°C vs -0.31°C).

Peak skin temperature showed a significant main effect for time ($F_{(1, 14)}$ = 7.094, p = 0.019), however, not for sex ($F_{(1, 14)}$ = 0.024, p = 0.879) or for sex X time interaction ($F_{(1, 14)}$ = 0.215, p = 0.650). Post-hoc analysis; however, did not show a statistically significant change in pre- to

post-intervention peak skin temperature for either the female group (p = 0.102) with moderate within-group effect size (0.891), or for the male group (p = 0.089) with small within-group effect size (0.555).

Plasma volume showed no significant main effect for sex ($F_{(1, 14)}$ = 2.848, p = 0.114), for time ($F_{(1, 14)}$ = 0.848, p = 0.373) or for sex X time interaction ($F_{(1, 14)}$ = 0.647, p = 0.435), though the male group did show a moderate effect size (0.729).

Sweat loss showed a significant main effect for sex ($F_{(1, 14)}$ = 11.511, p = 0.004), however, not for time ($F_{(1, 14)}$ = 0.008, p = 0.928) or for sex X time interaction ($F_{(1, 14)}$ = 0.272, p = 0.610). Post-hoc analysis revealed a statistically significant difference between groups pre- (p = 0.012) and post-intervention (p = 0.005).

Perceptual measures

Thermal sensation showed a significant main effect for time ($F_{(1, 14)}$ = 31.405, p < 0.001), however not for sex ($F_{(1, 14)}$ = 2.317, p = 0.171) or for sex X time interaction ($F_{(1, 14)}$ = 1.449, p = 0.258). Post-hoc analysis revealed a statistically significant improvement in pre- to post-intervention thermal sensation for the female group reducing from hot to warm (p = 0.003) with moderate within-group effect size (1.130) and greater than the smallest worthwhile change (0.91 vs 0.9), and also for the male group reducing from hot to warm (p = 0.003) with large within-group effect size (1.400).

Thermal comfort showed a significant main effect for time ($F_{(1, 14)}$ = 13.863, p = 0.005), however, not for sex ($F_{(1, 14)}$ = 0.192, p = 0.674) or for sex X time interaction ($F_{(1, 14)}$ = 1.385, p = 0.270). Post-hoc analysis revealed a statistically significant improvement in pre- to post-intervention thermal comfort for the female group reducing from slightly uncomfortable towards neutral (p = 0.049) with small within-group effect size (0.329), however, not for the male group (p = 0.063) with moderate within-group effect size (0.671).

RPE showed a significant main effect for time ($F_{(1, 14)}$ = 8.984, p = 0.010), however not for sex ($F_{(1, 14)}$ = 0.312, p = 0.586) or for sex X time interaction ($F_{(1, 14)}$ = 0.532, p = 0.479). Post-hoc analysis revealed a statistically significant improvement in pre- to post-intervention RPE for the female group reducing from light towards very light (p = 0.020) with moderate within-group effect size (0.842) and greater than the smallest worthwhile change (-1.1 vs -1), however, not for the male group (p = 0.188) with small within-group effect size (0.492).

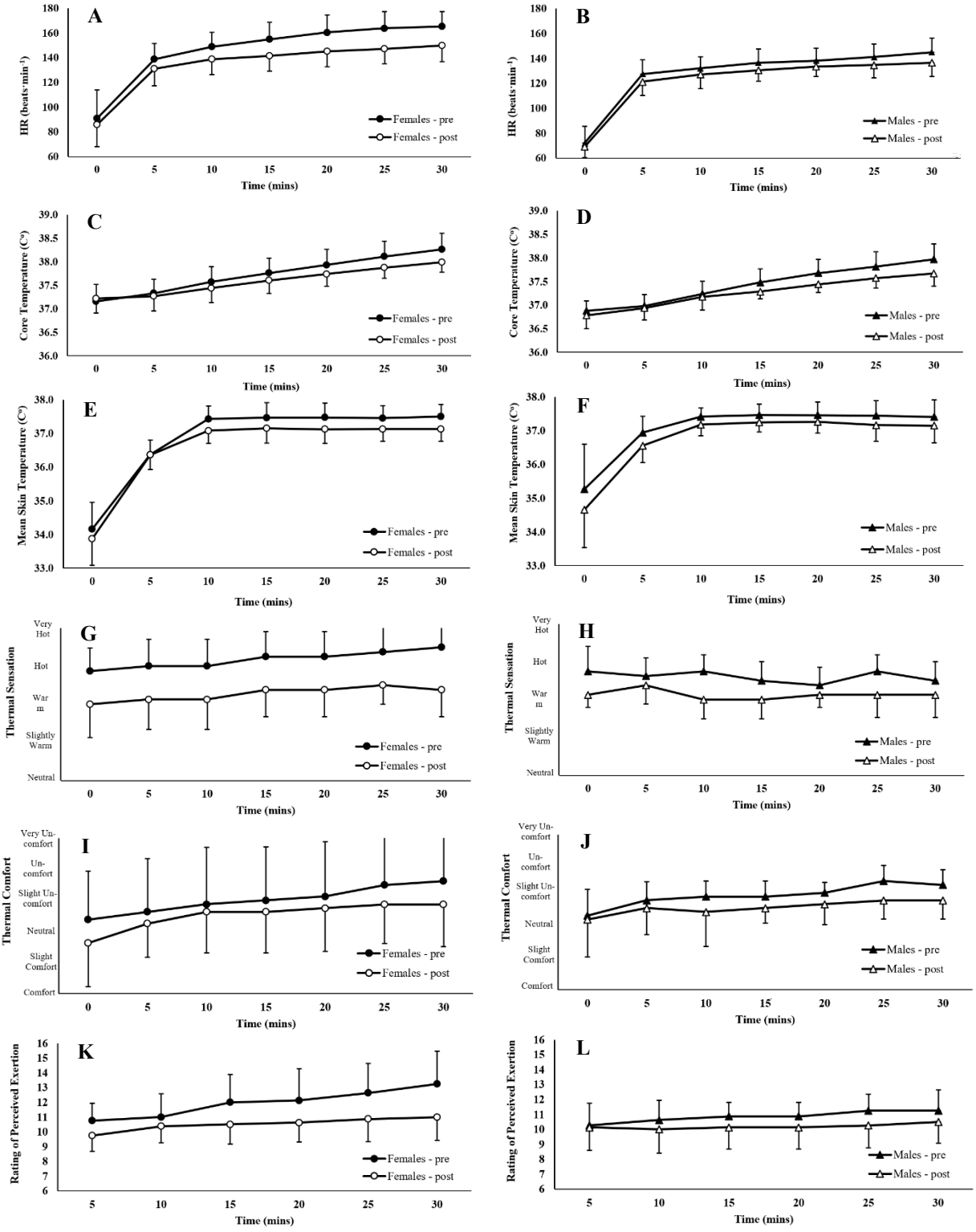

Figure 3. Group responses across pre- and post-intervention heat stress tests; **A**: Female HR (beats·min^{-1}), **B**: Male HR (beats·min^{-1}), **C**: Female core temperature (°C), **D**: Male core temperature (°C) **E**: Female mean skin temperature (°C), **F**: Male mean skin temperature (°C), **G**: Female thermal sensation, **H**: Male thermal sensation, **I**: Female thermal comfort, **J**: Male thermal comfort, **K**: Female rating of perceived exertion, and **L**: Male rating of perceived exertion. Data expressed as mean ± standard deviation.

