Effect of short-term heat acclimation with permissive dehydration on thermoregulation and temperate exercise performance

Running Head: Heat acclimation and temperate exercise

Original Investigation

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Abstract

We examined the effect of short-term heat acclimation with permissive dehydration (STHADe) on heat acclimation (HA) and cycling performance in a temperate environment. Ten trained male cyclists (Mean(SD) maximal oxygen uptake: 63.3(4.0)mL·kg^{-1}·min^{-1}; peak power output [PPO]: 385(40)W; training: 10(3)hours·week^{-1}) underwent a STHADe programme consisting of five-days of exercise (maximum 90mins·day^{-1}) in a hot environment (40°C, 50%RH) to elicit isothermic heat strain (rectal temperature 38.64(0.27)°C). Participants abstained from fluids during, and 30-minutes after, HA sessions. Pre- and post-STHADe HA was evaluated during euhydrated fixed-intensity exercise (60mins) in hot conditions; the effect of STHADe on thermoregulation was also examined under temperate conditions (20mins fixed-intensity exercise; 22°C, 60%RH). Temperate cycling performance was assessed by a graded exercise test (GXT) and 20-km time trial (TT). STHADe reduced thermal and cardiovascular strain in hot and temperate environments. Lactate threshold (Δ=16(17)W) and GXT PPO (Δ=6(7)W) were improved following STHADe ($P<0.05$), but TT performance was not affected ($P>0.05$), although there was a trend for a higher mean power ($P=0.06$). In conclusion, STHADE can reduce thermal and cardiovascular strain under hot and temperate conditions and there is some evidence of ergogenic potential for temperate exercise, but longer HA regimens may be necessary for this to meaningfully influence performance.

Introduction

Repeated frequent exposure to heat stress elicits adaptations enabling an individual to better accommodate the stressor (Fox, 1963; Nielsen *et al*., 1993). When this process occurs in the laboratory it is termed heat acclimation (HA) (Armstrong & Maresh, 1991). HA induces an array of adaptations including improved sweating, skin blood flow, and fluid balance, reduced cardiovascular strain and body temperatures, altered metabolism, and improved cellular protection (see recent reviews by Taylor, 2014 and Périard *et al*., 2015). HA is optimised when combined with exercise (Armstrong & Maresh, 1991). However, whilst endurance-trained athletes typically display many physiological adaptations similar to those seen with HA (Aoyagi *et al*., 1997), heat and exercise each induce specific transcriptional programmes (Kodesh *et al*., 2011) and, therefore, represent independent stressors; adaptive responses to heat are evident even in well-trained endurance athletes (Piwonka *et al*., 1965).

Although it is clear that HA improves exercise performance in a hot environment (Lorenzo *et al*., 2010; Garrett *et al*., 2012; Racinais *et al*., 2015), evidence is accumulating to suggest that adaptation to heat over a 7-21 day period confers ergogenic benefits under cooler conditions (Sawka *et al*., 1985; Hue *et al*., 2007; Scoon *et al*., 2007; Lorenzo *et al*., 2010; Buchheit *et al*., 2011; Buchheit *et al*., 2013; Racinais *et al*., 2014). For instance, Lorenzo *et al*. (2010) demonstrated that 10 days HA, consisting of 90 minutes cycling exercise per day (50% VO_{2max}) in a hot environment (40 °C, 30% RH), increased work done in a 60-minute cycling trial by a mean of 6% under cool (13 °C, 30% RH) conditions. Others have reported yo-yo run test distances were increased by an average of 7-44% in temperate ambient conditions (22-23 °C) following 7-14 day training camps in hot environments (29-40 °C, 27-50% RH) (Buchheit *et al*., 2011; Buchheit *et al*., 2013; Racinais *et al*,. 2014), whereas Scoon *et al*. (2007) reported a 32% increase in run time to exhaustion at 5-km run speed after a 21 day intervention consisting of frequent saunas. However, Corbett *et al*. (2014) have noted that these studies have seldom considered well-trained endurance athletes and have often used sub-optimal HA programmes (Scoon *et al*., 2007; Lorenzo *et al*., 2010) which might provide a diminishing level of thermal strain as HA progresses (Taylor, 2014). Furthermore some HA programmes include coincident hypoxic exposure (Buchheit *et al*., 2013) which may have opposing effects on changes in plasma volume (Levine & Stray-Gundersen, 1997) and some studies are without clear evidence of HA (Hue *et al*., 2007; Scoon *et al*., 2007; Racinais *et al*., 2014) and use exercise models where the 'true' performance effect as tested using a time trial, rather than a time to exhaustion test, is unclear (Sawka *et al*., 1985; Scoon *et al*., 2007;

