THE UNIVERSITY OF HULL

Effectiveness of Short-Term Heat Acclimation on Endurance Exercise with Moderately Trained Males

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Pulications and Presentations

Publications

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Garrett, A.T. (2019). https://theconversation.com/marathon-des-sables-how-we-rapidly-acclimatised-five-runners-for-the-gruelling-race-113973. Retrieved April 8, 2019

Conference Communications

International Conference on Environmental Ergonomics (ICEE) (2019), Amsterdam, Netherlands

Adaptation to the heat using short-term heat acclimation with dehydration in matched females and males
Andrew Garrett*, Edward Dodd, Victoria Biddlecombe, Damien Gleadall-Siddall, Rachel Burke, Jake Shaw, Cory Walkington, James Bray, Huw Jones, Grant Abt, & Jarrod Gritt

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Effectiveness of short-term heat acclimation on intermittent exercise in the heat with moderately-trained females controlling for menstrual cycle phase.
Andrew Garrett*, Edward Dodd, Victoria Biddlecombe, Damien Gleadall-Siddall, Rachel Burke, Richard Turpin, Jake Shaw, James Bray, Huw Jones, Grant Abt, & Jarrod Gritt

Abstract

Introduction – Exercising for an extended period in thermally demanding conditions places greater physiological and perceptual strain on the body than when exercising in temperate conditions (Galloway & Maughan, 1997; Moss et al., 2020; Tucker, Rauch, Harley, & Noakes, 2004). Heat acclimation (HA) has been suggested as one of the more effective interventions to be integrated into an athlete's preparation to reduce physiological strain and improve exercise performance in hot conditions (C. J. Tyler, Reeve, Hodges, & Cheung, 2016). Many studies have explored studied hydration status of participants exercising in the heat and it is generally accepted that hydration status influences physiological and performance responses in the heat (Sekiguchi, Filep, Benjamin, Casa, & DiStefano, 2020). However, this is disputed. *Aim* - To explore the effectiveness of a STHA protocol with no fluid intake over 4 d.

Method – Twelve moderately trained, male participants completed this study (mean±SD; age: 35±15years; height: 175.3±4.5cm; body mass: 79.7±11.2kg; VO_{2peak}: 47.2±9.9ml·kg·min). *Control:* Eleven of the 12 completed two 90-minute HST trials (35ºC; 60%RH) on a cycle ergometer at 40% PPO followed by a 2% PPO incremental test to exhaustion post 10-minute passive recovery, taken a week apart with no intervention. *Intervention:* Twelve completed a 90-minute HST trial on a cycle ergometer at 40% PPO followed by a 2% PPO incremental test to exhaustion post 10-minute passive recovery pre and post four consecutive days of isothermic (40ºC; 60%RH) HA with no fluid intake intervention.

Results – In the control trial, there was limited change for T_{re}, $\overline{T_{sk}}$, $\bar{T}_b$, f_c, %PV, and incremental performance trial. In the intervention trial, mean T_{re} lowered over time, specifically at 70-min (P = 0.03). Mean HR decreased over time, specifically at 10 (P = 0.05), 20 (P = 0.03), and 30-min (P = 0.03). There were significant effects over time for RPE, TC, and TS. Mean incremental performance time increased by 142 s (P = 0.04) and mean PPO by 76 W (P = 0.03). 90-min steady state exercise completion rate improved post intervention from 7 to 12 with 11 performing the incremental exercise to exhaustion trial.

Conclusion – A 4 d isothermic STHA protocol with no fluid intake was effective at reducing physiological and perceptual strain and improving performance during exercise in hot conditions.

Preface

First and foremost, in completing this thesis I wish to thank my supervisor Dr. Andrew Garrett for his unwavering support and guidance over the last 3 years. Also, I would like to thank you for your faith in me to offer me this opportunity. I have enjoyed every second of the last 3 years and I look forward to our future projects and adventuring into the promised land of a PhD!

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To Emma Andrews, the last 3 years have probably been the toughest of my life so far – both in my studies and in my personal life. You have been an absolute star and a rock for me when I have needed you and without you, I would not have been able to complete this thesis. I therefore dedicate this thesis to you and our friendship! Love you long time!

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Finally, to my father Sandy, this thesis will always be significant to me as at the time of data collection and subsequent write-up, we made contact after nearly a decade and a half! Thank you for the support and encouragement you have given me so far.

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f_c	Cardiac frequency [b·min^{-1}]
$\dot{Q}$	Cardiac output [L·min^{-1}]
HST	Heat stress test
$\dot{V}O_{2\,max}$	Maximum oxygen uptake [L·min^{-1}or mL·kg^{-1}·min^{-1}]
$\overline{T}_b$	Mean body temperature [^{o}C]
PPO	Peak power output (W)
MPO	Mean power output (W)
$\overline{T}_{sk}$	Mean skin temperature [^{o}C]
$\dot{V}O_2$	Oxygen consumption [L·min^{-1}or mL·kg^{-1}·min^{-1}]
$\dot{V}O_{2\,peak}$	Peak oxygen uptake [L·min^{-1}or mL·kg^{-1}·min^{-1}]
%PV	Percentage plasma volume (%)
$[alb]_p$	Plasma albumin (mg.mL^{-1})
$[aldo]_p$	Plasma aldosterone (pg.mL^{-1})
$[cortisol]_p$	Plasma cortisol (ug.dl^{-1})
$[Na^+]_p$	Plasma sodium (mmol.L^{-1})
$[K^+]_p$	Plasma potassium (mmol.L^{-1})
$[Cl^-]_p$	Plasma chloride (mmol.L^{-1})

$[TP]_p$	Plasma total protein ($mg.mL^{-1}$)
T_{re}	Rectal temperature [^oC]
RH	Relative humidity [%]
STHA	Short-term heat acclimation
$colour_u$	Urine colour (units)
osm_u	Urine osmolality (mOsm/kg)
SG_u	Urine specific gravity (units)
T_a	Ambient temperature
HA	Heat acclimation
CH	Controlled Hyperthermia
T_c	Core Body Temperature
Hct	Haematocrit
Hb	Haemoglobin
HSP	Heat Shock Protein (pg/ml)
CV	Cardiovascular
EUH	Euhydration
DEH	Dehydration
df	Degrees of freedom

1 Introduction

Exercising for an extended period in excessively hot conditions causes greater physiological and perceptual stress compared to exercising in mild environments (Galloway & Maughan, 1997; Moss et al., 2020; Tucker et al., 2004). This increased stress can frequently result in diminished exercise capacity (Ely, Martin, Cheuvront, & Montain, 2008) and possibly dangerous heat illnesses such as endotoxemia, heat exhaustion, and heat stroke (Moss et al., 2020; Wendt, van Loon, & Lichtenbelt, 2007). To achieve a heat-adapted phenotype, stress to the thermoregulatory and cardiovascular systems using hot and humid conditions coupled with physical work needs to occur (Gibson, Mee, et al., 2015; M. N. Sawka, Leon, Montain, & Sonna, 2011). This is termed as heat acclimation (HA) (Garrett, Rehrer, & Patterson, 2011).

Beneficial adaptations achieved by HA include reduced body temperature (Armstrong & Maresh, 1991; Buono, Heaney, & Canine, 1998; Garrett et al., 2011), improved cardiovascular stability (Frank, Belokopytov, Moran, Shapiro, & Epstein, 2001), reduced heat storage (Aoyagi, McLellan, & Shephard, 1997) enabled by increased sweat rates and concentration (Chinevere, Kenefick, Cheuvront, Lukaski, & Sawka, 2008; Lorenzo & Minson, 2010; Magalhaes Fde et al., 2006; Martinez, Jones, Hodge, & Buono, 2012), and decreased thermal and perceptual strain (P. Castle, Mackenzie, Maxwell, Webborn, & Watt, 2011) can be induced by repeated exposure to thermal stress (Moss et al., 2020; C. J. Tyler et al., 2016) – which initiates the heat-adapted phenotype (Gibson, Mee, et al., 2015). Approximately 75-80% of adaptations manifest within the first 4-7 days of exposure – termed short-term heat acclimation (STHA) – providing rapid but otherwise incomplete adaptation compared to longer durations of 7-14 days (medium-term heat acclimation (MTHA)) and $\geq$15 days exposure (long-term heat acclimation (LTHA)) (Garrett et al., 2011; Gibson, Mee, et al., 2015; Pandolf, 1979; C. J. Tyler et al., 2016). Although, not all adaptations can be attained within the period. An

example would be HR adaptations usually occurring before adjustments in exercise capacity and sweat responses are achieved (Moss et al., 2020; Periard, Travers, Racinais, & Sawka, 2016; C. J. Tyler et al., 2016).

In order to induce heat adaptations and therefore, a heat-acclimated phenotype, an increased core temperature and a sufficient stimulus is fundamental to generate a physiological strain greater than the adaptation threshold (Gibson, Mee, et al., 2015; Regan, Macfarlane, & Taylor, 1996; N. A. Taylor, 2014; N. A. S. Taylor & J. D. Cotter, 2006) and the scale of the adaptation seems to be dependent on the scale and regularity of the thermal strain and impulse applied (Moss et al., 2020). Isothermic HA – also known as controlled hyperthermia (CH) – is executed based on endogenous measures (P. C. Castle et al., 2013; Garrett, Creasy, Rehrer, Patterson, & Cotter, 2012; Garrett, Goosens, Rehrer, Patterson, & Cotter, 2009; Garrett et al., 2014; Hom et al., 2012; Magalhaes Fde, Amorim, et al., 2010; Magalhaes Fde et al., 2006; Magalhaes Fde, Passos, et al., 2010; Patterson, Stocks, & Taylor, 2004a, 2014) and may provide continued training and achievement of specific and individualised core body temperatures through a combination of active and passive HA (Fox, Goldsmith, Kidd, & Lewis, 1963; Gibson, Mee, et al., 2015). It has been suggested that the threshold for adaptation may be reaching and maintaining a T_c of approximately 38.5ºC as sweat and blood vessel responses to heat are strained and HSPs are produced (Fox, Goldsmith, Hampton, & Lewis, 1964; Gibson, Mee, et al., 2015; Gibson, Turner, et al., 2015). The equilibrium between work and rest to specify and reach a certain T_c guarantees a consistency, or a progression of endogenous heat stress to achieve adaptation – although this requires modifications in application during each session. By comparison, fixed intensity methods are very simple to implement, with participants sustaining a fixed workload throughout each individual active acclimation session (Amorim, Yamada, Robergs, Schneider, & Moseley, 2011; P. Castle et al., 2011; Cheung & McLellan, 1998; Houmard et al., 1990; Kresfelder, Claassen, & Cronje, 2006; Lorenzo,

Halliwill, Sawka, & Minson, 2010; Lorenzo & Minson, 2010; Marshall, Campbell, Roberts, & Nimmo, 2007; Nielsen et al., 1993; Nielsen, Strange, Christensen, Warberg, & Saltin, 1997; Sandstrom, Siegler, Lovell, Madden, & McNaughton, 2008; Watkins, Cheek, Harvey, Blair, & Mitchell, 2008; Yamada, Amorim, Moseley, Robergs, & Schneider, 2007). It can become problematic to attain and sustain a target T_c of 38.5°C using the more conventional continuous HA methods, nonetheless a controlled isothermic HA methodology defeats this problem by making sure that the target T_c is achieved and then sustained using passive and active heat stress (Moss et al., 2020).

Isothermic HA, where endogenous thermal stimulus is regularly targeted all the way through, may positively maintain the rate of adaptation, or progress adaptation should a gradual increase in T_c be employed (Gibson, Mee, et al., 2015; N. A. Taylor, 2014; N. A. S. Taylor & J. D. Cotter, 2006). Progressive isothermic approaches have, in the past, been applied using protocols where the exogenous conditions or amount of work during acclimation are increased (Burk et al., 2012; Chen, Tsai, Lin, Lee, & Liang, 2013; H. A. Daanen, Jonkman, Layden, Linnane, & Weller, 2011), more than likely to counter ongoing adaptation (Gibson, Mee, et al., 2015).

An advantage of isothermic HA protocols is that it is possible for adaptations to be attained with quicker exercise periods and lower workloads than fixed intensity methods (Gibson, Turner, et al., 2015), hence they may be beneficial leading up to a competitive event (C. J. Tyler et al., 2016). When individuals are not exposed to the heat, adaptations deteriorate at a rate of ~2.5% per day, therefore HA should be utilised as near to competition as possible to prevent decay and ultimately "de-acclimation" (H. A. M. Daanen, Racinais, & Periard, 2018; Moss et al., 2020). Though, a heavily intense HA protocol may negatively affect subsequent exercise performance as well as health through over-activating the hypothalamic-pituitary-thyroid axis (Reeve, Gordon, Laursen, Lee, & Tyler, 2019) and/or permeability of the gut (Lim

et al., 2009; Moss et al., 2020). The isothermic HA methodology has been explored either with an absolute T_c increase (Magalhaes Fde, Passos, et al., 2010) or the achievement of a target thermal strain (Garrett et al., 2012; Gibson, Turner, et al., 2015). One potential weakness when implementing the set increase in T_c method is that as adaptation occurs and resting T_c reduces, those undergoing the protocol may not be receiving an adequate thermal strain to prompt HA adaptations (Moss et al., 2020). Reaching a set thermal strain makes sure that an adaptation stimulus is continuously applied as adaptations occur (N. A. Taylor, 2014; C. J. Tyler et al., 2016). In recent publications, it is suggested that 90-minute daily isothermic bouts offer sufficient adaptive stimulus, although they note that it may be problematic to incorporate sessions of such duration into an athlete's preparation (Garrett et al., 2012; Gibson, Turner, et al., 2015).

Many studies have explored studied hydration status of participants exercising in the heat and it is generally accepted that hydration status influences physiological and performance responses in the heat (Sekiguchi et al., 2020). Restricting fluid intake exacerbates the strain applied on the body, encouraging cardiovascular and fluid regulatory adaptations that are key mechanisms of a heat-adapted phenotype (Fan et al., 2008; Garrett et al., 2012; Ikegawa et al., 2011; Judelson et al., 2008; Osterberg, Pallardy, Johnson, & Horswill, 2010; Sekiguchi et al., 2020). However, there is still continued debate on whether or not a dehydrated state has an impact on HA (Sekiguchi et al., 2020).

1.1 Aims and Hypothesis

The aim of the present study was to assess the effectiveness of a four daily consecutive 90-minute isothermic HA sessions with no fluid intake in relation to other, more established 5-7 d STHA protocols. This was achieved by observing changes in physiological markers of adaptation T_{re} and f_c indicating magnitude of physiological adaptation; psychological

markers RPE, TC and TS indicating perception of heat stress experienced by participants; and performance indicating the effectiveness of the applied HA protocol.