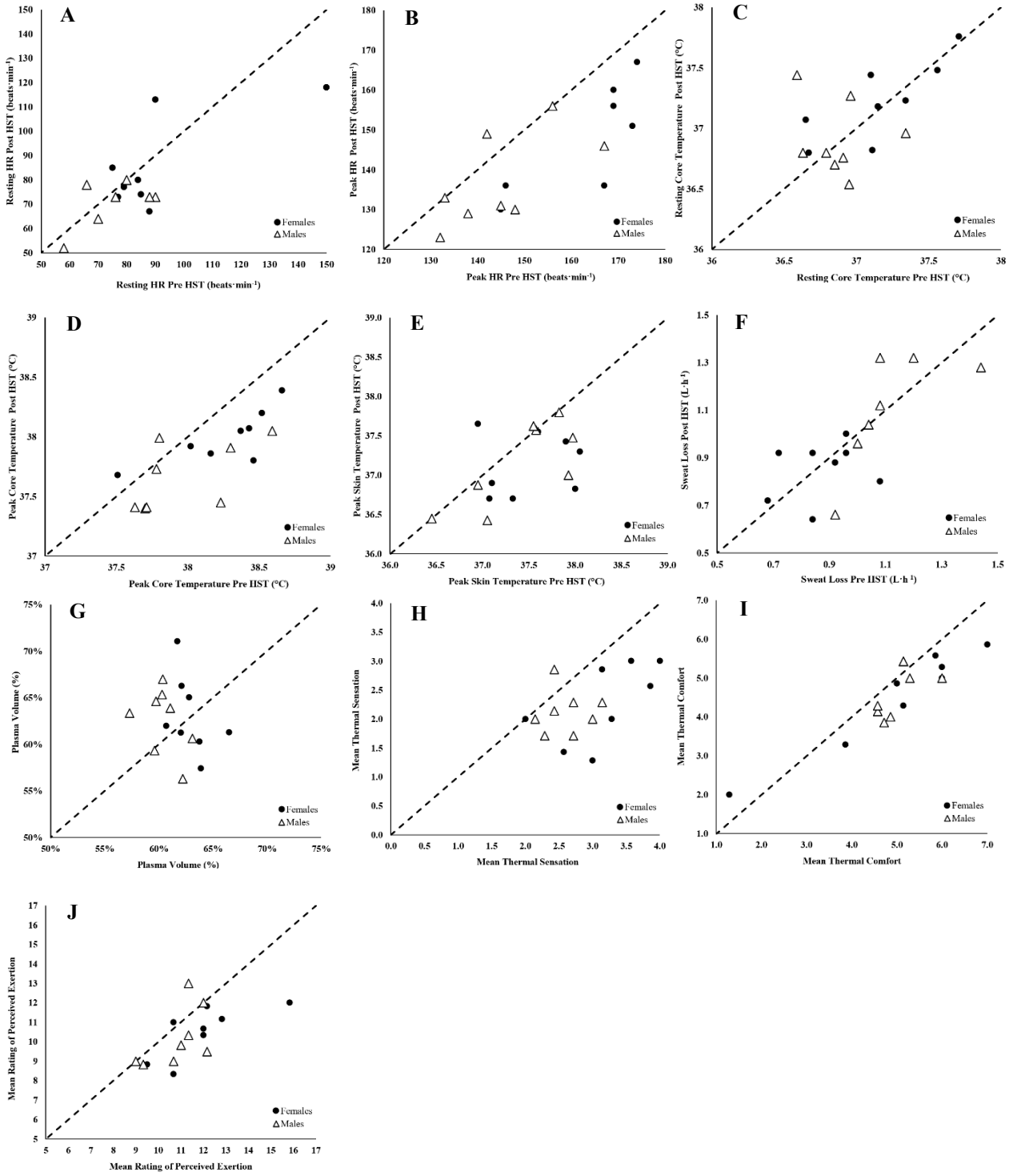

Figure 4. Individual comparison between pre- and post-intervention heat stress tests; **A**: Resting HR (beats·min^{-1}), **B**: Peak HR (beats·min^{-1}), **C**: Resting core temperature (°C), **D**: Peak core temperature (°C), **E**: Peak skin temperature (°C), **F**: Sweat loss (L·h^{-1}), **G**: Plasma Volume (%), **H**: Mean thermal sensation, **I**: Mean thermal comfort, and **J**: Mean rating of perceived exertion. Dotted line represents line of equality.

Table 4. Physiological and perceptual measures pre- and post-intervention during the 30 minute continuous heat stress test across sex. Data expressed as mean ± SD (95% CI), effect size (95% CI).