Buchheit *et al*., 2013; Racinais *et al*., 2014). Moreover, the mechanisms underpinning the ergogenic effect of HA in temperate conditions are not entirely clear. Aerobic exercise performance can progressively deteriorate as ambient temperature increases beyond ~10 °C (Ely *et al*., 2007), indicating a thermal limitation even under relatively cool conditions; it has been suggested that improved thermoregulatory capability following HA might attenuate this thermal decrement in a manner similar to that evident with hotter temperatures (Corbett *et al*., 2014). Alternatively, the adaptations elicited by HA can increase maximal oxygen uptake (VO$_{2max}$) (Sawka *et al*., 1985; Lorenzo *et al*., 2010), lactate threshold (LT) (Lorenzo *et al*., 2010) and economy (Sawka *et al*., 1983; Shvartz *et al*., 1977), and thus may be ergogenic even under conditions where performance is not thermally-limited.

In contrast to traditional guidelines for maintaining fluid and electrolyte balance during HA (Bergeron *et al*., 2012), it has recently been suggested that dehydration represents an independent stimulus for HA because of the influence of augmented fluid-regulatory hormone strain on plasma volume expansion and related effects on cardiovascular function, and that this may accelerate the time-course of HA (Garrett *et al*., 2011). Garrett *et al*. (2014) have shown that a 5-day controlled hyperthermia (target rectal temperature [T_{re}] of 38.5 °C) HA protocol with permissive dehydration (no fluid consumption during 90-minutes exercise at 40 °C, 60% RH) elicited greater plasma volume expansion and resting forearm perfusion, and lower end-exercise T_{re} and cardiac frequency (f_c), in comparison with the same HA programme in a euhydrated state. Although longer HA programmes are often used (Sawka *et al*., 1985; Hue *et al*., 2007; Scoon *et al*., 2007; Lorenzo *et al*., 2010; Buchheit *et al*., 2011; Buchheit *et al*., 2013; Racinais *et al*., 2014), it has been suggested that from a practical perspective short-term HA with permissive dehydration (STHADe) may be the preferred approach for trained athletes as it has been shown to be effective, is less expensive, and is less likely to disrupt tapering for competition (Garrett *et al*., 2009). Moreover, STHADe may be ergogenic, even in trained endurance athletes. Garrett *et al*. (2012) demonstrated a significant 4 s mean improvement in 2-km rowing times of trained rowers exercising at 30 °C (60% RH) following STHADe, as well as a 4.5% plasma volume expansion and reduced thermal and cardiovascular strain during a heat stress test (HST). However, whether STHADe affords any ergogenic benefit under temperate conditions is unclear. Accordingly, the aim of this study was to examine the effect of a STHADe protocol on cycling performance in a temperate environment and to provide insight into the mechanisms underpinning any beneficial effects. It was hypothesised that short-term heat acclimation accompanied by

permissive dehydration would: 1) be effective at inducing heat acclimation; 2) favourably influence thermoregulation under temperate conditions, and; 3) be ergogenic under temperate conditions.

Method

Participants

Ten trained (performance level 3 [De Pauw *et al.,* 2013]) male cyclists and triathletes (10(3) hours·week^{-1} training) participated in this study which was approved by the University's Ethics Committee (Mean(SD) age: 24(4) years; height: 1.76(0.04) m; mass: 70.87(7.30) kg; body fat: 10.0(3.5)%; VO$_{2max:}$ 63.3(4.0) mL·kg^{-1}·min^{-1}; PPO: 385(40) W). All participants provided written informed consent.