2 Literature Review

2.1 Physiological Adaptation to the Heat

2.1.1 Core Body Temperature

Exercising in the heat imposes a greater physiological strain than exercising in temperate conditions at the same intensity and this added strain causes a decreased ability to exercise (Galloway & Maughan, 1997; C. Tyler & Sunderland, 2008; C. J. Tyler et al., 2016). There are two main forms of heat adaptation that can be achieved to counter this physiological strain: (i) heat acclimation (HA) which is achieved using a laboratory-based environmental chamber and (ii) heat acclimatization achieved through natural exposures such as warm-weather training camps or domestic heat waves (Blatteis et al., 2001; C. J. Tyler et al., 2016). Heat acclimation or acclimatization is developed through regular exposure to exercise and hot environmental conditions $\geq 30^{o}C$ (Pryor, Minson, & Ferrera, 2018).

Exposure to repeated heat stress sufficient to result in physiological strain (N. A. Taylor, 2014) disturbs homeostasis – producing morphological, chemical, functional and genetic alterations in an attempt to reduce physiological strain in future exposures to heat stress (C. J. Tyler et al., 2016). Thermal adaptations occur when a sufficiently overloading thermal impulse is applied which surpass an adaptation threshold (N. A. Taylor, 2014) however, an insufficient thermal impulse will cause adaptations to either occur sub-optimally or not at all (Moss et al., 2020; C. J. Tyler et al., 2016)

Heat acclimation triggers numerous adaptations that improve thermoregulation, cardiovascular stability, thermotolerance, fluid-electrolyte balance leading to a reduced physiological strain and, therefore, an enhanced submaximal and maximal aerobic performance in the heat (Pryor et al., 2018). However, there is some debate as to which adaptations indicate a successful HA. M. N. Sawka et al. (2011) suggested “four classic markers of HA” (lower

heart rate (HR), lower core body temperature (T_c), higher sweat rate, and improved exercise performance in hot conditions), whereas N. A. Taylor (2014) suggested seven: HR, stroke volume, sodium (Na^+) loss, urine loss, water loss, sweat rate, and skin blood flow (C. J. Tyler et al., 2016). A common (Adolph, 1947; Buono et al., 1998; Patterson, Stocks, & Taylor, 2004b) but not universal (Creasy, 2002; Garrett et al., 2012; Garrett et al., 2009; Turk & Worsley, 1974; Weller & Harrison, 2001) feature of short-term heat acclimation (STHA) is a reduction in resting T_c (Garrett et al., 2014).

Changes in T_c receive increased attention from HA literature and a reduced T_c during rest and exercise is a vital adaptation indicative of a successful HA regimen (C. J. Tyler et al., 2016) and has the potential for increasing work capacity in heat stressful conditions where thermal compensability is restricted (Cheung & McLellan, 1998; Garrett et al., 2014; Nielsen et al., 1993). In a recent meta-analysis, it was reported that rectal temperature (T_{re}) was the "most commonly measured" means of estimating T_c among aural and gastric (C. J. Tyler et al., 2016). While the effect sizes are comparable across measurement sites, the authors refuse to endorse the use of aural or gastric temperatures as it is well established that these methods are invalid estimates of T_c (Casa et al., 2007; Ganio et al., 2009; C. J. Tyler et al., 2016).+

Despite this, moderate to large benefits from HA were observed on reducing resting (Beaudin, Walsh, & White, 2012; Bonner, Harrison, Hall, & Edwards, 1976; Brade, Dawson, & Wallman, 2013; Brazaitis & Skurvydas, 2010; Buono et al., 1998; Burk et al., 2012; P. Castle et al., 2011; Cheung & McLellan, 1998; Febbraio et al., 1994; Flouris et al., 2014; Fujii et al., 2012; Fujii, Tsuji, Honda, Kondo, & Nishiyasu, 2015; Garrett et al., 2012; Garrett et al., 2009; Gibson, Turner, et al., 2015; Henane & Bittel, 1975; Kanikowska et al., 2012; Kuennen et al., 2011; Lee, Miller, James, & Thake, 2016; Magalhaes Fde, Amorim, et al., 2010; Magalhaes Fde et al., 2006; Magalhaes Fde, Passos, et al., 2010; Maruyama, Hara, Hashimoto, Koga, & Shido, 2006; Neal, Corbett, Massey, & Tipton, 2016; Nielsen et al., 1997; Patterson et al.,

2004a; Petersen et al., 2010; Racinais et al., 2012; Regan et al., 1996; Saat, Sirisinghe, Singh, & Tochihara, 2005; L. C. Senay, Jr., 1972, 1975; Shido, Sakurada, Sugimoto, Hiratsuka, & Takuwa, 2001; Shido, Sugimoto, Tanabe, & Sakurada, 1999; Shin, Lee, Min, & Yang, 2013; Sunderland, Morris, & Nevill, 2008; Voltaire et al., 2002; Watkins et al., 2008; Yamazaki & Hamasaki, 2003; Yeargin et al., 2006; Zurawlew, Walsh, Fortes, & Potter, 2016), mean (Aoyagi, McLellan, & Shephard, 1995; Armstrong et al., 2005; Flouris et al., 2014; Garrett et al., 2012; Griefahn, 1997; Lee et al., 2016; Magalhaes Fde, Passos, et al., 2010; Neal et al., 2016; Racinais et al., 2014; Rowell, Kraning, Kennedy, & Evans, 1967; A. G. Willmott, Gibson, Hayes, & Maxwell, 2016) and comparable time point (Burk et al., 2012; Cheung & McLellan, 1998; Cheuvront et al., 2008; Fujii et al., 2012; Garrett et al., 2009; Griefahn, 1997; Henane & Bittel, 1975; McClung et al., 2008; Nielsen et al., 1997; Racinais et al., 2012; Regan et al., 1996; Saat et al., 2005; Shido et al., 2001; Watkins et al., 2008) T_c irrespective of measurement method (C. J. Tyler et al., 2016).

Moss et al. (2020) support the notion that T_c is indicative of a successful HA protocol, reporting a -0.38 $\pm$ 0.26°C decrease in resting T_{re} after 5 d of isothermic heat exposure. A lower resting T_{re} can delay the attainment of high T_c's frequently observed to limit exercise capacity in the heat (Gonzalez-Alonso et al., 1999; Tucker et al., 2004) by maximising the range between dangerously high T_c and resting T_{re}, therefore increasing exercise capacity (C. J. Tyler et al., 2016). In contrast, Garrett et al. (2014) found an unsurprising absence of any reduction in resting T_c and attributes this to the time of day stating that T_c adaptations are specific to the time of day of habitual heat exposures (Shido et al., 1999). This occurred due to logistical complications restricting the ability to acclimate participants at the same time as their HST's (Garrett et al., 2014). Despite this, Garrett et al. (2014) observed lower exercising T_c

and this is credited to improved heat loss as opposed to a reduced initial heat content or reduced heat production during exercise as resting T_{re} was not lowered.

Garrett et al. (2019) observed a decrease in T_{re} of -0.2°C after 5 d of isothermic HA albeit in a female cohort and has previously reported similar decreases in previous work involving male participants (Garrett et al., 2012; Garrett et al., 2009; Garrett et al., 2014). Mee, Gibson, Doust, and Maxwell (2015) contradict Garrett et al. (2019) and their work with a female cohort, reporting a -0.07 $\pm$ 0.18°C decrease in attenuated T_{re} during a 30-min run in the heat after 5 d of controlled hyperthermia HA – but observed a -0.39 $\pm$ 0.36°C decrease in a male cohort, supporting previous data mentioned above.

2.1.2 Cardiovascular Stability

Cardiovascular insufficiency contributes to an impaired ability to exercise in the heat more so than hyperthermia (Gonzalez-Alonso & Calbet, 2003; Moss et al., 2020; Periard, Cramer, Chapman, Caillaud, & Thompson, 2011), reinforcing the notion that any cardiovascular adaptations to HA would be of immense potential benefit to an athlete exercising in the heat (C. J. Tyler et al., 2016). A decreased HR and increased stroke volume at the same relative-intensity exercise in the heat (Garrett et al., 2011; Pryor et al., 2018), and better maintenance of the arterial pressures are classic markers of an effective HA protocol (Moss et al., 2020; C. J. Tyler et al., 2016) – enabling increased heat transfer to and from the skin (Garrett et al., 2011).

Heat adaptations to HR are frequently described to occur quickly and is often reported that the majority of the adaptations takes place within the first 4-5 days and that complete adaptation takes approximately 7 days (Moss et al., 2020; Periard, Racinais, & Sawka, 2015; C. J. Tyler et al., 2016). Data from a recent meta-analysis by C. J. Tyler et al. (2016) supports this as short-term (Brazaitis & Skurvydas, 2010; Fujii et al., 2012; Kuennen et al., 2011;

Racinais et al., 2012; Yamazaki & Hamasaki, 2003; Zurawlew et al., 2016) and medium-term effects (Bonner et al., 1976; Burk et al., 2012; Cheung & McLellan, 1998; Flouris et al., 2014; Gibson, Turner, et al., 2015; Kanikowska et al., 2012; Lee et al., 2016; Magalhaes Fde, Amorim, et al., 2010; Magalhaes Fde et al., 2006; Maruyama et al., 2006; Poh et al., 2012; Saat et al., 2005; Shido et al., 2001) on resting HR were comparable. Despite the fact that there is still debate over the time course of HR adaptations, the reduction in HR during exercise at the same relative-intensity as adaptation occurs is well established and supported by the data provided by C. J. Tyler et al. (2016) – but is not universally supported (Moss et al., 2020).

Moss and colleagues (2020) reported in their findings that resting HR was unaffected by HA, however, they did find that mean exercising, and end-exercise HR was reduced following STHA. This is in contrast to numerous studies which reported a lower resting HR (Bonner et al., 1976; Brazaitis & Skurvydas, 2010; Burk et al., 2012; Cheung & McLellan, 1998; Flouris et al., 2014; Fujii et al., 2012; Gibson, Turner, et al., 2015; Griefahn, 1997; Kanikowska et al., 2012; Kuennen et al., 2011; Lee et al., 2016; Magalhaes Fde et al., 2006; Magalhaes Fde, Passos, et al., 2010; Maruyama et al., 2006; Patterson et al., 2004a; Petersen et al., 2010; Poh et al., 2012; Racinais et al., 2012; Saat et al., 2005; Shido et al., 2001; Yamazaki & Hamasaki, 2003; Zurawlew et al., 2016) whilst also concurring with studies that found a reduced exercising (Aoyagi et al., 1995; Armstrong et al., 2005; Febbraio et al., 1994; Flouris et al., 2014; Garrett et al., 2012; Kenefick, Cheuvront, Elliott, Ely, & Sawka, 2012; King, Costill, Fink, Hargreaves, & Fielding, 1985; Lee et al., 2016; Magalhaes Fde, Passos, et al., 2010; Neal et al., 2016; Racinais et al., 2014; Racinais, Periard, Karlsen, & Nybo, 2015; Rowell et al., 1967; Voltaire et al., 2002; A. G. Willmott et al., 2016) HR.

This could have occurred due to possible fitness differences in the populations used, as highly trained athletes behave physiologically as if they were already acclimated (Garrett et al., 2012) or method variances such as exposure time where Moss and colleagues (2020)

implemented a 60-minute bout compared to longer bouts of 90-minutes or more (Garrett et al., 2012; Garrett et al., 2019; Garrett et al., 2009; Garrett et al., 2014; Garrett et al., 2011; Gibson, Turner, et al., 2015; Neal et al., 2016; Racinais et al., 2014; Racinais et al., 2012; Sunderland et al., 2008). Protocols of 7-14 days appear to be no more effective than those lasting ≤7 days, although the effect sizes increase with longer protocols (C. J. Tyler et al., 2016). In another study, albeit with a female cohort, Garrett *et al.* (2019) reported that the increased cardiovascular stability was due to increased heat loss as opposed to a lower resting core temperature. This could suggest that while the they did find indications of CV adaptation to the heat, participants had adapted the heat loss mechanisms during exercise and not necessarily adapted in preparation for intense heat stress – although this concept has yet to be thoroughly discussed.

Maintaining a sufficient thermal impulse can be difficult depending on the methods used. For example, Griefahn (1997) explored the difference between different ambient conditions: warm-humid, hot-dry and radiant-heat. Participants were exposed to 15 consecutive days of HA in their respective condition, completing four successive 25-minute periods of walking on a treadmill at 4km/h after a 10-minute initial rest in the environmental chamber – resting for 3-minutes in between each bout with the first rest being 5-minutes. In total, subjects spent 100-minutes walking and 22-minutes at rest. Imposing an equal wet-bulb-globe-temperature (WBGT) between 33.4 and 33.6^{o}C across the three different environments alongside a control group in 21.1^{o}C WBGT, it was reported that HR adaptations had occurred rapidly at the beginning of each HA protocol regardless of condition. Notably, it was reported that CV adaptations had plateaued at 7 days in warm-humid, 7 days in hot-dry, and 11 days in radiant-heat conditions. While this appears to be successful, exogenous heat stress was heavily relied upon as the mode of exercise was inherently standardised across all participants walking

at the same pace, therefore imposing varying thermal impulses to each individual respectively (Garrett et al., 2009; Garrett et al., 2011).

An example of maintaining the thermal impulse can be found in the work of Patterson et al. (2004a). Using the CH technique, work rate was modified to sustain the thermal strain – preventing a progressive decline in strain as acclimation progressed – by ensuring the target T_c of 38.5ºC was achieved within approximately 30-minutes. Participants underwent 6 days of humid HA for 3 weeks in 40ºC, 60%RH for 90-minutes. Heat stress tests conditions were 28ºC and 60%RH and were conducted before (day 1) and after 1 week (day 8) and 3 weeks (day 22). Due to the constant modification to work rate, resting HR was reduced after 8 days (-5 bpm) and continued to reduce after 22 days of HA (-5 bpm). Adjusting the work rate is a common theme in more recent publications, following the concept of maintaining the thermal strain in order to illicit a more completed adaptation to the heat (Garrett et al., 2012; Garrett et al., 2019; Garrett et al., 2009; Garrett et al., 2014; Garrett et al., 2011; Moss et al., 2020; C. J. Tyler et al., 2016) by stressing fluid regulatory mechanisms.