	Females				Males				ANOVA *p* values		
	Pre-Intervention	Post-Intervention	Change	Within-group ES	Pre-Intervention	Post-Intervention	Change	Within-group ES	Sex	Time	S x T
Resting HR (beats·min^{-1})	82.6 ± 5.7	81.3 ± 15.1	-1.3 ± 14.2 (-14.4, 11.9)	0.177 (0.87, 1.10)	72.1 ± 14.3	69.2 ± 9.5	-2.9 ± 10.8 (-11.9, 6.6)	0.248 (-0.75, 1.22)	0.049	0.531	0.810
Peak HR (beats·min^{-1})	165.8 ± 13.4	149.9 ± 14.1	-15.9 ± 8.0* (-22.6, -9.2)	1.020 (0.10, 2.22)	145.1 ± 11.9	137.1 ± 11.6	-8.0 ± 9.7 (-16.1, 0.1)	0.662 (-0.33, 1.69)	0.015	<0.001	0.099
Resting CT (°C)	37.16 ± 0.38	37.22 ± 0.33	0.06 ± 0.23 (-0.14, 0.26)	0.290 (-0.81, 1.15)	36.88 ± 0.23	36.91 ± 0.30	0.03 ± 0.41 (-0.32, 0.38)	0.000 (-0.87, 1.10)	0.042	0.591	0.861
Peak CT (°C)	38.37 ± 0.22	38.04 ± 0.21	-0.33 ± 0.17* (-0.49, -0.18)	1.120 (0.42, 2.65)	37.97 ± 0.36	37.67 ± 0.28	-0.30 ± 0.29* (-0.55, -0.05)	0.865 (-0.10, 1.96)	0.010	<0.001	0.791
Peak ST (°C)	37.50 ± 0.45	37.13 ± 0.39	-0.37 ± 0.55 (-0.83, 0.09)	0.891 (-0.15, 1.90)	37.41 ± 0.5	37.15 ± 0.54	-0.26 ± 0.37 (-0.57, 0.05)	0.555 (-0.50, 1.50)	0.879	0.019	0.650
Plasma Vol (%)	62.9 ± 1.8	63.1 ± 4.2	0.1 ± 5.3 (-4.3, 4.5)	0.046 (-0.92, 1.04)	60.5 ± 1.8	62.6 ± 3.5	2.1 ± 4.5 (-1.7, 5.9)	0.729 (-0.26, 1.77)	0.114	0.373	0.435
Sweat Loss (L·h^{-1})	0.88 ± 0.13	0.85 ± 0.12	-0.03 ± 0.15 (-0.15, 0.10)	0.203 (-0.74, 1.22)	1.14 ± 0.18	1.16 ± 0.28	0.02 ± 0.17 (-0.13, 0.16)	0.076 (-0.90, 1.07)	0.004	0.928	0.610
Thermal Sensation	3.18 ± 0.67	2.27 ± 0.69	-0.91 ± 0.58* (-1.39, -0.43)	1.130 (0.25, 2.42)	2.63 ± 0.37	2.02 ± 0.24	-0.61 ± 0.35* (-0.93, -0.29)	1.400 (0.77, 3.15)	0.171	<0.001	0.258
Thermal Comfort	5.02 ± 1.77	4.52 ± 1.27	-0.50 ± 0.59* (-1.00, -0.00)	0.329 (-0.66, 1.31)	5.04 ± 0.48	4.64 ± 0.64	-0.39 ± 0.50 (-0.81, 0.03)	0.671 (-0.30, 1.72)	0.674	0.005	0.270
RPE	11.41 ± 1.16	10.31 ± 1.28	-1.10 ± 0.92* (-1.94, -0.25)	0.842 (-0.13, 1.93)	10.85 ± 1.15	10.19 ± 1.53	-0.67 ± 1.30 (-1.75, 0.42)	0.492 (-0.51, 1.48)	0.586	0.010	0.479

Abbreviations: HR = heart rate, ST = skin temperature, CT = core temperature, Vol = volume, RPE = rating of perceived exertion, ES = effect size. * Significant within group difference $p \leq 0.05$.

4.3. Training sessions

All sixteen participants were able to complete each 110 minute training session in 40°C and 40% R.H. across the five-day intervention. Figure 5 displays mean power across first and last session by sex and Table 5 presents a summary of measures and changes from the first to last session. There were significant differences for females at timepoints from 30 minutes to 90 minutes, and for males from 45 minutes to 110 minutes. Comparison of mean power responses to the first and final training sessions also showed significant between-session differences for both females 14.8% ± 11.6% (5.1%, 24.4%) ($t_{(8)}$ = 2.930, p = 0.022), and males 11.2% ± 6.2% (6.1%, 16.4%) ($t_{(8)}$ = 4.161, p = 0.004). Mean tympanic temperature responses to the first and final training sessions from the 45 minute to 110 minute timepoints reduced significantly for females -0.5% ± 0.5% (-0.9%, -0.1%) ($t_{(8)}$ = -2.901, p = 0.023), however not for males 0.8% ± 2.1% (-1.0%, 2.5%) ($t_{(8)}$ = 1.035, p = 0.335). Sweat loss responses to the first and final training sessions increased significantly for females 16.9% ± 17.6% (2.2%, 31.6%) ($t_{(8)}$ = 2.594, p = 0.038), however not for males 10.0% ± 10.8% (0.9%, 19.0%) ($t_{(8)}$ = 2.361, p = 0.051).

The HA sessions resulted in a total duration of 550 mins (5 x 110 mins) and a total heat dose of 22,000°C ·min (40°C x 550 mins). The mean total energy expenditure for the female group was 15,051 ± 4,404 kJ (91.2 ± 26.7 W x 550 mins with 20% efficiency), and the mean rate of energy expenditure was 27.4 ± 8.0 kJ·min^{-1}. While mean total energy expenditure for the male group was 24,989 ± 5,616 kJ (151.5 ± 34.0 W x 550 mins with 20% efficiency), and the mean rate of energy expenditure was 45.4 ± 10.2 kJ·min^{-1}.

Table 5. First and last training session measures. Data expressed as mean ± SD (95% CI).

	Females			Males		
	Session 1	Session 5	Difference	Session 1	Session 5	Difference
Power (W)	87.6 ± 25.6 (64.7, 110.5)	100.3 ± 27.9 (75.4, 125.2)	12.6 ± 12.2* (2.4, 22.8)	144.0 ± 31.0 (116.3, 171.7)	160.9 ± 34.7 (129.8, 192.0)	16.9 ± 8.6* (9.3, 24.5)
Mean HR (beats·min^{-1})	151.6 ± 12.5 (140.4, 162.8)	144.8 ± 14.8 (131.5, 158.1)	6.9 ± 5.4* (2.1, 11.7)	143.6 ± 13.4 (131.6, 155.6)	141.9 ± 13.2 (130.1, 153.7)	1.8 ± 2.9 (-0.9, 4.4)
Mean TT (°C)	38.33 ± 0.23 (38.10, 38.56)	38.18 ± 0.33 (37.85, 38.51)	-0.15 ± 0.15* (-0.30, 0.00)	38.32 ± 0.56 (37.82, 38.82)	38.35 ± 0.48 (37.92, 38.78)	0.03 ± 0.39 (-0.32, 0.38)
Sweat Loss (L·h^{-1})	0.78 ± 0.12 (0.55, 1.01)	0.92 ± 0.19 (0.59, 1.25)	0.13 ± 0.10* (-0.02, 0.28)	1.28 ± 0.25 (0.78, 1.78)	1.42 ± 0.34 (0.99, 1.85)	0.13 ± 0.15* (-0.22, 0.48)

Abbreviations: W = watts, HR = heart rate, TT = tympanic temperature, L·h^{-1} = litres per hour. * Significant difference for group between session 1 and 5, p ≤ 0.05.