Experimental design

A within-participant, pre-post, design was employed. Participants undertook a short-term HA programme (target ambient conditions of 40 °C, 50% RH), preceded and followed by a HST under the same ambient conditions, a temperate (target ambient conditions of 22 °C, 60% RH) graded exercise test (GXT), and a temperate 20-km cycling time trial (TT) (figure1).

*****************INSERT FIGURE 1 NEAR HERE*****************************

Experimental procedures

Heat Acclimation

The HA consisted of a 5-day protocol with exercise (up to 90 min·day^{-1}), isothermic heat strain (target T_{re} of 38.5-38.7 °C) and permissive dehydration. Isothermic heat strain is a preferred model for heat acclimation relative to traditional fixed work-rate approaches (Garrett *et al.,* 2012; 2014; Gibson *et al.,* 2015). Permissive dehydration is defined as *purposefully* allowing a person to dehydrate, through restricting fluid intake (Garrett *et al*., 2011); no fluid consumption was permitted during exercise and for 30 minutes after to promote dehydration. Participants exercised on a calibrated (Davison *et al*., 2009) Computrainer cycle ergometer (RacerMate Inc., Seattle, WA, USA), initially selecting a work rate eliciting a rating of perceived exertion (RPE [Borg, 1982]) of 15. This was maintained until T_{re}=38.3°C, at which point cooling was facilitated by a fan in front of the participant and external power output adjusted as appropriate to maintain the target T_{re}. This degree of

convective cooling (~2-3 m·s^{-1} [anemometer thermometer, Meterman TMA10, Amprobe, USA]) was used in order to facilitate the exercise component during the heat exposure and to provide some perceptual benefit, whilst maintaining a high mean skin temperature ($\bar{T}_{sk}$). The fan was only utilised once the participant reached the target T_{re}, and was switched-off if their T_{re} exceeded the upper limit of the target range. Thirty minutes after the exercise participants consumed 1.75 L of 3.6% carbohydrate-electrolyte fluid (Science In Sport, Nelson, UK) and drunk *ad libitum* thereafter, to promote euhydration upon arrival on subsequent days.

Heat Stress Test

Participants cycled on a Computrainer for 60 minutes at 35% PPO (determined from initial GXT). 1.25 L of 3.6% carbohydrate solution (drink temperature 20 °C) was ingested to replace fluid losses, divided into five equal boluses (250 mL) and consumed immediately prior to commencing exercise and every 15 minutes thereafter. Convective cooling was provided at a rate of 3.5 m·s^{-1}; pilot work indicated that the addition of a small amount of convective cooling facilitated the completion of the HST (*i.e.* stopped most participants achieving T_{re} withdrawal criteria) whilst maintaining an acceptably high $\bar{T}_{sk}$.

Temperate GXT

All GXTs were performed on a Velotron cycle ergometer (RacerMate Inc., USA). Participants exercised for 20 minutes at 85-110 W, dependent upon the estimated fitness of the participant and fixed within-participant. Thereafter, work-rate was incremented by 25 W every 3 minutes until blood lactate concentration [Lac] was ≥ 4 mmol·L^{-1}, following which work-rate increased 25 W·min^{-1} until volitional exhaustion. [Lac] was determined from fingertip capillary blood obtained at the end of each exercise stage (Biosen C-line, EKF Diagnostic, Cardiff, UK). Convective cooling was provided at a rate of 3.5 m·s^{-1}.

Temperate TT

Following 10 minutes warm-up at 100 W on a calibrated Velotron cycle ergometer, participants undertook a 20-km self-paced TT constructed with Velotron 3D software (RacerMate Inc., USA). Our previous experiments have shown that under conditions where participants are blinded to all feedback, with the exception of distance elapsed, the coefficient of variation for Velotron cycling is 1.1% (Corbett *et al.,* 2009) and 1.6% for short and longer TTs, respectively. Only distance completed was displayed to the participant and convective cooling was provided at a rate of 3.5 m·s^{-1}.

General procedures

Participants wore the same clothes each day, abstained from alcohol throughout the experimental period or caffeine for 12 hours before exercise, consumed a similar diet before each test and drank 500 mL of water 2 hours before every attendance. Training and food diaries were completed detailing the participant's typical training and diet prior to and during the experimental period. Participants were instructed to maintain their normal high-intensity training (except 24 hours before HSTs, GXTs or TTs) and replace an equivalent duration of low/moderate training with that completed in the laboratory to maintain usual training volume.