2.1.3 Plasma Volume and Body Water

Fluid regulatory adaptations have been established as an important factor in HA (Bass, Kleeman, Quinn, Henschel, & Hegnauer, 1955; Garrett et al., 2011; Patterson et al., 2004b; Wyndham et al., 1968) and it is suggested that through stressing fluid regulation, thermal adaptations can be optimised (Garrett et al., 2011; N. A. Taylor & J. D. Cotter, 2006). The result of stressing fluid regulation is the expansion of plasma volume (PV) and is one of the main features of heat stress and exercise (Garrett et al., 2014). Patterson et al. (2004b) suggests that an expanded PV is facilitated by increased sodium retention which brings with it an increased total blood volume whereby cardiac filling pressures increase and provide a more stable $\dot{Q}$ during exercise in the heat (Pryor et al., 2018) – however this has previously been

debated (Garrett et al., 2009; Harrison, 1985; M. N. Sawka, Convertino, Eichner, Schnieder, & Young, 2000; L. C. Senay, Mitchell, & Wyndham, 1976; Shapiro, Hubbard, Kimbrough, & Pandolf, 1981).

While Patterson and colleagues (2004b) propose that sodium retention is the main driver in retaining body water, the mechanisms remain unclear (Pryor et al., 2018). The fluid conserving hormone aldosterone ($[aldo]_p$) – produced by the adrenal cortex – enables the reabsorption of sodium ions (Na^+) in the kidneys and sweat glands (Garrett et al., 2011; Moreira, Johnson, Forbes, & Consolazio, 1945; C. J. Tyler et al., 2016) with $[aldo]_p$ itself is regulated by potassium (K) concentrations (Garrett et al., 2011) is considered one of the primary pathways to initially expand total body water (Pryor et al., 2018). Tyler and colleagues' (2016) review supports this by exploring sweat loss in the literature. Their meta-analysis appears to confirm that HA reduces sodium sweat concentrations (Chinevere et al., 2008; Houmard et al., 1990; Kirby & Convertino, 1986; Neal et al., 2016; Nielsen et al., 1997; Petersen et al., 2010; Racinais et al., 2014; Racinais et al., 2012; C. J. Tyler et al., 2016) with no effect on sweat K levels (Chinevere et al., 2008; Houmard et al., 1990; Petersen et al., 2010; Saat et al., 2005; C. J. Tyler et al., 2016). This seems logical, as mentioned previously, aldosterone release tracks K levels without reabsorbing it hence the lack of decrease. The other suggested primary pathway for the retention of body water and, subsequently, an increased PV is through arginine vasopressin (AVP) during exercise heat stress (Pryor et al., 2018).

Plasma AVP helps to conserve water by reabsorbing solute-free water (Fellmann, 1992) and the adjustment of secretion supports body water regulation by reducing water excretion and manipulating the feeling of thirst, thereby prompting water consumption (Mudambo, Coutie, & Rennie, 1997; Ramsay, 1989). However, the latter is particularly difficult in protocols that utilise a dehydration acclimation strategy as there is little or no fluid intake. Arginine vasopressin is secreted from the posterior pituitary gland, is also known as an anti-

diuretic, and absorbs solute in the kidneys (Garrett et al., 2011). Elevated sodium concentrations are considered to be the main impetus for secretion during exercise (Convertino, Keil, Bernauer, & Greenleaf, 1981; Garrett et al., 2011; Hew-Butler, 2010). Through this, AVP is believed to retrieve water from the interstitial space into the intravascular space, thereby increasing PV (Pryor et al., 2018).

This may be where the debate takes place. If aldosterone and AVP are considered to be primary pathways while both being regulated by sodium concentrations, then it becomes nearly impossible to discern exactly which fluid regulatory hormone is working. Specifically, if $[aldo]_p$ reacts to high K triggering the reabsorption of sodium, and if AVP is mediated by sodium concentrations, then the two should cancel out one another. If K levels are high, then $[aldo]_p$ could reabsorb enough Na^+ to limit the response of AVP. Conversely, is if AVP reacts to high enough levels of Na^+ then the additional water retrieved this way could prompt a sufficient sweat response to reduce levels of K enough to limit the secretion of $[aldo]_p$ – however, this is merely speculation. Regardless of the mechanism, PV expansion can produce beneficial physiological and functional outcomes (Garrett et al., 2014; Lorenzo et al., 2010; Racinais et al., 2012; Scoon, Hopkins, Mayhew, & Cotter, 2007) such as CV and thermoregulatory responses (Pryor et al., 2018). As mentioned previously, HA induces CV adaptations to heat stress, and it is believed that PV expansion is a contributor to this – specifically increasing or maintaining stroke volume and lowering exercise HR (Garrett et al., 2011; Pryor et al., 2018).

Several methods for measuring PV expansion exist but the more common method is the Dill and Costill (1974) technique (Garrett et al., 2014). This measures the percentage of haemoglobin (Hb) and haematocrit (Hct) in the blood samples routinely taken by finger prick. By measuring the change in percentage change between the two, it can be determined whether PV has expanded or contracted (Dill & Costill, 1974). Patterson and colleagues (Patterson et

al., 2004b) used the Evans blue dye dilution technique in their study to determine PV expansion. However, this is an invasive technique which required participants to have a catheter while exercising whereas the Dill and Costill (1974) technique is substantially less invasive. Garrett et al. (2014) utilised carbon monoxide (CO) dilution – which measures Hb mass – but this method can be noisy when measuring PV expansion. Irrespective of method, measuring Hb and Hct is the fundamental way to assess PV expansion (Garrett et al., 2014; Racinais et al., 2012).

2.1.4 Cellular Mechanisms

Many of the cellular mechanisms behind heat adaptations are not fully understood (Pryor et al., 2018), although an important cellular adaptive response to heat is improved cellular protection from heat stress (Garrett et al., 2012; Garrett et al., 2014; Garrett et al., 2011; C. J. Tyler et al., 2016). This is termed "heat shock response" (HSR) and is facilitated mainly by heat shock proteins (HSP) (Garrett et al., 2014; Garrett et al., 2011; C. J. Tyler et al., 2016). These are thought to protect cells from short-term effects that can cause damage by various stressors (Pryor et al., 2018) - which include hyperthermia, hypoxia, starvation and oxygen stress (Garrett et al., 2011; Gething & Sambrook, 1992).

Heat shock proteins are grouped according to their molecular mass as well as the location of their expression (C. J. Tyler et al., 2016) with the 70 kDa (HSP70) group being the most inducible – sharing common protein sequences but responding to different stimuli (Garrett et al., 2011). Data regarding HSP in response to HA is scarce, but existing data reviewed by C. J. Tyler et al. (2016) seems to indicate that HA can have a trivial effect on extracellular HSP72 levels (+17.7%) (Magalhaes Fde, Amorim, et al., 2010). In contrast, intracellular concentrations reported by Jeukendrup, Saris, Brouns, and Kester (1996) and (Armstrong et al., 2005) showed 110% and 320% increases respectively – suggesting that

intracellular HSP may be more responsive to heat stress coupled with HA than extracellular HSP (C. J. Tyler et al., 2016). In another study, indirectly measuring HSR, resting plasma concentrations of HSP70 doubled across 5-days of acclimation as the acute response was notably reduced by day 5 (Garrett et al., 2014). More work dedicated to the role of HSP in adaptation needs to be completed as there is a lack of conclusive evidence to characterise this aspect of heat adaptation (Garrett et al., 2014; Garrett et al., 2011; McClung et al., 2008; Pryor et al., 2018; C. J. Tyler et al., 2016).

2.2 Protocols

Numerous laboratory protocols have been created with exercise-heat induced hyperthermia as the core principle. Traditionally, continuous workload protocols using exercise in the heat for 100 minutes per day at 40-50% $\dot{V}O_{2max}$ for 10-14 days was suggested to prompt HA adaptations in both untrained and trained athletes (Pandolf, 1998). Considering that most HA generated adaptations are achieved within 4-7 days, researchers are investigating the bare minimum dose of exercise-heat stress to achieve thermal adaptations and performance gains (Pryor et al., 2018). High intensity, short duration exercise-heat stress – for example 75% $\dot{V}O_{2max}$, 30-35 minutes – can achieve similar adaptations in 9 days (Houmard et al., 1990). On the other hand, CH techniques require as few as five days in elite level athletes (Garrett et al., 2009). Current HA best practice guidelines imply at least 60-minute training sessions for 7 d in the heat, preferably longer (14 d) to achieve further performance benefits. Concurrently exercise intensity and rest intervals should be sport specific whilst inducing hypovolemia and hyperthermia (Pryor et al., 2018).

2.2.1 Controlled Hyperthermia

Heat acclimation methods are an important factor to consider and are categorised into three variations: constant work-rate; self-regulated; and CH (Garrett et al., 2019; Garrett et al., 2011;

Nigel AS Taylor, 2000; N. A. Taylor & J. D. Cotter, 2006; C. J. Tyler et al., 2016). The constant work-rate method is commonly used among military institutions due the ease of acclimating larger groups simultaneously however, this is a more standardised method as the relative thermal load will differ between individuals in the same conditions (Garrett et al., 2011; C. J. Tyler et al., 2016). Self-regulated models also allow for the difference in relative thermal load, not due to the conditions, but to the exercise intensity throughout HA, specifically because it relies on the participant to set their own work-rates based on their own individual levels of perceived discomfort (C. J. Tyler et al., 2016). However, this method does have greater practical implications than research focus as it can be difficult to ascertain whether or not the same work load is being placed upon all participants within a cohort (Garrett et al., 2011). Due to difficulties with the constant work-rate and self-regulated methods in identifying the relative thermal strain, and to ensure the successful application of a progressive thermal overload, is it suggested that they may be less efficient compared to the controlled hyperthermia model for inducing optimal adaptations (C. J. Tyler et al., 2016).

Controlled hyperthermia, when compared with the constant work-rate and self-regulated methods, has received an increasing amount of attention in the literature over the past decade. Garrett and colleagues (2011) had stated that CH had received limited attention but recently there has been a surge in studies that have utilised this technique during HA. One reason for this is that CH is believed to offer a more complete adaptation to heat stress than the other two methods (Garrett et al., 2019; Garrett et al., 2011) by reaching and maintaining an internal core temperature of 38.5^{o}C through a combination of active and passive heat stress (Moss et al., 2020). Thus, overcoming the shortfalls of the constant work-rate and self-regulated methods by making it possible to observe and adapt the thermal stress accordingly during acclimation bouts (Garrett et al., 2011; Hessemer, Zeh, & Bruck, 1986; Moss et al., 2020).

Within the more recent literature, a CH approach has been explored with either an absolute increase in T_c (Magalhaes Fde, Passos, et al., 2010) or by reaching a set thermal strain (Garrett et al., 2012; Gibson, Turner, et al., 2015; Moss et al., 2020). Moss et al. (2020) suggest that the issue with using a set increase in T_c – for example, an increase of 1°C – is that because of continuous progressive adaptation, individuals may not exhibit the desired level of heat adaptation. They continue by stating that the use of a set thermal strain overcomes this by ensuring that as adaption occurs, an adaptation stimulus can continue to be provided. That being said, there continues to be some debate over the necessary time needed to prompt a more complete adaptation to the heat. Given that the majority of heat adaptations as a result of HA are achieved within 4-7 days, the recent focus of researchers has been the minimum dose of exercise-heat stress to illicit beneficial thermal adaptations and performance gains (Pryor et al., 2018).

Heat acclimation protocols typically take place over 5 to 14 days (Sekiguchi et al., 2020) and vary in regards to the length of bouts used in a particular protocol. For example, Moss et al. (2020) argues that a 60-minute per day of acclimation model can induce heat adaptations whilst maintaining less-intense exercise throughout the bout – claiming it would be beneficial to athletes in the tapering phase of their training in the lead up to competition. Conversely, other HA literature has indicated a 90-minute HA protocol offers a more complete adaptation as well as a sufficient stimulus to achieve heat adaptation (Garrett et al., 2012; Garrett et al., 2019; Garrett et al., 2009; Garrett et al., 2014; Garrett et al., 2011; Gibson, Turner, et al., 2015; Racinais et al., 2012). Evidence provided by Garrett et al. (2014) showed a reduced CV and thermal strain during extended exercise in the heat after 5 consecutive days of 90-minute CH HA, as well as an adaptive response at a cellular level. A 2 hour per day CH protocol has also been shown to be effective by Turk and Worsely (1974) in a cohort of army personnel (n = 51) in a hot environment (36°C WGBT) and resulted in an estimated 80% of participants

achieving a satisfactory level of adaptation, indicated by an increased CV stability. However, it can be difficult to integrate a regimen of this calibre into an athletes preparation (Garrett et al., 2009; Moss et al., 2020).

2.2.2 Exercise Intensity

Deciding on an appropriate exercise intensity during CH to achieve an approximate 1.5°C rise in core body temperature poses its own challenge. Gibson, Willmott, James, Hayes, and Maxwell (2017) suggest 40°C and 40%RH is enough to achieve the necessary rise in T_c in above-average fit males. When exercise is of equal relative intensity, trained females perform comparable to men in dry heat (Horstman & Christensen, 1982) and a recent publication found CH with dehydration and controlling for menstrual phase produced the desired rise in T_c over 45-minutes in hot conditions (35°C, 60%RH) (Garrett et al., 2019). However, when preferring exercise intensity measures not relative to individual fitness, such as power, thermal sensation (TS), velocity, and rate of perceived exertion (RPE), a slightly lower exercise intensity for females and lesser trained males may be necessary until more data becomes available for these groups (Pryor et al., 2018).

2.2.3 Comparing Acclimation Methods

Determining whether one protocol is better than another is challenge for researchers and practitioners – with very few studies directly comparing HA protocols (Pryor et al., 2018). For example, Gibson, Mee, et al. (2015) found similar adaptations between the constant workload and CH methods in a moderately trained ($\dot{V}O_{2\,max}$ range: 45-50 $mL \cdot kg^{-1} \cdot min^{-1}$) cohort of males ($n$ = 8). Comparing protocols between studies is difficult due to population sample differences (fitness, gender, and sample size), varying environments, and exercise attributes (intensity, duration, mode) (Pryor et al., 2018). As mentioned previously, the factors depict the scale and extent of the adaptation stimuli and, therefore, the degree and time to achieve full acclimation.

In addition, logistical factors such as the number of athletes to acclimate or available equipment may influence the decision to use one HA technique over another (Pryor et al., 2018).