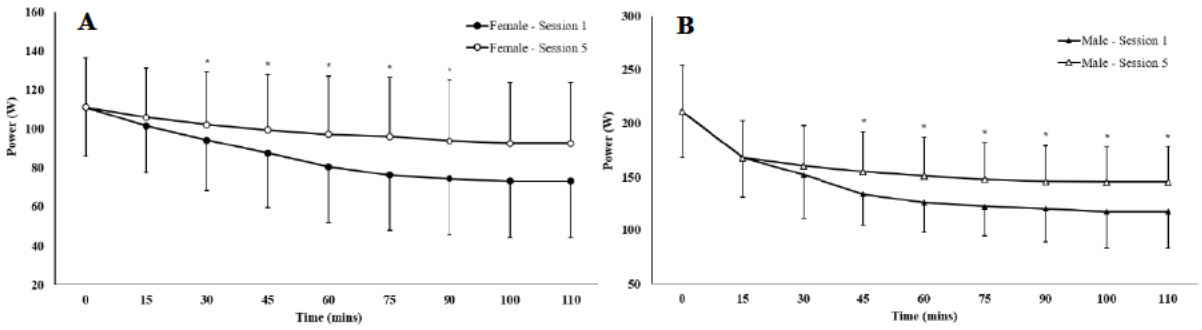

Figure 5. Mean power measured for each group during the first and last sessions; **A**: Females, **B**: Males. Measurements were taken at the start where participants started at the same power for every session, then every 15 minutes until 90 minutes, then at 100 minutes and at end of 110 minutes. Data expressed as mean ± standard deviation. * Significant difference at timepoint for group between sessions, $p \leq 0.05$.

5. Discussion

The current study examined the effects on physiological, perceptual and 20 km time trial performance outcomes on non-heat acclimatised tier-two trained female and male cyclists, enduring five consecutive days of heat acclimation sessions using a controlled heart rate model. The main findings are that while both groups improved performance and markers of HA, males tended towards a greater performance improvement while females showed a more complete improvement in markers of HA. The protocol also led to both groups progressively increasing intensity and sweat rate across sessions. These findings being relevant for athletes wanting to improve performance in the heat through use of a heart-rate based HA protocol.

5.1 Performance measures

The intervention proved effective, with both females and males significantly increasing power output and reducing time trial duration to a similar extent. Females saw an improvement in power of 4.9 ± 7.1% and a reduction in duration of 2.0% ± 3.3%, with improvements of 6.7 ± 10.0% and 2.6% ± 4.0%, respectively, for males. Mechanisms for the improvement in performance, which will be discussed in more detail across the following sections for the measured markers of HA, are likely driven by expansion of plasma volume providing cardiovascular stability and an ability to maintain stroke volume to direct blood flow to the working muscles, an increase in sweat rate through cutaneous vasodilation and reduction in onset threshold of sweating to attenuate the rise in core temperature through evaporative cooling, and maintaining a core to skin temperature gradient to promote heat exchange via

convection where the ambient temperature is less than skin temperature (Ely et al., 2009; Cuddy et al., 2014; Periard et al., 2012; Taylor, 2014).

Findings were in line with that found by Stephenson et al. (2019) of a 4.5% increase in power output across a mixed cohort with similar fitness levels (61.5 ± 6.4 $ml \cdot kg^{-1} \cdot min^{-1}$) using a controlled heart rate protocol. The results in the current study exceeded the improvement in mean power output found by Kirby et al. (2019) of 2.8% in females at four days, potentially due to the additional day of heat acclimation, longer session duration and higher humidity during sessions in the current study. The improvements seen in the current study were not to the same extent as that in research by Wingfield et al. (2016), who using a fixed intensity STHA protocol, found a 23 ± 35% improvement in mean power output and a 5.9 ± 7.1% reduction in duration for males in the low intensity group. Reasons for the considerable improvement seen in the research by Wingfield et al. (2016) compared to the current study may be related to the greater capacity for improvement due to a lower level of participant fitness (Cheung et al., 1998). As higher trained individuals already tolerate high core temperatures better from physiological responses to thermal strain exposure during training (Périard et al., 2012), and therefore, may see less decrement to performance in pre-intervention testing. The mean participant $\dot{V}O_{2max}$ in that study of 44.3 ± 6.0 $ml \cdot kg^{-1} \cdot min^{-1}$ was much lower compared to 61.13 ± 9.24 $ml \cdot kg^{-1} \cdot min^{-1}$ in the current study, and likely manifested lower pre-intervention time trial mean power output of 124 ± 32 W and completion time of 44.68 minutes compared to mean power output of 257.6 ± 45.2 W and duration of 33.12 minutes in the current study for males. Similarly, the greater reduction in time trial duration of 5.5% seen in research by Guy et al. (2016) at four days of HA compared to the current study, may also be due to the lower fitness level of male participants in that research (45.0 ± 5.0 $ml \cdot kg^{-1} \cdot min^{-1}$) providing for greater capacity for improvement.

Experimental design factors may have contributed to differences across research regarding the decrement experienced in pre-intervention testing. For example, while mean power data was not published to compare intensity, the time trial test employed by Guy et al. (2016) had a higher wet bulb temperature of 30.3°C (35°C and 70% RH) when compared to the wet bulb temperature for the test in the current study of 22.9°C. Moreover, with the time trial test also preceded by 40 minutes of progressive sub maximal exertion in the same conditions. These factors may have resulted in a greater decrement to performance in pre-intervention testing that was better mitigated after HA in post testing.

The reduction in duration above the coefficient of variation of the test (1.0%) when applied to well-trained cyclists (Borg et al., 2018) means that performance improvement can be attributed

to the heat acclimation sessions with the magnitude of change large enough to be considered worthwhile to improve results competitive road time trials (Paton & Hopkins, 2006), however, not to the more recent worthwhile change identified in (Borg et al., 2018). This improvement would be highly advantageous for athletes competing in hot conditions who are wanting to lessen the decrement experienced to performance from travelling to compete in hot environments (Racinais et al., 2015b; Voltaire et al., 2002) with improvement to performance from HA being greater in hot environments than temperate (Lorenzo et al., 2010).

While there were no significant differences between groups regarding the improvements in performance, there were, nonetheless, small effect sizes in favour of males for both power output (0.418) and duration (0.569). The male group alone displayed a tendency towards significant plasma volume expansion and this may explain their greater improvement in performance. Improved cardiovascular stability through plasma volume expansion limits the reduction in stroke volume caused by a decline in venous pressure from increased blood flow to the periphery during exposure to the heat, with cardiac output subsequently maintained to deliver oxygen to working skeletal muscle (Nybo et al., 2014). An additional factor may have been the lower resting and peak core temperature seen in the male group during the heat stress tests. Starting at a lower core temperature than the female group may have delayed the effect of central fatigue to maintain muscle activation during the prolonged exercise of the time trial and provided greater capacity (González-Alonso et al., 1999). Other markers of HA expected to improve performance such as a reduction in peak skin temperature leading to a greater core to skin gradient (Périard et al., 2015) and improved thermal comfort promoting volitional behaviour with regard to exercise performance (Flouris & Schlader., 2015; Tyler et al., 2016) were similarly reduced for both groups.

5.2 Physiological & perceptual measures

The physiological changes found to elicit heat acclimation manifest primarily in a lower heart rate, lower core temperature, lower skin temperature, higher sweat rate, and plasma volume expansion compared to an un-acclimatised person (Sawka et al., 2011). Carrying on from the main findings, the intervention successfully induced HA as evidenced by significant changes to peak HR, peak core temperature, thermal sensation, thermal comfort and rating of perceived exertion during heat stress tests pre- to post-intervention. Between the sex groups; peak HR reduced for females only, peak core temperature reduced for both females and males, thermal sensation was improved for both females and males, thermal comfort was improved for females while males had a tendency to see improvement, rating of perceived

exertion reduced for females, and males had a tendency towards significant plasma volume expansion.

Cardiovascular measures

Cardiovascular changes are early responses to a successful HA and are expected within seven days, with a meaningful change to exercising and resting heart rate considered to be a reduction of 12 and 6 beats·min^{-1}, respectively (Périard et al., 2015; Tyler et al., 2016). After only five days of HA in the current study, males experienced a 5.5 ± 12.2% reduction in exercising heart rate (8.0 ± 9.7 beats·min^{-1}) and 4.0 ± 19.0% reduction to resting heart rate (2.9 ± 10.8 beats·min^{-1}), both not significant though exercising heart rate was tending towards a significant change ($p = 0.053$). Conversely, females experienced a 9.6 ± 14.4% reduction in exercising heart rate (15.9 ± 8.0 beats·min^{-1}) , which is considered to be meaningful, and 1.6 ± 19.0% reduction to resting heart rate (1.3 ± 14.2 beats·min^{-1}), with only the change to exercising heart rate significant. Comparable STHA for males saw a significant 7 - 13 beats·min^{-1} reduction to exercising heart rate and significant 5 beats·min^{-1} reduction to resting (Garrett et al., 2009; Neal et al., 2016; Patterson et al., 2004a). The absolute reduction for males in exercising heart rate was within the range of comparable studies and was close to significance, however, resting heart rate was slightly below comparable studies and may have required another HA session with Periard et al. (2015) suggesting full heart rate adaptation occurs at 7 days. In comparable STHA research in females exercising heart rate was reduced by 4 - 13 beats·min^{-1} and resting heart rate was reduced by 5 - 13 beats·min^{-1} (Garrett et al., 2019; Mee et al., 2015; Mee et al., 2018). Changes to exercising heart rate in the current study were at the top of this range and higher than both the sauna and temperate conditions of 4 and 10 beats·min^{-1}, respectively, in Mee et al. (2018). The lack of significant change to resting heart rate is not uncommon in female STHA outside of additional treatments such as sauna exposure pre-HA session and may require MTHA in females to see significant change (Kirby et al., 2019; Mee et al., 2015; Mee et al., 2018; Wickham et al., 2021). Further, changes in resting heart rate may have been impacted by hormonal variances during menstrual phase timing which can see small increases in the luteal phase (Moran et al., 2000; Teixeira, et al., 2012). Three female participants who were assessed as completing post-intervention testing in their mid or late luteal phase all had increased post-intervention resting heart rate. Ranging from 2 – 23 beats·min^{-1}, with the highest being in mid luteal. It is likely this was driven by hormonal influences, given all three experienced reduced post-intervention peak heart rates. Further, the magnitude of heart rate change from resting to peak was less post-intervention (38 – 59 beats·min^{-1}) than pre-intervention (70 – 88 beats·min^{-1}), indicating for the same intensity and conditions the participants had greater cardiac stability. While changes to resting heart rate trended towards improvement for both groups, the lack of significant change may

have been impacted by the method of measurement. Resting heart rate was recorded in the heat chamber at 5 minutes of rest ahead of the commencement of heat stress test, which may have caused elevation due to nervousness regarding final time trial test (Ayuso-Moreno et al., 2020). Also the lack of significant plasma volume expansion which effects a lower heart rate through increased stroke volume (Tyler et al., 2016), may have limited the size of change.

Plasma volume has been reported to increase markedly within the first five days of HA, with the magnitude of expansion varying by heat acclimation model employed (Périard et al., 2015). Plasma volume expansion in the current study was not significant in either males (3.5 ± 10.9%) or females (0.2 ± 8.6%). While the plasma volume expansion for males approached significance ($p = 0.068$) it was less than that reported across studies of 4.3 ± 4.7% irrespective of length (Tyler et al., 2016). Findings that intensity of the thermal strain drives the magnitude of plasma volume expansion (Senay et al., 1980), it is interesting to note that Travers et al. (2020) in applying the same controlled heart rate model also did not find a significant plasma volume expansion at either five days (4%) or after ten. This is despite the protocol allowing workload to increase each session minimising contraction of plasma volume that may be seen in fixed intensity models (Patterson et al., 2004a). STHA using controlled hyperthermia in conditions of greater relative humidity (60% RH vs 40% RH) yet same temperature to the current study were able to achieve plasma volume expansion of between 4.5 - 10.9% with both also seeing significant increase in sweat rate in contrast to the current study (Garrett et al., 2012; Patterson et al., 2004b). While other STHA research involving males with conditions of 35°C and 60% RH and 40°C and 50% RH, respectively, saw non-significant plasma volume changes of 1.2 - 4.2% accompanied by no significant change in sweat rate (Garrett et al., 2009; Neal et al., 2015). It may be the additional thermal impulse of higher humidity with high temperature is required to see significant changes using the controlled heart rate model. The lack of change in plasma volume for females is in contrast to changes in other STHA studies with females of 7.5 – 9.5% (Garrett et al., 2019; Mee et al., 2018; Pethick et al., 2017) though similar to that in Campbell et al. (2022). However, our results may not be completely unexpected given a recent meta-analysis by Kelly et al. (2023) found no significant change in plasma volume for females, which the authors concluded may be due to the small number of studies including a measure of PV that met inclusion criteria and the impact of the menstrual cycle on fluid shifts (Aguree et al., 2020). Regarding fluid shifts; in the current study three of the female participants may have seen a lack of plasma volume expansion due to post-intervention measurements occurring when estrogen and/or progesterone levels were lower compared to pre-intervention measurements. Due to the effect of these hormones on attenuating albumin capillary escape and subsequent fluid to shift to interstitial space, and also promoting renal sodium retention driving extra-cellular fluid and in turn plasma volume

expansion (Stachenfeld, 2008). Noting that in Mee et al. (2018) the plasma volume expansion of 9% was only seen where sauna was used prior to HA sessions. Notwithstanding variability between individuals undertaking the same intervention, and also within individuals, is common in short term HA studies and may have impacted results at a group level for both males and females (Kissling et al., 2021). In addition to female plasma volume results being impacted by hormonal induced fluid shifts; females have been found to have a faster rate of returning to baseline fluid levels post intervention than males (Hahn, 2016). Regardless, the moderate effect (d = 0.728) observed for males in the current study here may have assisted to lower peak heart rate and improved performance by reducing cardiovascular strain and improving heat capacity of the blood in subsequent exercise in the heat (Périard et al., 2021a).