2.2.4 Hydration Strategies

The addition of restricting fluid intake during HA has received an increasing amount of attention in recent years (Akerman, Tipton, Minson, & Cotter, 2016) however the benefits are not universally understood (Garrett et al., 2019; Neal et al., 2016). Nevertheless, many HA studies have explored hydration status of cohorts (H. A. M. Daanen et al., 2018; Garrett et al., 2012; Patterson et al., 2014; Pethick et al., 2019; Schleh, Ruby, & Dumke, 2018) and it is well known that hydration status does have an influence on physiological and performance responses while exercising in the heat (American College of Sports et al., 2007; Sekiguchi et al., 2020). Dehydration whilst exercising in the heat increases the fluid-regulatory, CV, and thermal strain, resulting in increased responses (Kenefick et al., 2007) as well as thirst (Engell et al., 1987; Garrett et al., 2014; Maresh et al., 2004). However, there is still debate over whether or not dehydration strategies positively affect HA (Sekiguchi et al., 2020).

Previous work conducted by Garrett et al. (2014) suggests, through the increased strain on fluid-regulatory mechanisms and their related effects to CV stability, that dehydration during HA increases the stimulus for the body to conserve more sodium and, therefore, retain more water in a dehydrated state. This presumably would force the body to work with the water it has, as opposed to seeking fluid intake and, therefore, when the body has increased body water levels can work more efficiently. However, with guidelines advocating for sufficient hydration, it is the reality for those that partake in HA that some dehydration occurs (Garrett et al., 2014; Greenleaf, 1992; Noakes et al., 1988). This progressive dehydration could in-fact enhance the scale of HA adaptations and so could be of benefit – however prolonged and

excessive dehydration could possibly impair exercise capacity and as well as harm the health of participants (C. J. Tyler et al., 2016).

A recent review by Sekiguchi et al. (2020) exploring hydration (euhydration) and dehydration found four studies that directly compared euhydration and dehydration strategies. They concluded that, regardless of protocol, there was not enough evidence to support dehydration as a benefit as only one study showed greater changes in HR with dehydration bouts compared to euhydration (Sekiguchi et al., 2020). All four studies failed to show differences in PV, core body temperature, and skin temperature – as well as two of the remaining three studies failing to show differences in HR in euhydration versus dehydration trials.

Neal, Corbett, Massey, and Tipton (2016) explored whether restricting fluid intake changed the induction or decay of HA and performance over 7 d using a cross-over design. They concluded that permissive dehydration adequate to prompt a mild, temporary hypohydration did not affect the attainment or deterioration of HA or performance variables, which included an increased peak power output (PPO). However, Garrett and colleagues (2014) determined that permissive dehydration (~1.8%), compared to euhydration, did not impair adaptative responses but added some increased functional adaptations. As a collective, very few well-controlled studies exist to truly ascertain whether adding planned dehydration throughout HA protocols result in an increased or decreased adaptive benefit and performance effect (Akerman et al., 2016).

2.3 VO_{2peak} versus VO_{2max}

$\dot{V}O_{2\,max}$ is defined as the maximum oxygen delivered and utilized during exercise and is characterised by a plateau in oxygen consumption ($\dot{V}O_2$) as exercise intensity increases – and if this plateau is absent, this is considered $\dot{V}O_{2\,peak}$ (Azevedo, 2018; Green & Askew, 2018).

$\dot{V}O_{2\,peak}$ is closely related to $\dot{V}O_{2\,max}$ as it is the highest rate of O_2 uptake during a single "maximal" test (Green & Askew, 2018). Both methods are a means to assess an individuals' cardiorespiratory fitness, however there is debate over which one is the more favourable.

Recently, there has been debate over the use of $\dot{V}O_{2\,peak}$ versus $\dot{V}O_{2\,max}$ and whether one is more effective than the other. For example, as already mentioned, determination of $\dot{V}O_{2\,max}$ is dependant of presence of a plateau in $\dot{V}O_2$ but this plateau is not evident in ramp or step protocols (Azevedo, 2018; Rossiter, Kowalchuk, & Whipp, 2006). Ramp or step protocols would usually be a singular, maximal effort to exhaustion as used in previous works by Garrett *et al.* (2009, 2011, 2014, 2019) and would result in a peak $\dot{V}O_2$. However, Bhammar and Babb (2018) question whether taking the highest $\dot{V}O_2$ and labelling it $\dot{V}O_{2\,peak}$ is methodologically sound without supporting evidence that a higher $\dot{V}O_2$ is not actually possible. That being said, Brugniaux and Perry (2018) endorses the use of using $\dot{V}O_{2\,peak}$, stating that with sufficient familiarisation and motivation, $\dot{V}O_{2\,peak}$ can provide valuable insight into an individuals' cardiorespiratory fitness as well as being an effective method of determining exercise intensity (Perry et al., 2016).

It is said that $\dot{V}O_{2\,max}$, mode-specific $\dot{V}O_{2\,max}$, and $\dot{V}O_{2\,peak}$ provide estimates of individual functional limits with $\dot{V}O_{2\,max}$ representing *the* upper functional limit; mode-specific $\dot{V}O_{2\,max}$ representing *an* upper functional limit; and $\dot{V}O_{2\,peak}$ representing *an* upper functional limit during a single bout (Green & Askew, 2018). From this, it seems reasonable to assume that for studies where cardiorespiratory fitness is either the main focus – if not an important factor – a $\dot{V}O_{2\,max}$ protocol would be appropriate due to the more thorough examination of $\dot{V}O_2$ (Azevedo, 2018). In contrast, for studies where cardiorespiratory fitness is required to

determine whether participants are similar in fitness, it would be reasonable to utilise $\dot{V}O_{2\,peak}$ in a singular test where time and/or logistical problems could occur with appointments, or where there are a larger number of participants to screen (Green & Askew, 2018). Mode-specific $\dot{V}O_{2\,max}$ would be appropriate where the sport is not functionally similar to a traditional treadmill or cycle ergometer trial.

2.4 Rationale

Based on previous research at the University of Hull as well as the current literature, four consecutive days of 90-minutes CH with dehydration was selected for this study. Due to researchers focusing on the minimum dose of HA needed to illicit satisfactory heat adaptation and the majority implementing five consecutive days of HA, four consecutive days was the logical next step to directly compare methods. Controlled hyperthermia was selected as it made it possible to ensure the relative thermal strain was consistent across all bouts of acclimation and between participants – overcoming any progressive adaptive responses that may occur. Rectal temperature was chosen as the measure for T_c as it is the most valid means. As there is limited research into whether or dehydration has an impact on heat adaptations, it was utilised in this study to further add to this lack of available data, although some existing data infers dehydration exacerbates thermal impulse and therefore adaptive response. The Dill and Costill (1974) technique through finger prick was used to assess PV as this method is far less invasive and much more comfortable for the participant. This methodology was adopted to better compare with past studies. An incremental $\dot{V}O_{2\,peak}$ protocol was implemented to assess cardiorespiratory fitness as this was only an indicator of fitness and was not a measured outcome of the HA protocol.

2.5 Hypothesis

The primary hypothesis was that the current protocol would lower physiological markers of adaptation T_{re} and f_c. Secondly, it was hypothesised that the current protocol would reduce the perceived heat stress indicated by a reduction in RPE, TS and TC. Thirdly, it was hypothesised that, as a result of the first and second hypothesis, that performance would improve, indicated by an increased TTE, PPO, and distance achieved and a reduction in time to target temperature (39.5^{o}C).

The fundamental theme for this work was the health and safety of male athletes undergoing acclimation protocols, particularly the reduced heat exposure (4 d) reducing the risk of potential heat illness or physical injury.

3 Methods

3.1 Experimental Design and Overview

Twelve moderately trained males completed this intervention study of four consecutive of short-term heat acclimation with no fluid intake during each bout. Figure 1 presents the experimental period structure.

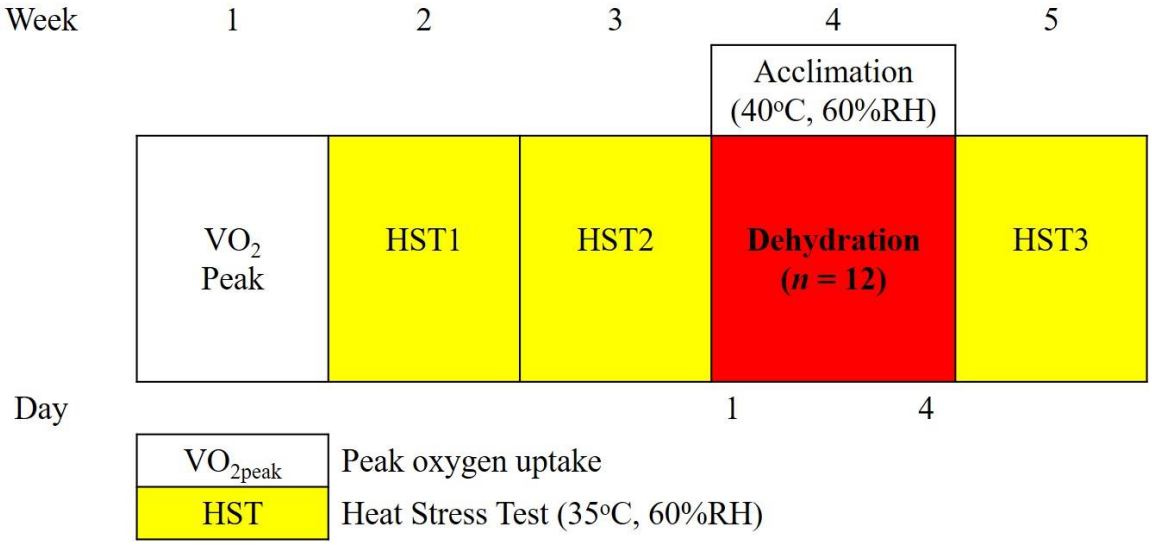

Figure 1: Overview of STHA protocol for moderately trained males.

3.1.1 Experimental Controls

All participants were informed fully of all experimental procedures by both oral and written means. All participants were free from injury and unacclimated to the heat. Experimental periods were conducted outside of the British summer time to minimise seasonal acclimation effects. Heat Stress Tests and acclimation bouts were occurred at the same time of day (8am) to minimise circadian rhythm effects. Participants were asked not to partake in strenuous exercise immediately prior to, and 24h before each session (Garrett et al., 2011). Participants were asked to refrain from caffeine and alcohol consumption at least 12h before each session. Food diaries were not requested as each participant was internally recruited with a vested interest in data produced.

As an added control, participants completed HST1 and HST2 a week apart with HST3 being completed within 7 d of the final acclimation bout to ensure any effects gained had completely dissipated.

3.2 Intervention Study

The intervention study took place over weeks 3-5 (**Figure 1**) with the intervention on week 4.

3.2.1 Participants

Twelve (n = 12) males (mean±SD; age: 35±15yrs; 175.3±4.5cm; 79.7±11.2kg; vo2peak: 47.2±9.9mL·kg^{-1}·min^{-1}) from a varied background of sports – including football and cycling – who took part in training at least twice per week took part in this study. Pre-exercise medical questionnaires and informed consent forms were completed before being recruited – all of which were in good health. Ethical approval was provided by the University of Hull Ethics Committee following World Health Organisation declaration of Helsinki guidelines (No. 1516177 [AMMENDED]).

3.2.2 4-d Heat Acclimation Protocol

The experiment tool place in the University of Hull's Environmental Chamber (Type SSR 60-20H, Design Environment, Gwent, Wales) in 40^{o}C and 60%RH – for 90-minutes per day with no fluid intake. Participants cycled (Monark 824E, Monark Exercise AB, Varberg, Sweden) against a self-selected resistance at 60rpm until reaching the target T_{re} of 38.5^{o}C as quickly as possible. Once achieved, participants ceased exercise and were seated within the environmental chamber either until the 90-minutes was completed, or T_{re} had fallen to 38.4^{o}C. If the latter occurred, the participant resumed cycling until the target temperature was achieved. Elevation of T_{re} to the same point each bout intended to increased workload gradually throughout the acclimation week.

3.2.3 Urine

Urine samples were obtained pre and post exposure on both days one and four of acclimation. From these, urine specific gravity (SG_u) was determined using a calibrated refractometer (Unicron-N, Urine specific gravity refractometer, Atago Co., Tokyo, Japan) (Armstrong *et al.*, 1998); urine colour ($colour_u$) was measured using a urine colour chart (Armstrong *et al.*, 1994); and urine osmolality (osm_u) was measured using an osmometer (Model 3320, Advanced Instruments Inc., Massachusetts, USA). All measures were collected in duplicate and the mean value reported. In the case of two values being substantially different, a third was measure was taken and the mean of the three was reported as the value. All measures were analysed immediately after they were obtained by the same experienced experimenter. When appropriate, the equipment was cleaned with an alcohol wipe between measurements.

3.2.4 Blood

3.2.4.1 Venous Blood Samples

Venous blood samples were taken form an antecubital vein (Vacutainer Precision Glide 21-gauge needle, Becton Dickinson Vacutainer Systems; Wokingham, UK) for the measurement of plasma aldosterone ($[aldo]_p$), plasma cortisol ($[cortisol]_p$), plasma albumin ($[alb]_p$), and heat shock protein 72 (HSP72). Samples were analysed later; hence they were stored using chilled K-EDTA tubes (1.6 $mg \cdot ml^{-1}$) in a -80^oC freezer located in the Sport, Health, and Exercise Science building at the University of Hull.

Analysis of $[aldo]_p$ and $[cortisol]_p$ used the Coat-A Count procedure, using respective assay kits (Aldosterone ELISA kit, Abcam, ab136933; Cortisol ELISA kit, Abcam, ab108665). All samples were analysed in duplicate within the same respective assay kits. Intra-assay coefficient of variation was 8.8% for duplicate measures of $[aldo]_p$ and 12.1% for duplicate measures of $[cortisol]_p$.

3.2.4.2 Plasma Volume

To determine ΔPV, changes in concentrations of haemoglobin (Hb) and haematocrit (Hct) were used, as defined below by Dill and Costill (1974) – where PV_B refers to before dehydration and PV_A to after dehydration:

$$\text{“}\Delta PV, \% = 100\ (PV_A\text{-}PV_B)/PV_B\text{”}$$

Both Hct and Hb were obtained through finger prick pre- and post-bout. Haematocrit was analysed using a microhaematocrit centrifuge (Hawksley & sons, Lancing, UK), and Hb was analysed using a Hb analyser (Hemocue 201+, Radiometer Ltd, Crawley, UK). Each measure was collected in duplicate and the mean value was recorded. In the case of two substantially different values, a third measure was taken, and the mean value determined from the three samples. Both were analysed immediately after they were obtained.

3.2.5 Heat Stress Test

The HSTs were conducted in the same environmental chamber, set to 35ºC 60%RH. The HST consisted of 90-minutes continuous exercise on a cycle ergometer (Daum Electronic Gmbh, Furth, Germany) – using individualised workloads at 40% PPO achieved in the $\dot{V}O_{2\,peak}$ trial. Upon completion, participants were given 10-minutes passive recovery before performing an incremental ramp protocol to exhaustion. Increments were 2% of the original PPO achieved in the $\dot{V}O_{2\,peak}$ trial applied every 30 s, commencing from the individualised workload. Recorded measures from the performance trial were End T_{re}, time to exhaustion, power achieved, and end f_c. Heat stress tests were conducted 1 week apart in order to prevent heat acclimation effect (Barnett & Maughan, 1993).