Temperature measures

Similar to cardiovascular measures, HA induced changes to core and skin temperature are expected to occur within seven days as early cardiovascular changes allow for increased skin blood flow, improving the ability to remove heat from working muscles (Tyler et al., 2016). In the current study, while there was no change to resting core temperature in males, they did experienced a significant reduction of 0.30 ± 0.29°C in core temperature and a tendency towards a significant reduction of 0.26 ± 0.37°C in peak skin temperature, with the change in core temperature very close to a meaningful change. Females saw similar changes of no change to resting core temperature, a significant reduction of 0.33 ± 0.17°C in peak core temperature and non-significant reduction of 0.37 ± 0.55°C in peak skin temperature, with the change in core temperature a meaningful change.

In a selection of STHA studies in males, researchers found that males did not reduce resting core temperature, however, they did reduce core temperature during exercise (Garrett et al., 2009; Garrett et al., 2012). These findings, which are supported by the current study, contrast to a systematic review conducted by Tyler etl al. (2016) that found reduced resting core temperature across STHA research of 0.17°COther STHA research did see significant changes in resting core temperature of 0.24°C in males (Mee et al., 2015; Neal et al., 2016). Peak skin temperature changes in research on males has atbeen significant at 0.7°C (Wingfield et al., 2016) and also not great enough to be significant in males at 0.5°C (Mee et al., 2015).

Regarding female temperature measures in STHA research; while Garrett et al. (2019) found a significant reduction in peak core temperature (0.2°C) and mean skin temperature (0.5°C), changes in resting core temperature, peak core temperature and peak skin temperature were

seldom found (Garrett et al., 2019; Kirby et al., 2019; Mee et al., 2015; Mee et al., 2018). However with the addition of sauna prior to HA session Mee et al. (2018) were able to achieve a significant reduction across resting core temperature (0.28°C), peak core temperature (0.42°C), and peak skin temperature (0.89°C). The change in peak core temperature for females from the current study was comparable to these studies, and greater than the smallest worthwhile change.

While Garrett et al. (2019) and Mee et al., (2018) completed HA sessions and testing during the follicular phase and didn't see changes in resting core temperature, there is the potential hormonal impact of progesterone elevating core temperature during testing in the current study (Lei et al., 2017). Three female participants who were assessed as completing post-intervention testing in their mid or late luteal phase all had increased post-intervention resting core temperature. Increases ranging from 0.05 – 0.42°C. It is likely this was driven by hormonal influences, given all three experienced reduced post-intervention peak exercising core temperatures ranging from 0.10 – 0.36°C. Further, the magnitude of core temperature change from resting to peak was less post-intervention (ranging 0.48 – 1.02°C) than pre-intervention (ranging 0.92 – 1.78°C) for all three, indicating for the same intensity and conditions the participants had enhanced cooling ability. One participant was determined to have completed pre-intervention testing in their late luteal phase, which could "enhance" the intervention effect on temperature when comparing measures. However hormone analysis combined with the participant being in the placebo section of their monophasic oral contraceptive indicated that their basal core temperature was not likely elevated at that point in time (Salkeld et al, 2001).

It's not clear why in the current study changes were not seen on the measure of resting core temperature. Diurnal changes to core temperature can impact measurement, however, measurements were taken prior to the commencement of heat stress tests at equivalent times of the day to avoid these impacts.The length of intervention may also be a reason for lack of significant change to resting core temperature, with MTHA more likely to see changes through greater total heat dose (Kelly et al., 2023; Kirby et al., 2019; Wickham et al., 2021). Despite a lack of change to resting core temperature in the current study, changes to resting core temperature are inconsistent across the literature, with overall changes seen by both groups across temperature measures fairly consistent to prior research in each sex.

Sweat rate changes

Despite a significant increase in sweat rate between the first and last training sessions for both females (0.13 ± 0.14L·h^{-1}) and males 0.13 ± 0.16L·h^{-1}) and similar body mass loss to that

during sessions in research by Kirby et al. (2019), the lack of change in sweat rate during the HST for either sex may in part be attributed to the short duration and low intensity of the HST (30 minutes and, 40% of MAP). Moreover there may be a combination of enhanced cooling through skin-core temperature gradient changes such that a higher sweat rate wasn't required during post-intervention testing. While Mee et al. (2018) did see a significant increase in sweat rate in the non-sauna condition, it is also likely the intervention was too short to see significant changes in sudomotor function with LTHA required for significant sweat response adaptations (Kelly et al., 2023; Tyler et al., 2016; Wyndham et al., 1968). Patterson et al. (2004a) found the onset of sweating follows a reduction in resting core temperature, which as above section covers, was not seen in the current study and therefore earlier sweating could not contribute to demonstrate increased sweating for the same heat stress conditions pre- and post-intervention. Additionally the heat stress test wet-bulb temperature was slightly less (28.6°C) than the recommended 30°C+ (Daanen et al., 2018). Thus considering the increase in sweat rate for both groups across HA sessions, it could be concluded that the combination of STHA, intensity and heat load of the heat stress test was insufficient to drive an increase sweat rate across the entirety of the session relative to pre-intervention.

Perceptual measures

Though impact of HA to thermal sensation may be limited by available data (Tyler et al., 2016) there may be an effect during exercise and thermal comfort's impact via volitional behaviour is considered a main driver of exercise performance in hot environments (Flouris & Schlader., 2015; Tyler et al., 2016). In the current study there were significant small to moderate effect size changes across all perceptual measures of thermal sensation (-0.9 ± 0.6), thermal comfort (-0.5 ± 0.6) and RPE (-1.1 ± 0.9) for females with thermal sensation (-0.6 ± 0.4) improving for males and thermal comfort approaching significance ($p = 0.063$) with moderate effect size. Males in the current study experienced similar or greater change on perceptual measures to STHA research with similar length heat stress test (Garrett et al., 2012), however less when compared to longer heat stress tests (Neal et al., 2016). This may be due to the low intensity (40% of MAP) and short nature of the heat stress test not eliciting enough thermal strain to show beneficial changes once adapted. For females the changes were similar to that reported by both Mee et al. (2018) and Garrett et al. (2019), although the change to RPE was less in the current study which may have been due to the lower intensity used in the heat stress tests. Comparing to Garrett et al. (2019) the effect size for thermal sensation was higher in the current study ($d = 1.130$ vs $d = 0.670$), while thermal comfort had a lower effect size ($d = 0.329$ vs $d = 0.890$). Suggesting that while the participants felt the conditions were not as hot post-intervention, the shift in comfort was less pronounced potentially due to the level of intensity limiting potential metabolic heat production.

Markers of HA between sexes

Between females and males there were similar changes in markers of HA, with change in peak HR approaching significance ($p = 0.053$) for males. Though not significant, the increase in plasma volume in males was greater than that of females, as discussed below. Peak skin temperature changes were also not significant across sexes though results suggest there was a tendency towards change. It would be expected that an expansion in plasma volume would help facilitate this given the reduction in peak HR, however, the female group saw no change in plasma volume which may be attributed to fluid shifts from measures being taken during the luteal phase of the menstrual cycle. While all three perceptual measures were found to have significantly changed in females, only thermal sensation ($p = 0.003$) was found to be significantly improved in males with thermal comfort approaching significance ($p = 0.063$). It's not entirely clear why females had greater perceptual changes than males, it's postulated that the intensity and thermal strain during the HST was not enough to elicit evidence of beneficial changes in males, which is supported in part by males seeing a moderate effect size increase to plasma volume and tendency towards increase sweat rate across training sessions. Females were found to have a larger reduction in exercising heart rate between pre- and post-intervention HSTs than males, and this may explain why RPE was significantly lower for that group.

5.3 Training sessions

The purpose of using a controlled heart rate model during the heat acclimation sessions was to provide progressive overload that cannot be achieved when using a fixed intensity model (Périard et al., 2015; Tyler et al., 2016). An additional benefit in using controlled heart rate being the application of a model that can be expected to be less impacted than a controlled hyperthermia model by known hormonal impacts to core temperature which impacts magnitude of change from resting to exercising core temperature (Lei et al., 2017; Wickham et al., 2021). The rationale being that resting heart rate is less impacted than resting core temperature by oral contraceptive use (Lei et al., 2019) and between menstrual cycle phase (Moran et al., 2000; Teixeira et al., 2012). Comparison of mean power output responses between the first and final training sessions supported the use of this model, with significant between-session differences for both females (14.4% ± 13.9%) and males (11.7% ± 5.9%). The changes in mean session power output in the current study were greater than changes seen in controlled hyperthermia research not controlling for menstrual cycle phase 8.3% (Kirby et al., 2019). However slightly less than controlled hyperthermia research, when comparing post 15 minute female change in power output in the current study (17.3%), with training

sessions conducted in the follicular phase 21% (Garrett et al., 2019). And similar to that found in other controlled heart rate research 10-14% (Stephensen et al., 2019; Travers et al., 2020). The change in power output over sessions would indicate that progressive overload was experienced by all participants.

In regards to the thermal impulse from training sessions; the total duration (550 minutes), total heat dose (22,000°C·min), total mean energy expenditure (15,051 ± 4,404 kJ) and mean exercise intensity (27.4 ± 8.0 kJ·min^{-1}) of the current study were largely adequate according to the numbers calculated in a recent meta-analysis of female physiological adaptations and exercise performance in the heat (Kelly et al., 2023) which found a total duration of 451-900 minutes, a total heat dose >= 23,000°C·min, total mean energy expenditure >= 12,719 kJ and exercise intensity >= 14.7 kJ·min^{-1} was required to achieve desired outcomes in females. Males in the current study endured a total duration greater than the recommended minimum of 420 minutes to see main physiological changes (Racinais et al., 2015a). Their total heat dose (22,000°C·min), total mean energy expenditure (24,989 ± 5,616 kJ) and mean exercise intensity (45.4 ± 10.2 kJ·min^{-1}) were greater than the 15,750 - 18,000°C·min, 13,905 - 17,766 kJ, and 30.9 - 39.5 kJ·min^{-1} respectively in other STHA studies (Garrett et al., 2009; Mee et al., 2015; Neal et al., 2016). While the specific combination of frequency, duration, and intensity will affect the magnitude of change (Périard et al, 2015), it can be concluded that the protocol used in the current study provided what would be considered an effective combination on these measures.

As high levels of cardiovascular strain leads to greater heat acclimation, especially in moderately trained participants (Campbell et al., 2022), it is important to understand if the combined stimulus of metabolic heat from active exercise and the hot environment utilised in the current study elicited this. Females in the current study experienced a mean heart rate over the five sessions of 148.3 ± 14.3 beats·min^{-1} and males 143.4 ± 12.3 beats·min^{-1}. Noting that mean session heart rate reduced across sessions as participants' cardiovascular strain was reduced in the first 15 minutes as they underwent HA. Comparing to STHA research that was effective in HA; the figures from the current study are higher against females 142 beats·min^{-1} (Garrett et al., 2019), lower to mixed cohort 153 beats·min^{-1} (Stephenson et al., 2019) and much lower compared to only males 150 - 159 beats·min^{-1} (Guy et al., 2016; Mee et al., 2015; Wingfield et al., 2016). In contrast, comparing to STHA research that was less effective the mean heart rate experienced by females in the current study was similar 146 – 151 beats·min^{-1} ([at 4 days]Kirby et al., 2019); (Mee et al., 2015); [non-sauna]Mee et al., 2018), and slightly higher compared to males 139 beats·min^{-1} (Neal et al., 2016). When compared to Travers et al., 2020 who employed the same HA model in males, the heart rate in the current

was slightly lower compared to 147 beats·min^{-1}, while performance improvement was sig at 10 days (there was no performance test after 5 days), physiological measures at 5 days were sparing. It may be that cardiovascular strain was not quite adequate across the five days for some participants and this may be reflected in the lack of significant changes to plasma, peak skin temperature and to a lesser degree resting heart rate given changes on that measure are less likely during STHA.