3.2.5.1 Urine

Urine measures were obtained and analysed as outlined in section **1.2.3**

3.2.5.2 Blood

Blood measures were obtained and analysed as outlined in section **1.2.4.2**.

3.2.5.3 Cardiac Frequency

Cardiac frequency was measured using HR monitor (Polar FS1, Polar Electro, OY, Finland) at baseline and every 10-minutes throughout the trial.

3.2.5.4 Body Temperature

Core body temperature was measured using a rectal thermistor (U thermistor, Grant Instruments Ltd, Cambridge, UK) inserted 10cm past the anal sphincter. Skin thermistors (Type EUS-U-V5-V2, Grant Instruments Ltd, Cambridge, UK) were placed on four sites on the left-hand side of the body before entering the chamber: chest, bicep, thigh, and calf (Ramanathan, 1964). Micropore tape was used to secure the thermistors during exercise. Mean skin temperature ($\overline{T}_{sk}$) and mean body temperature ($\overline{T}_b$) were calculated as:

$$\overline{T}_{sk} = (0.3 \times T_{chest}) + (0.3 \times T_{bicep}) + (0.2 \times T_{thigh}) + (0.2 \times T_{calf})$$ (Ramanathan, 1964)

$$\overline{T}_b = (0.9 \times T_{re}) + (0.1 \times \overline{T}_{sk})$$ (M. N; Sawka, Wenger, & Pandolf, 2011)

Temperature data was recorded at 10-minute intervals from rest using a portable data logger (2020 series data logger, Grant Instruments Ltd, Cambridge, UK).

3.3 Control Study

The control study took place on weeks 2 and 3 (**Figure 1**) with no intervention. HST1 and HST2 were conducted following the methodology outlined in section **1.2.5**.

3.4 Aerobic Fitness Testing

Prior to conducting the trial, height (cm) and mass (kg) were recorded. Participants performed an incremental ramp protocol on a cycle ergometer to determine $\dot{V}O_{2\,peak}$. Starting at 50W, resistance increased by 25W every minute until volitional exhaustion. Breath by breath expired air was collected via a metabolic cart system (Cortex Metalyzer 3B, Cortex Biophysic, Leipzig, Germany) calibrated using a 3L calibration syringe (Hans Rudolph 3L, Cranlea and Co., Birmingham, UK) and calibration gas (5% CO_2, 15% O_2, Cranlea and Co., Birmingham, UK). Participants' RPE and f_c were recorded every minute. All participants received verbal encouragement in waning stages of the test.

The trial concluded when either of 2 conditions were met:

1. The participant voluntarily ended the test.
2. The participant could not maintain >60rpm.

If the participants' rpm fell below 60, they were given the chance to reach >60rpm. Upon falling below 60rpm once more, the trial was concluded.

3.5 Data Analysis

The stress response of dependant measures in STHA and HSTs were analysed using the Shapiro-Wilk test to determine normal distribution. A two-way repeated measures ANOVA was used to determine main effects between day one and day four of acclimation, pre vs post HSTs as well as interaction and effect over time for all dependant measures. Pairwise comparison, LSD correction *t*-tests were used when appropriate through SSPS (IBM SPSS Statistics, Version 25, IBM Corp, Armonk, New York, USA). Significance was defined at <0.05 and reported with *F*-values (*F* [df, Error] = *F*-value, *P*-value) or *T*-values (*T* [df] = *T*-value, *P*-value) where appropriate to define variation among groups through SSPS. The change

in thermal markers on day one to day four of acclimation were analysed using one-way ANOVA, with repeated measures and LSD correction, pairwise comparison *t*-tests to isolate differences between days. Where appropriate, data is reported as mean differences±SD with 95% confidence intervals (95%CI) and the magnitude of effect using Cohen's *d* effects sizes (0.2-0.59 small; 0.6-1.19 moderate; 1.2-1.99 large, 2.0-4.0 very large) using the following equation

$$d = (M - \mu_0) / s$$

where s is the sample SD, and μ_0 is the original number from which the measure of *d* is determined (Cumming, 2013).

4 Results

Eleven of the 12 completed the Control study consisting of HST1 and HST2 with no intervention. Data is representative of 11 of the 12 participants unless specifically stated otherwise. All twelve participants completed the Intervention study, comprising of HST2 and HST3 with four consecutive days of heat exposure in between. All participants provided venepuncture blood samples. Data is representative of n=12 unless otherwise stated due to logistical complications during analysis.

4.1 Acclimation

4.2 Thermal Stress and Strain

Mean±SD for Thermal stress and strain are presented in **Table 1** and total work completed in Figure 1. Measures of T_a and RH indicated thermal stress was consistent across the four days of acclimation. Likewise, T_{re} responses show consistent thermal strain. Time to target temperature was longer on day four than on day one (**Table 1**) therefore, more work was performed on day four than on day one (**Table 1** and **Figure 2**).

Table 1: Mean±SD thermal stress and strain on day one and four of short-term heat acclimation for ten moderately trained males (n = 10).

	Day 1	Day 4	Cohens D	p-value
T_a (°C)	40°C	40°C	-	-
RH (%)	60%	60%	-	-
Mean T_{re} (°C)	38.28±0.19	38.14±0.16	0.70	0.06
Time to T_{re} 38.5°C (min)	37.27±6.89	40.90±8.13	0.43	0.09
Work (KJ)	39.08±8.33	39.56±10.52	0.04	0.92
BM Change (%)	-1.3±0.1	-1.3±0.1	0.01	0.89
%PV Change	-5.78±6.55	-4.85±11.39	0.09	0.82

T_a=ambient temperature; RH=relative humidity; T_{re}=rectal temperature; min=minutes; KJ=kilojoules; %=percentage; PV=plasma volume; °C=degrees celcius. Data presented as mean±SD for ten male participants.

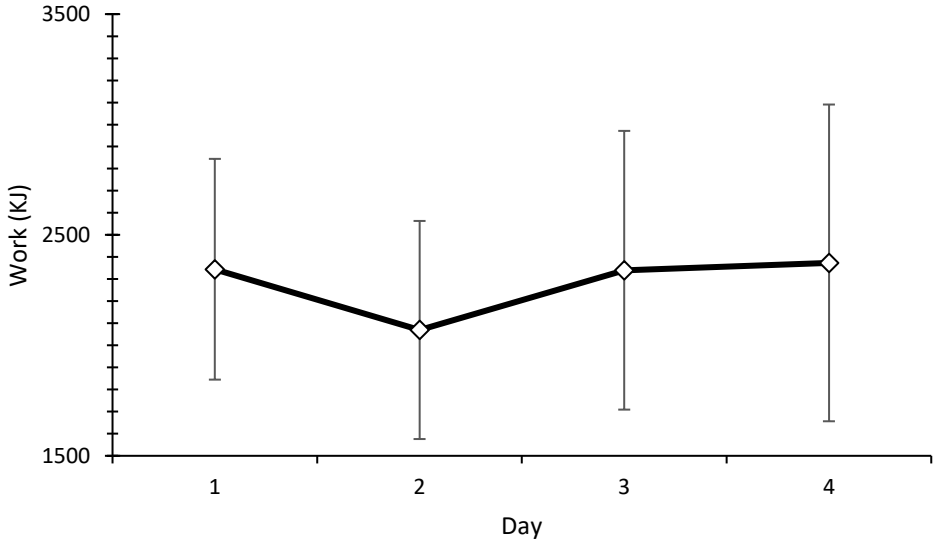

Figure 2: Mean±SD for work completed on days one to four of acclimation after 90-min exposure for 10 moderately trained males.

4.2.1 Urinary Measures

Mean±SD for hydration markers (**Table 2**) $colour_u$, osm_u, SG_u, and BM were measured pre- and post-exercise on day one and four of acclimation (**Table 2**). A significant effect across time (F [1,9] = 140,798, P = <0.001) and a significant interaction effect (F [1,9] = 23.4, P = 0.001) was detected in BM measures. Pairwise comparisons showed significance between post-measures on days one and four (T [9] = 2.8, P = 0.02), as well as in day one pre-post (T [9] =10.9, P = <0.001) and day four (T [9] = 12.3, P = <0.001). A significant effect across time was detected in $colour_u$ (F [1,9] = 22,959, P = 0.001) and further analysis indicated significance on day one pre-post (T [9] = -3.9, P = 0.004) and day four (T [9] = -3.3, P = 0.009). A significant main effect (F [1,9] = 0.041; P = 0.004) and significant interaction (F [1,9] = 0.002; P = <0.001) was detected for osm_u. Further analysis failed to identify specific significance. A significant main effect (F [1,9] = 0.009, P = 0.001) and a significant interaction (F [1,9] = 0.055; P = 0.006) was detected in SG_u measures. Further analysis identified a significant difference between pre- and post-measures on day one (T [9] = -2.3; P = 0.047).

Table 2: Mean±SD for urinary measures of hydration ($colour_u$; osm_u; SG_u) and nude body mass, pre- and post-exposure, on days one and four of acclimation (n = 10)

	Day 1		**Day 4**	
	Pre	Post	Pre	Post
$colour_u$ (units)	2±1	4±1	3±1	4±1
osm_u (mOsm/kg)	401±333	465±286	424±326	485±263
SG_u (units)	1.0110±0.0105	1.0131±0.0099	1.0109±0.0103	1.0125±0.0081
BM (kg)	76.8±6.4	75.0±6.3	76.4±6.4	74.3±6.2

$colour_u$=urine colour; osm_u=urine osmolality; SG_u=urine specific gravity; kg=kilograms; STHA=short-term heat acclimation. Data presented as mean±SD for ten male participants. A two-way repeated measures ANOVA and LSD correction t-tests was used when appropriate to determine differences between pre- and post-exposure, on day one and day four of STHA.

4.2.2 Blood Measures

Mean±SD for blood measures, including percentage change, on day one and day four of acclimation after 90-minutes exposure are presented in **Table 3**.

Table 3: Mean±SD for blood measures and percentage change from pre- to post-exposure on day one and day four of acclimation (n = 10)

	$[aldo]_p$ ($pg{\cdot}mL^{-1}$) (n = 8)	**$[Na^+]_p$ ($mmol{\cdot}L^{-1}$) (n = 10)**	**$[TP]_p$ ($mg{\cdot}mL^{-1}$) (n = 10)**	**$[alb]_p$ ($mg{\cdot}mL^{-1}$) (n = 10)**	**$[cortisol]_p$ ($_ug{\cdot}dL^{-1}$) (n = 8)**	**HSP70/TP ($ng{\cdot}mg^{-1}$)**
Day 1 Acclimation						
Rest	457±201	140.5±2.8	75.6±3.1	757±35	-11±18	0.09±0
End	654±269	144.6±4.7	83.5±2.4	827±45	-10±31	0.08±0
%Change	43%	3%	10%	9%	14%	-10%
Day 4 Acclimation						
Rest	553±427	139.8±3.0	76.1±2.2	763±33	4±26	0.09±0
End	1200±755	141.2±4.4	85.6±4.3	860±84	-15±22	0.08±0
%Change	117%	1%	12%	13%	-503%	-12%

$[aldo]_p$=plasma aldosterone; $pg{\cdot}mL^{-1}$=pictograms per millilitre; $[Na^+]_p$=plasma sodium; $mmol{\cdot}L^{-1}$=millimoles per litre; $[TP]_p$=total protein; $mg{\cdot}mL^{-1}$=milligrams per millilitre; $[cortisol]_p$=plasma cortisol; $_ug{\cdot}dL^{-1}$=micrograms per decilitre. Data is presented mean±SD for eight to ten moderately trained males. A two-way repeated measures ANOVA and post-hoc LSD correction t-tests when appropriate was used to determine differences from pre- and post-exposure, on day one and day four of acclimation.

$[Na^+]_p$ analysis showed a significant effect across time (F [1,9] = 23.1, P = 0.001) as well as a significant main effect (F [1,9] = 9.4, P = 0.013). Post-hoc pairwise comparisons indicated day one measures to be significantly different (T [9] = -2.5, P = 0.033). There was significant effect across time (F [1,7] = 22.5, P = 0.002) detected in $[aldo]_p$ measures. Further analysis showed a significant difference on day four pre-post (T [7] = -4.1, P = 0.005). Similarly, TP analysis indicated a significant effect across time (F [1,9] = 68.4, P = <0.001), with further analysis indicating significance on day one pre-post (T [9] = -7.8, P = <0.001) and day four pre-post (T [9] = -7.2, P = <0.001). Analysis of $[alb]_p$ measurements indicated a significant effect across time (F [1,9] = 26.7, P = 0.001) and further post-hoc tests showed significance for day one pre-post (T [9] = -6.7, P = <0.001) and day four pre-post (T [9] = -4.2, P = 0.002). Cortisol measures showed no significant main, time, or interaction effect from day one to four (P = >0.05). Heat shock protein 70/total protein analysis showed no significant main, time, or interaction effects from day one to four (P = >0.05).

4.3 Heat Stress Test

Measurements were taken at rest and across the 90-minutes protocol at 10-minutes intervals throughout the HSTs. Data is presented for ten males unless stated otherwise.

4.3.1 Control Study

The HST1 versus HST2 was the control trial taken one week apart with no intervention. There was limited change for T_{re}, $\overline{T_{sk}}$, $\overline{T_b}$, f_c, and %PV (P = >0.05). In the incremental performance test, there was limited change in TTE, PPO (P = >0.5).

4.3.2 Intervention Study

The HST2 trial took place one week before the STHA (4-days) with no fluid intake intervention. The post HST3 occurred within seven days of the last acclimation bout.