When exercising in uncompensable heat, the rise in core and skin temperature become the main drivers of HA physiological adaptations through cardiovascular strain (Cheung et al., 1998; Periard et al., 2015). As skin temperature was not measured during HA sessions, tympanic temperature was used to understand if the current protocol allowed for an adequate magnitude change in core temperature. Noting that there are limitations in the accuracy of tympanic temperature measurement (Ganio et al., 2009), with the device used found to underestimate rectal temperature by 0.1°C (Otani et al., 2020). In the current study both males and females had a mean tympanic temperature for the final 65 minutes of the session of 38.3°C. With the mean magnitude of changes in temperature (HA session tympanic temperature from post 45mins to pre-intervention heat stress test core temperature) approximately 1.1°C for females and 1.5°C for males. While difficult to get exact reports of these values, the figures for females are comparable to 1.2°C in Kirby et al. (2019) who did not see significant changes at day 4, less than 1.3°C using controlled hyperthermia in Garrett et al. (2019), similar for males but lower for females from the current study compared to the clamp of 1.5°C used by Campbell et al. (2022) in a mixed cohort, and similar for males compared to the 1.5°C seen in Travers et al. (2020). It's likely that detail is lost in group means, especially for females where menstrual cycle can play a role in the magnitude of change and impact effect size of HA interventions especially for controlled hyperthermia models (Kelly et al., 2023).

While the 16 participants were each able to complete their five intervention sessions, anecdotal feedback was that completion was more challenging for those with a higher $\dot{V}O_{2max}$, as the application of 65% of $\dot{V}O_{2max}$ for starting power and target HR meant those participants were exercising at a much higher intensity generating greater metabolic heat. Though to an extent the power and HR during training sessions should have been relative to an individual participants fitness, both females and males with a lower $\dot{V}O_{2max}$ required a relatively lighter intensity to keep HR at target. Likely due to lower fitness impacting ability to attenuate the effects of the heat (Alhadad et al, 2019). A lower $\dot{V}O_{2max}$ may have actually been a $\dot{V}O_{2peak}$ where a participant did not reach their $\dot{V}O_{2max}$ due to neuromuscular limitations, though it is expected in trained individuals that given the protocol employed they would have reached, or

come close to, $\dot{V}O_{2max}$ (Beltz et al., 2016). Mean power across sessions did steadily increase for all participants, the male group who exercised at a higher intensity (first session mean power: 144.0 ± 31.0 W, last session mean power: 160.9 ± 34.7 W), both increased power and tympanic temperature across sessions suggesting heat stimulus was maintained at a high level, while the female group at lower intensity (first session mean power: 87.6 ± 25.6 W, last session mean power: 100.3 ± 27.9 W) reduced mean tympanic temperature potentially as a result of lower stimulus (Cramer & Jay, 2014). Noting there are limitations in interpreting tympanic temperature readings during exercise in the heat due to bias (Ganio et al., 2009). Using an approach suggested by Gibson et al. (2016) when examining session mean power by body mass, females held 1.5 ± 0.4 $W.kg^{-1}$ while males held 2.0 ± 0.5 $W.kg^{-1}$. As a result the session intensity may have impacted the extent of heat acclimation, with further research warranted to understand the application of this protocol to different levels of trained status.

6. Limitations

While the current study has expanded the knowledge regarding the application of a controlled heart rate model and HA in females, there are a number of limitations which may inform direction of future research. The lack of a thermoneutral control group impacted the ability to compare changes in performance during the time trial that were directly related to the HA intervention. However, the impact of thermoneutral training sessions to performance in the hot time trial would be considered negligible due to the training eligibility requirements for participation meaning that participants were already participating in regular aerobic training. Future research may wish to include a control group or cross-over design to isolate training effect on performance. Additionally, while in keeping with the methodology of other researchers (Keiser et al., 2015; Racinais et al., 2015b), participants were afforded access to power, heart rate, time and distance during the time trial test. Though this method can provide information to assist a trained athlete in how to best pace their all-out effort; more recent research has suggested that in order to minimise replication of prior efforts, only distance based data be made available to trained participants (Borg et al., 2020).

Testing was carried out in part outside of winter, which may have influenced the magnitude of results due to partial heat acclimatisation. However, it is expected that this will have minimal impact as participants were advised to avoid exposure to heat and especially exercise in the heat, efforts were made to understand potential exposure to heat by participants, and weather conditions in the lead up to and through testing were monitored.

Given the importance of adequate cardiovascular strain, employing additional measures in order to quantify the level of cardiovascular strain such as blood pressure in addition to heart rate would help elucidate the HA kinetics. A larger sample size to allow for exploration of the relationship between relative rate of energy expenditure by body mass to HA would also assist in understanding thermal impulse from the controlled heart rate model and influence protocol design, potentially allow for individualisation.

An area for future research is to accurately understand the magnitude of change in core temperature during a session, with comparison between participants grouped to different phase of menstrual cycle. In the current study, due to cost of core temperature pills, HA session core temperature was not measured outside of tympanic measurement. Should future research find an economical, non-invasive way to accurately measure core temperature during training sessions, it would assist in determining how effective the controlled heart rate model is for ensuring an adequate magnitude of change in core temperature in relation to hormonal elevated basal core temperature.

7. Conclusion

The application of a controlled heart rate model of HA to trained cyclists using a STHA protocol saw power progressively increase across sessions along with sweat rate, resulting in significant improvement to performance during a 20 km time trial in hot conditions. The protocol was effective in demonstrating positive and significant improvements to peak core temperature and thermal sensation across males and females, with females additionally experiencing significant improvement to peak heart rate (males showing a moderate effect size), thermal comfort (males showing moderate effect size) and RPE. The protocol did not elicit significant plasma volume expansion though males tended towards significant expansion with moderate effect size. There was a small effect size towards males in time trial performance however no significant differences were revealed between sexes regarding power and duration.

The current study provides a practical, effective protocol for athletes wishing to obtain HA through progressive means delivering consistent cardiovascular stimulus. Demonstrating that the controlled heart rate STHA protocol was effective for both males and females, where previously this had only been tested via a MTHA protocol in males, opens up opportunity for athletes to prepare for events in hot environments with less disruption to training. With a mix of hormonal levels either via natural menstruation or contraceptive, the study has also

expanded knowledge regarding female HA protocols. Future research can build on this knowledge to further the field and find optimal protocols relative to hormonal influences. Overall the findings of the current study are relevant for athletes wanting to improve performance in the heat through use of a heart-rate based HA protocol.

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Appendix A.

VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

EXERCISE PHYSIOLOGY UNIT

MENSTRUAL CYCLE QUESTIONNARIE

1. Are your menstrual cycles regular?

Yes/No

2. What is the average length of your cycle (in days)?

days

3. What was the start date of your previous two menstrual periods?

Last period: / /

Second last period: / /

4. Do you use any contraceptive product?

Yes/No

If 'Yes', please provide details:

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