4.3.2.1 Body Temperatures

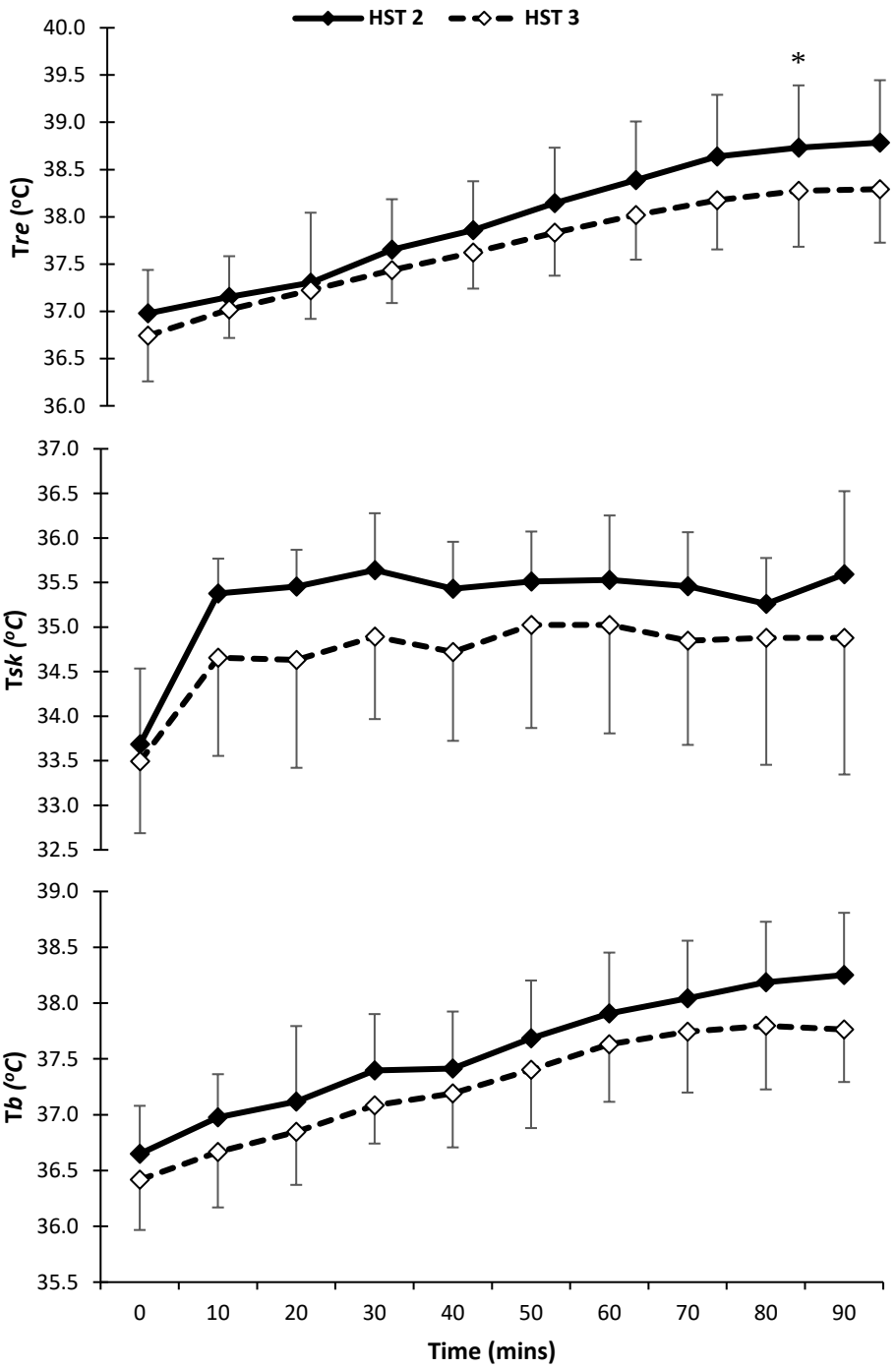

Figure 3: Mean±SD for RPE (upper), Tsk (middle), and Tb (lower) pre- to post short-term heat acclimation in hot conditions (35oC; 60%; n = 12). *P = <0.05 LSD correction t-tests.

Figure 3 presents mean±SD T_{re}, $\overline{T}_{sk}$, $\overline{T}_b$ pre-to-post short-term heat acclimation, in hot conditions (35ºC; 60%RH; n=12).

There was no main effect for T_{re} (F [1, 7] = 3.1; P = 0.121) however, there was a significant effect across time (F [9, 63] = 81; P = <0.001). LSD corrected post-hoc comparisons showed a significant mean difference at 70 (T [11] = 2.5; P = 0.03) min. At rest, T_{re} reduced by 0.24 (-0.54 to 0.07ºC; d = 0.50: small) and at 90 min, reduced by 0.49 (-1.16 to 0.18; d = 0.80: moderate). There was no main effect for $\overline{T}_{sk}$ (F [1, 7] = 2.5; P = 0.16) however, there was a significant interaction across time (F [9, 63] = 13.9; P = <0.001). LSD corrected post-hoc comparisons failed to show a significant mean difference at any time point (P = >0.05). At rest, $\overline{T}_{sk}$ reduced by 0.19 (-0.94 to 0.56ºC; d = 0.23: small) and at 90 min reduced by 0.98 (-2.66 to 0.70ºC; d = 0.55: small). There was no main effect for $\overline{T}_b$ (F [1, 7] = 2.9; P = 0.132) however, there was a significant effect across time (F [9, 63]) = 61.1; P = <0.001). LSD corrected post-hoc comparisons failed to show a significant difference at any time point (P = >0.05).

4.3.2.2 Cardiac Frequency and Percentage Change in Plasma Volume (%PV)

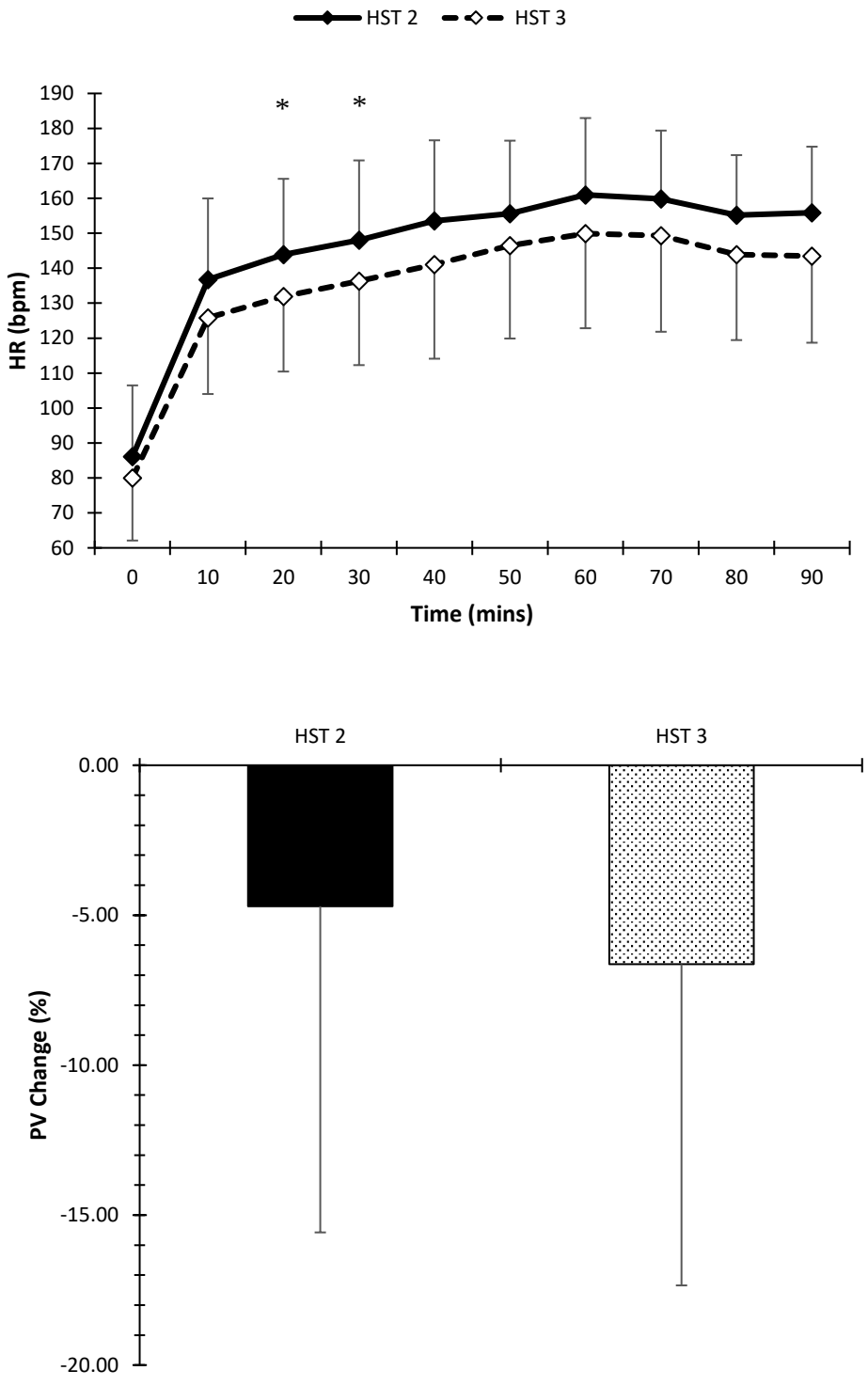

Figure 4: Mean±SD for cardiac frequency (upper) and %PV (lower) pre- to post short-term heat acclimation in hot conditions (35oC; 60%RH; n=12). *P = <0.05 post-hoc LSD correction t-tests.

Figure 4 presents mean±SD for f_c and %PV pre- to post acclimation in hot conditions (35ºC; 60%RH).

There was no main effect for f_c (F [1, 7] = 2.6; P = 0.154) however, there was a significant effect across time (F [9, 63] = 96.1; P = <0.001). LSD corrected post-hoc comparisons indicated significant differences at 20 (T[11] = 2.6; P = 0.026) and 30 (T [11] = 2.5; P = 0.028) minutes. Resting f_c reduced by 6 (-19 to 6 b·min^{-1}; d = 0.32: small), while end-exercise f_c reduced by 12 (-33 to 8 b·min^{-1}; d = 0.56: small). There was a decrease from pre- to post acclimation for %PV by 47% (-13.14 to 0.17; d = 0.18; moderate; P = 0.617) although insignificant.

4.3.2.3 Perceptual

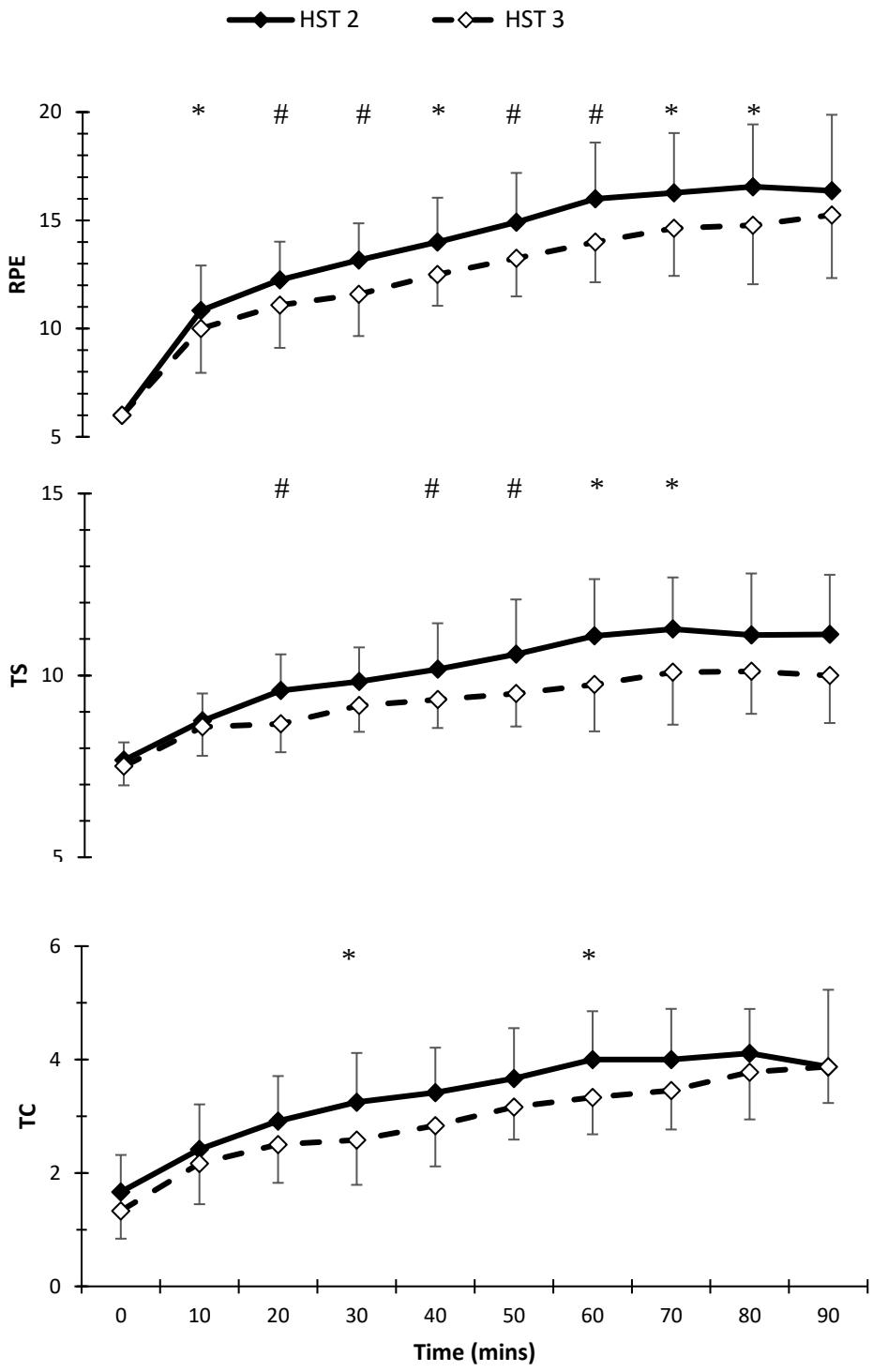

Figure 5: Mean for RPE (upper), TS (middle), and TC (lower) pre- to post short-term heat acclimation in hot in conditions (35oC; 60% RH; n=12). *P = <0.05; # = P = <0.01 LSD correction post-hoc t-tests

Figure 5 presents mean±SD for RPE, TS, and TC pre- to post acclimation in hot conditions (35^{o}C; 60%RH).

There was a significant main effect for RPE (F [1, 7] = 6.2; P = 0.042). There was also a significant effect across time (F [9, 63] = 58.2; P = <0.001). LSD corrected post-hoc comparisons indicated significant differences at 10 (T [11] = 2.3; P = 0.044), 20 (T [11] = 3.4; P = 0.006); 30 (T [11] = 3.5; P = 0.005), 40 (T [11] = 2.9; P = 0.014), 50 (T [11] = 3.9; P = 0.003); 60 (T [11] = 3.4; P = 0.006), 70 (T [10] = 2.8; P = 0.02), and 80 (T [8] = 2.5; P = 0.035) minutes. Rate of perceived exertion reduced at 90-min by 1 (-2 to 0; d = 0.35: small).

There was no main effect for TS (F [1, 7] = 4.8; P = 0.065). However, there was a significant effect across time (F [9, 63] = 22.1; P = <0.001). LSD correction post-hoc comparisons showed significant differences at 20 (T [11] = 4.8; P = 0.001), 40 (T [11] = 3.1; P = 0.01); 50 (T [11] = 3.5; P = 0.005), 60 (T [11] = 3.1; P = 0.01), and 70 (T [10] = 2.6; P = 0.029) minutes. Thermal sensation reduced at 90-min by 1 (-1 to 0; d = 0.76: moderate).

There was no main effect for TC (F [1, 7] = 1.3; P = 0.293). However, there was a significant effect across time (F [9, 63] = 36.8; P = <0.001). LSD correction post-hoc comparisons showed significant differences at 30 (T [11] = 1.4; P = 0.013) and 60 (T [11] = 2.6; P = 0.025) minutes.

4.3.2.4 Incremental Performance Trial

The incremental performance trial (**Table 4)** was performed post 90-minutes exercise and 10-minute rest interval, pre- to post STHA in hot conditions (35^{o}C; 60%RH).

Table 4: mean+SD TTE, PPO, HR End, and T_{re} End for twelve male participants (n = 12)

	HST 2	**HST 3**	**p-value**	***d***
TTE (s)	323±333	465±235	0.04*	0.83
PPO (W)	137±128	213±77	0.03*	0.72
HR End ($b \cdot min^{-1}$)	177±14	178±17	0.25	0.03
T_{re} End	38.64±0.32	38.52±0.56	0.31	0.25

*TTE=time to exhaustion; PPO=peak power output; HR=heart rate; T_{re}=rectal temperature. Significant differences by paired t-test are shown by *.*

There was a significant main effect for TTE (T [11] = -2.4; P = 0.04) and PPO (T [11] = -2.5; P = 0.03) but not T_{re} End or HR End (P = >0.05).

4.3.2.5 Heat Stress Test Performance Overview

Figure 3 presents HST performance for twelve moderately trained males in hot conditions, pre- and post- STHA (35°C; 60%RH; *n* = 12). The percentage of participants that completed the 90-min continuous exercise period increased from ~60% to 100%, with 92% progressing to the incremental exercise trial.

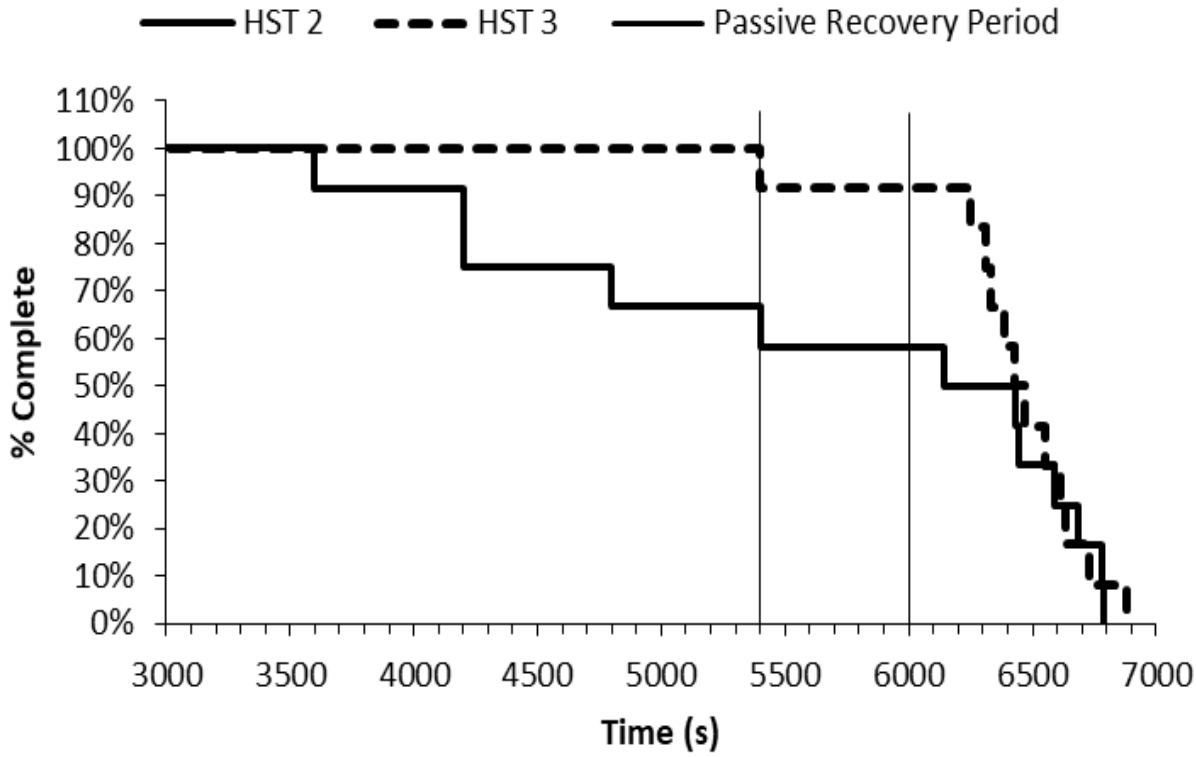

Figure 6: Completion rate from pre- to post short-term heat acclimation for twelve moderately trained males in hot conditions (*n* = 12; 35°C; 60%RH).

5 Discussion

The aim of the current study was to determine the effectiveness of a 4 d HA regime with no fluid intake on performance in hot and humid conditions. Physiologically, participants demonstrated partial adaptation to the heat evidenced by a reduced resting rend T_{re} and lowered exercising T_{re} (**Figure 3**). Mean exercising f_c reduced over time at 20 and 30 minutes while perceptual measures indicated a substantial reduction in the perception of thermal stress. Exercise performance (**Figure 6**) indicates that the main improvement was found during the 90-minute continuous exercise stage of the HST – where all participants reached at least the 90-minute mark compared to pre-acclimation. However, there was a minor change in incremental performance. Heat-stress response measures indicated negligible adaptation pre to post-intervention. Based on the current data, and previous work, adaptive response appears to vary despite similar durations of heat exposure.

5.1 Effectiveness of Short-Term Heat Acclimation

5.1.1 Body Temperatures

This HA protocol successfully reduced resting T_{re} (-0.24; -0.54 to 0.07°C) and resting f_c (-6; -19 to 6bpm) after 4 d of heat exposure, at the time of the HSTs. Both responses echo previous work which reported that these adaptations occurred rapidly (Tyler *et al.*, 2016; Moss *et al.*, 2020). A lower resting T_{re} has been suggested as an important indicator of successful HA regimens, chiefly as it delays critical exercise capacity limiting high-core body temperatures in the heat (Gonzalez-Alonso et al., 1999; Moss et al., 2020; Tucker et al., 2004). Inducing a lower resting T_{re} through HA is important for individuals in uncompensable heat stress by widening the core temperature band at the time of day heat-exercise bouts occur (Akerman *et al.*, 2016).

The reductions in resting T_{re} (-0.24±0.48°C) are greater than mean changes reported in a recent meta-analysis (0.17±0.12°C) (Tyler *et al.,* 2016), as well as previous 5 d isothermic studies (Garrett *et al.,* 2012, 2014; Neal *et al.,* 2016; Moss *et al.,* 2020), 5 d high-intensity (Reeve *et al.,* 2019), and 5-7 d fixed intensity STHA (Reeve et al., 2019; Schleh et al., 2018). One possibility for this occurrence – as well as the larger variation within the measurements in this study – is considerable reductions in resting T_{re} which may be explained by a lower training status of one, or several participants in this study compared to previous literature, where minor reductions in resting T_{re} were reported in higher trained individuals (Garrett *et al.,* 2012, 2014; Neal *et al.,* 2016; Moss *et al.,* 2020). Additionally, where Moss and colleagues (2020) indicate a 5 d programme, it could be perceived as a 6 d programme as their first HST occurs the day immediately before the first acclimation bout and utilises the same conditions as their acclimation sessions (40°C; 50%RH).

Exercising T_{re} indicated a delta change across time, specifically at the 70-minute (**Figure 3**: upper) time-point. This is indicative of a reduced physiological strain experienced by participants post-intervention (Moss *et al.,* 2020). The notable change observed could possibly be due to the lower resting T_{re} increasing the range of core temperature available during exercise in the heat (Akerman *et al.,* 2016). More efficient heat loss mechanisms, such as sweating, occurring at a lower core temperature adds to the reduction in thermal strain (Moss *et al.,* 2020). This is evidenced by the number of participants in the current study who did not complete the 90-min continuous exercise bout in HST2 but did in HST3 (**Figure 2**). This echoes the findings of Mackay, Patterson, Jeffries, and Waldron (2018) who found that 5 d of HA was adequate to improve time-to-exhaustion in a rugby league specific HA regimen – albeit this was observed in temperate conditions. Compared to a previous 5 d study, more significance was observed across time (45-min), as well as main effects between trials (Moss *et al.,* 2020).

5.1.2 Cardiac Frequency

Resting f_c was unaffected by HA, however there was an effect across time, specifically at the 20 (P = 0.026), and 30 (P = 0.028) minute time points. Data from a recent meta-analysis infers that HA offers moderate benefits on lowering resting f_c (Tyler *et al.,* 2016), while data from the current study indicates a large effect on resting f_c (-6; -19 to 6 bpm; d = 1.49: large). It is widely accepted that f_c adaptations occur rapidly, typically within 4-5 d (Tyler *et al.,* 2016). As with T_c, participants' training status affects the magnitude of adaptation that occurs, with lesser trained individuals undergoing a greater degree of adaptation (Tyler *et al.,* 2016; Garrett *et al.,* 2014).

Exercising f_c was stabilised across the 90-min (**Figure 4**: upper). Similar 5 d work shows a significant difference at early time points in relation to the current study. Although Moss and colleagues (2020) only recorded 45-min of exercise, their significant values occur at similar time-points to the present data – 20, 30, and 40-min compared to the 10, 20, and 30-min time points in the current study. This is despite the current protocol duration of 4 d. Although, as mentioned previously, Moss and colleagues' (2020) 5 d regimen could arguably be considered as 6 d of exposure.

Typically, increased f_c stability is accompanied by PV expansion which aids with the reduction of CV strain during exercise-heat bouts (Tyler *et al.,* 2016; Moss *et al.,* 2020). In addition, data from the meta-analysis indicates that PV expansion is common following HA regardless of methodology (Tyler *et al.,* 2016). However, this is not the case in the current study where mean PV decreased further, contradicting previous work. Training status of participants has been known to affect the magnitude of adaptation as higher trained, endurance athletes already possess expanded PV (Akerman *et al.,* 2016). Conversely, it is suggested that HA does not improve the retention of PV during exercise in the heat (Akerman *et al.,* 2016;

Pethick *et al.,* 2019; Neal *et al.,* 2016; Garrett *et al.,* 2014), potentially justifying the findings in the current study.

Schleh and colleagues (2018) describe the relationship between PV expansion and the hormone $[aldo]_p$. They observed a significant decrease in aldosterone levels both pre- and post-exercise following acclimation contradicting previous beliefs that HA would promote the release of the hormone enabling PV expansion. They attributed this to an already increased PV at rest and therefore limited further expansion (Schleh *et al.,* 2018). The current study contradicts the work of Schleh and colleagues (2018) as increases in resting and post-exercise (P = <0.01) $[aldo]_p$ concentrations on day four. Similar previous work conducted by Garrett *et al.* (2014) found a comparable relationship between increases in aldosterone and PV expansion, suggesting a positive feedback mechanism because of increasing plasma osmolality caused by dehydration on $[aldo]_p$ (Schleh *et al.,* 2018; Garrett *et al.,* 2014).

5.1.3 Perceptual Adaptations

Participants in the current study felt more thermally comfortable and perceived their efforts as less strenuous post HA compared to pre (**Figure 4**). This is consistent with previous work which suggests participants felt more thermally comfortable after 5 d of heat exposure (Moss *et al.,* 2020). It also suggests that T_{re} and $\overline{T}_{sk}$ are key factors of thermal perceptions. This is true for the current study, as the affects can be clearly seen in **Figure 4**, concurrently with the observed decreases in T_{re} and $\overline{T}_{sk}$ seen in **Figure 3**. The current data indicates the 4 d programme is just as effective previous 5 d isothermic data, although longer durations of >7 d offer an additional benefit (Moss *et al.,* 2020).

Reducing the perception of effort has the potential to increase the capacity of exercise, particularly in subsequent heat-exercise bouts (Tyler *et al.,* 2016). In a recent meta-analysis, it is suggested that a lower RPE empowers participants to tolerate steady-state – or continuous

exercise-bouts such as in the current study – for longer, as can be seen in **Figure 2**. Data showing the lowering of RPE was only present in three studies (Tyler *et al.*, 2016), although now with the addition of Moss and colleagues (2020), and the current study, RPE is shown to be lowered consistently throughout STHA (4-7 d). Willmott and colleagues (2017) support this as during their four session acclimation period – which took place with four daily sessions and two twice daily session groups – observed a lowering in RPE with concurrent reductions in T_{re}. In addition, they partly attribute performance improvements to improved comfort levels related to lower RPE and the same fixed exercise intensity in the respective groups' final sessions (A. G. B. Willmott et al., 2017). The perceptual adaptations found in previous STHA work – as well as this study – could be representative of a reduced tendency to select lower exercise intensities in the heat, as well as possibly maintaining decision-making and cognitive functions during race conditions, underscoring the importance of conscious behavioural thermoregulation during endurance performance in the heat (Flouris & Schlader, 2015; A. G. B. Willmott et al., 2017).

5.1.4 Performance

5.1.4.1 HST Performance

Short-term heat acclimation was effective at increasing the mean performance of participants as seen in **Figure 2**. The completion rate of the 90-min continuous exercise part of the trial increased from ~60% to 100% from pre- to post acclimation. **Figure 2** was generated by using the Kaplan-Meier survival trend analysis. Whilst this statistical tool is more commonly used to examine differing methods while observing for a particular outcome, this is not the case for **Figure 2**. The way the Kaplan-Meier survival analysis has been used in this context is not as a statistical tool, but as a unique way to visualise the performance of the group from the first second to the very last. To the authors knowledge, this has not been used for acclimation studies

in this way before, therefore, contributing to already existing knowledge and practices. As a new form of data presentation within heat-acclimation at this stage, no statistical data was generated from this method and is used solely as a visual aid.

5.1.4.2 Continuous Exercise Performance

Although not an outcome measure, continuous exercise performance showed the greatest improvement post-acclimation. **Figure 2** shows clearly that pre-acclimation performance was hampered in the hot conditions of the HSTs (35ºC; 60%RH) whereas post-acclimation, performance improved to the extent that all participants completed the 90-mins steady state exercise. Two possible explanations can be applied to the improvement observed in the current study.

Firstly, training status of the participants varies within the group. Those who are lesser trained within the current group may have experienced a greater magnitude of physiological adaptation compared to their higher trained counterparts (Garrett *et al.*, 2012, 2014; Neal *et al.,* 2016; Moss *et al.*, 2020). Secondly, the lesser trained participants may have adapted their behaviour more radically compared to the higher trained individuals as they may have been more unfamiliar and less experienced with the demand of endurance exercise. Whereas those who are endurance trained possess some adaptations before they undergo acclimation (Pryor *et al.,* 2018; Garrett *et al.,* 2012; 2014; Moss *et al.,* 2020; Tyler *et al.*, 2016). Both these statements reflect previous work that physiological adaptations provide an increased buffer before reaching performance compromising core temperatures (Akerman *et al.,* 2016) and perceptual/behavioural adaptations enhance participant performance during steady-state exercise performance in the heat (Tyler *et al.*, 2016).

5.1.4.3 Time to Exhaustion Performance

Performance measures obtained post-intervention (**Table** 4) showed significant increases in TTE (P = 0.04) and PPO (P = 0.03) but not in end f_c (P = 0.24) or end T_{re} (P = 0.30). While there is no change in mean end T_{re} and f_c, this is in fact indicative of improved performance as the participants are achieving the same core temperatures and f_c as in the pre-intervention HST just over an increased time period and at a higher work rate than pre-intervention. Due to the varying methodologies used in the application of HA (Pryor *et al.*, 2018), it is difficult to compare directly to other performance data. However, other previous STHA (≤7 d) performance data indicate improved performance capability in varying hot conditions. Garrett and colleagues (2012) observed a mean decrease of 4 s in time to completion in untrained and moderately trained males (P = 0.02) while Garrett *et al.* (2009) observed a 106 s increase in TTE post-STHA (5 d) (P = 0.001) and Neal *et al.* (2016) found no increase in time to completion (P = 0.38) but did observe an increase in mean PPO (P = 0.056) albeit this was deemed insignificant. The studies used isothermic methodology like the current study but utilised different performance methods.

5.2 Heat-Stress Response

5.2.1 Cortisol

Cortisol is frequently described as an indicator of physical and psychological strain (Moss *et al.*, 2020). Previous work that observed $[cortisol]_p$ responses noted increased concentrations post initial exercise-heat stress (Moss et al., 2020; Silva et al., 2019) and that was the case in the present study (+14%). In contrast to several previous studies that found an attenuated increase in session $[cortisol]_p$, the current study observed a decrease in mean $[cortisol]_p$ concentrations of over 500%. Moss and colleagues (2020) advise evaluating other more established methods of determining heat adaptation as the variation between and within studies

is considerable, as evidenced with the figures in the current study. With a decrease of over 500% (**Table 3**), the values presented offer speculation to the reliability of cortisol as a measure of adaptation because of the variety of within-subject differences, or because of a technical difficulty obtaining a measurement.

5.2.2 Heat Shock Proteins

The findings in the present study found limited change in HSP72/TP response to exercise-heat stress. This is consistent with previous work – albeit data is very limited – which suggests HA has a trivial effect on increasing extracellular concentrations of HSP72 (Tyler *et al.*, 2016). However, when exploring intracellular concentrations of HSP, increases of 110% (Jeukendrup et al., 1996) and 320% (Armstrong et al., 2005) have been reported (Tyler *et al.*, 2016). The present study contributes to this lack of existing data by suggesting HA has very little effect on extracellular HSP concentrations as an adaptive response. For more HSP data to be contributed, researchers must secure funding to analyse HSP, as the equipment and consumables are expensive.

5.2.3 Fluid Retention

The fundamental component of using the CH technique is that participants experience the same thermal load, and that was the case in the current study. Experiencing mild hypohydration of ~1.3% BM in both the pre- and post-intervention trials (**Table 1**), participants had not yet adapted to retain more fluid. Blood data during acclimation (**Table 3**) indicates a meaningful change in $[Na^+]_p$ – which is responsible for aiding fluid retention (Garrett et al., 2019) – on day one and not on day four. This is despite an increase in resting $[aldo]_p$ on day four. Also, post-exercise $[aldo]_p$ increased significantly from resting concentrations on day four ($P = 0.005$). To see this increase in $[aldo]_p$ concentrations without increases in $[Na^+]_p$ is inconsistent with previous data (Allsopp, Sutherland, Wood, & Wootton, 1998; Brandenberger, Candas,

Follenius, & Kahn, 1989; Francesconi et al., 1993; Garrett et al., 2019) but is not universal (Garrett et al., 2012). This is unexpected as the notable effects of $[aldo]_p$ is the retention of $[Na^+]_p$, thereby retaining water from the urine to maintain extracellular volume and, consequently, blood volume (Garrett *et al.,* 2012; 2019). Garrett and colleagues' (2012) observation of PV increase without a concurrent $[Na^+]_p$ increase was attributed to the increase in PV itself. As PV had already increased (4.5%), it may not have been a necessary function to then attempt to retain more water. Interestingly, the absence of such a change on day four similar to day one when taking into account $[aldo]_p$ and PV measures could be related to the reduced heat exposure compared to previous studies (Allsopp et al., 1998; Brandenberger et al., 1989; Francesconi et al., 1993; Garrett et al., 2019). Although, as mentioned previously, there is evidence to suggest this may not be the case (Garrett *et al.,* 2012).

5.3 Relationship between Adaptive Response and Exposure Duration

With the wide variety of protocols, the question is raised regarding the duration and structure of the heat exposure imposed. The more obvious variation between studies is the number of daily exposures outlined by Tyler and colleagues (2016). For example 4 d (Petersen et al., 2010; Piwonka & Robinson, 1967; A. G. B. Willmott et al., 2017), 5 d (Garrett *et al.,* 2009; 2012), 6 d (Fujii et al., 2012; Fujii et al., 2015; Racinais et al., 2012)and 7 d (Buono et al., 1998; Houmard et al., 1990) have been used previously. However, the less obvious variation between studies is the duration of the daily exposure. For example, Garrett and colleagues (2009; 2012) employ a 90-min duration – the same as the current study – whereas other studies range from 30-min to 120-min (Tyler *et al.,* 2016), with one study imposing a 240-min duration over 5 d (Racinais et al., 2012). This then raises the question about what is more important when designing and implementing an acclimation protocol: Is the number of daily exposures more important than the duration of the exposure itself? Or rather a combination of the two?

Pryor and colleagues (2018) suggest exercise intensities and rest periods should be sport specific – consequently, should duration and number of daily exposures follow suit? If the participant(s) are highly trained, endurance athletes then it would be futile to design a short duration (<60-min) with fewer daily exposures (<5 d). This would be due to the training status of the participants as higher trained athletes already possess physiological and behavioural adaptations to cope with the heat (Garrett *et al.,* 2012; 2014; Neal *et al.,* 2016; Moss *et al.,* 2020). Hence, it may be difficult to induce an adaptive stimulus enough to encourage adaptation – particularly if incorporating the CH technique which requires achieving the target temperature of 38.5^{o}C (Pryor *et al.,* 2018; Moss *et al.,* 2020). It may be practical to increase the duration upwards of 60-min – as well as additional daily exposures – to allow more time to achieve either the target temperature, or to induce a sufficient adaptive stimulus (Pryor *et al.,* 2018; Moss *et al.,* 2020; Tyler *et al.,* 2016).

On the other hand, for lesser trained individuals, it may be more practical to reduce the duration of heat exposure at first as the magnitude and rate of adaptations are greater in lesser trained individuals (Garrett *et al.,* 2012; 2014; Pryor *et al.,* 2018; Moss *et al.,* 2020; Tyler *et al.,* 2016). Therefore, shorter sessions of ≤60-mins over ≤5 d may be enough to induce physiological and perceptual benefits. In the current study, 90-min per day over 4 d was sufficient to encourage partial adaptation in moderately trained athletes – comparable with previous work over 5 d which showed greater magnitude of adaptations (Garrett *et al.,* 2012; 2014; Moss *et al.,* 2020; Tyler *et al.,* 2016).

When considering the duration and number of exposures, it may be worth noting the total exposure time. For example, in the current study, participants were exposed for a total of 6 h over 4 d (90-min per day). In other studies, such as Moss and colleagues' (2020) recent publication, participants were heat exposed for a total of 5 h over 5 d, arguably 5 h 45 min over 6 d due to study layout. Similarly, previous work by Garrett *et al.* (2012; 2014) implemented a

total of 7.5 h over 5 d. For the current study to produce results comparable to previous work with more daily exposures would seem to indicate that the length of a single exposure and number of repeated daily exposures are linked. Hence, it would be conceivable to explore and compare various acclimation regimens by length of total exposure time as opposed to number of daily doses – as shown by the current 6 h over 4 total exposure time compared to 5 h over 5 d/5 h 45 min over 6 d.

6 Limitations

Compared to a vast majority of previous work, it is rare find a study with a large number of participants similar to that in the current study. Due to the logistics of undertaking studies of this calibre, it can be difficult to accumulate a large enough data set to truly identify benefits of an acclimation protocol. The current work was conducted over a 2 year period, which emphasises the commitment needed to produce this study and others like it. Each individual was required to commit to a total of approximately 24 h in the laboratory – raising the issue of working around other commitments. In some cases, researchers come and go midway through a project. Luckily, the core researchers were responsible for the complete data collection process from start to finish. This made data collection seamless due to a developed routine.

Participant recruitment was handled internally, with participants being individuals based or associated with the University of Hull – either through being a current student or being associated with a member of staff. This reduced the likelihood of participant drop-outs as there was a personal or professional interest in the data being collected. Without this, the current work would have struggled to recruit externally as other commitments such as work, family etc. would have made completion within the required time frame to be able to efficiently control the experiment would have been much more difficult. On the other hand, in the future, recruitment could be improved in terms of demographics such as fitness, and anthropometric aspects such as training status, and overall similarity between participants.

Due to spatial limitations of the environmental chamber, testing periods were staggered. Groups of participants were limited to four participants per testing period. Ideally, and to increase participant numbers in the future, a bigger chamber would be useful in allowing more participants to be undergo acclimation at a given time. For safety, however, more researchers

would be required for participant monitoring. Additional costs would be more than likely incurred the likelihood of acclimating a group of 10+ participants would prove challenging.

7 Practical Applications

Due to the reduced exposure throughout acclimation using 4 d, there is a reduced risk to participant safety as opposed to using a 5 d protocol. This offers participants an extra day of rest to recover from the stress of undertaking acclimation, with post-intervention HST's occurring the following week. This also reduces the commitment needed to complete the intervention as 4 d can be easier to accommodate during a working week compared to a 5 d protocol typically requiring a full weeks' commitment.

For events such as the Marathon des Sables, a typical HA protocol of 5-7 d may be stressful on the athletes in the lead up to the event itself. Typically, athletes taper in their training in the lead up to an event while HA is most efficient as close to an event as possible. This protocol offers sufficient adaptive potential – be it physiologically or perceptually – and does not require a full week of training the week before an event. Ideally, participants competing in the Marathon would travel to the location as early as possible in to acclimatise naturally. However, this is not always possible. This protocol could be conducted the week before – on the Monday for example – and athletes can travel to the location on the Friday and still be able to acclimatise naturally for the days leading up to the event. Essentially, this 4 d protocol could be used as a "kick-start" for adaptations and experience of exercise in the heat before the event itself. While a 5-7 d, or even a 7-15 d protocol has their advantages with increased exposure time and, therefore, increased adaptive potential, the current 4 d protocol could be considered the bare minimum in the event of cost, logistical or commitments issues hampering an athletes preparation.

A 4 d protocol would also reduce the strain on researchers. While the focus of each acclimation project is focused on the participants, and obviously so, the researchers are still required to enter the chamber with the participants. This imposes similar stresses onto the researchers

themselves without the luxury of exercise to induce an adaptive stimulus. While this may not be important in terms of data collection, a 4 d protocol will also reduce the likelihood of researchers experiencing heat-related illness or discomfort. After all, researchers are not simply immune to environmental stressors due to not exercising. With testing periods based in the British summertime, for example, researchers do not acclimate as well, if at all, while conducting the intervention. Hence, a 4 d programme would reduce the strain on researchers too and therefore reducing risk of any health and safety concerns for everyone involved compared to HA protocols of a longer duration.

8 Future Research

Based on the current work, there a several avenues available for future research prospects. Such as comparing varying age groups of similar training status. Little research exists that directly compares different age groups that have completed identical protocols. This would add to the understanding of the effect of age on performance in hot conditions. Similarly, exploring the differences in heat response between genders is particularly limited in availability. There are studies that exist that explore female responses to heat-stress such as Garrett *et al.* (2019). However, there is a lack of direct comparison between males and females undergoing the same protocol, especially with no fluid intake. This would be of interest to the scientific community, particularly to female athletes that are receiving an increasing amount of media exposure and support from professional organisations. An exploration into the effects of aging in females' response to exercise would be also warranted. Garrett and colleagues' (2019) study involving females who use contraception found an effect of certain contraceptive pills that varied between certain brands of pill. However, there exists little to no data involving an older, post-menopausal female population.

Another avenue of future research would be to directly compare varying methods of acclimation to truly determine whether a certain method if more productive compared to another with the same population. An example may be a twice daily protocol (Wilmott *et al.*, 2017), a 4 d isothermic protocol with no fluid intake - such as the current study, or a 5 d protocol with fluid intake using a fixed intensity workload contrary to the current study. Varying populations could be recruited ranging from sedentary to highly trained individuals, varying sports could be examined, or various ethnicities could be recruited and compared against each other through various protocols.

9 Conclusion

In summary, STHA (4 d) with no fluid intake was effective in enhancing performance capacity in hot and humid conditions in moderately trained males. Classic markers of physiological heat adaptation indicated partial adaptation to the heat, although the training status of one or more participants may have influenced the magnitude of adaptations observed. Short-term HA induced much greater perceptual changes in all participants**.** Restricting fluid intake was adequate in increasing the physiological and perceptual stress experienced by participants thereby encouraging adaptation to the environment. In terms of cellular heat stress response, additional exposures may be required to prompt this level of adaptation, Regardless of the level of physiological adaptations, the behavioural adaptations coupled with the perceptual benefit brought about by 4 d isothermic STHA was enough to illicit improved performance and, by association, improved thermotolerance in hot conditions.

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