Prior to every exercise bout hydration was assessed from a urine sample (Osmometer 3320, Advanced Instruments Inc., Norwood, MA, USA), in order to ensure that hydration status was similar before HSTs and temperate exercise tests, and to ascertain the extent to which participants were able to maintain hydration status across the course of the STHADe programme; nude body mass was measured immediately pre- and post-exercise (Industrial Electronic Weight Indicator, Model I10, Ohaus Corporation, USA). Ambient conditions were measured by a WBGT logger (Squirrel 1000, Grant Instruments, Cambridge, UK), T_{re} by a thermistor (Grant Instruments, Cambridge, UK) self-inserted approximately 15 cm beyond the anal sphincter and cardiac frequency (f_c) by short range telemetry (Polar RS800, Polar Elector, Kempele, Finland). Participants were withdrawn from an exercise trial if T_{re} >40 °C. During HSTs and GXTs, skin temperature (T_{sk}) was measured using thermistors on the chest, biceps, thigh and calf (Grant Instruments, Cambridge, UK) and local sweat rate (upper right back [Q-Sweat, WR Medical Electronics, Maplewood, MN, USA]) and skin blood flow (forearm [MoorLAB, Moor Instruments, Devon, UK] were recorded. During HSTs expired gases (Douglas bag method), rating of perceived exertion ([RPE] Borg, 1970), thermal sensation, and thermal comfort (Zhang & Zhao, 2008) were measured at 15 minute intervals; a sample of sweat was collected using a custom patch constructed from Parafilm® (Bemis NA, Neenah, WI, USA) for determining sodium concentration ([Na^+]) by flame photometry (Corning 400, Essex, UK). During GXTs and TTs VO_2 was measured breath-by-breath throughout (Quark B2, COSMED, Rome, Italy).

Immediately before and after HSTs venous blood samples were obtained from the antecubital vein following supine rest for the measurement of [Hb] (201$^+$ HemoCue, Sweden) and Hct (Hawksley, England), in triplicate.

Data analysis

Mean skin temperature ($\bar{T}_{sk}$) and mean body temperature ($\bar{T}_b$) were calculated according to Ramanathan (1964) and Sawka *et al.* (1996), respectively. For GXT data the LT was defined as power output at [Lac] of 4 mmol·L^{-1}, gross mechanical efficiency (GME) was calculated at 160 W (highest work rate below LT achieved by all participants), and VO$_{2max}$ was defined as the highest 15 s VO$_2$. Oxygen pulse was defined as VO$_2$/f_C and plasma volume shifts were determined according to Dill & Costill (1974). Metabolic heat production (MHP) was determined as:

MHP ($W{\cdot}m^{-2}$) = MHP / BSA

When, MHP (W) = (heat production × 1000) / 60

And, heat production ($kcal$) = ((100 − ME / 100) × energy input

BSA = Body Surface Area (m^2) (Calculated using Dubois and Dubois, 1916)

ME = Mechanical Efficiency ($\%$)

Statistical analysis was undertaken using SPSS (IBM Version. 20). Significance was set at $P{\leq}0.05$, with a statistical trend defined as $P{\leq}0.10$ and effect sizes presented using Cohen's *d* for *t*-tests (*d* $\leq$0.2: trivial; 0.2-0.5: small; 0.5-0.8: moderate; <0.8: large) and partial eta squared for analysis of variance (η_p^2 $\leq$0.02: small; 0.02-0.13: medium; 0.13-0.26: large) (Cohen, 1988); data are presented mean(SD). Repeated measures ANOVA was used to analyse changes in daily responses during STHADe with the Greenhouse-Geisser statistic employed to account for violations of sphericity; *post-hoc* analysis utilised the Bonferroni correction for multiple pair-wise comparison tests. Similarly, a two-way repeated measures ANOVA with post-hoc pair-wise comparisons (Bonferroni corrected) was used to compare the main and interaction effects during the TTs. Two-tailed paired samples *t*-tests were used to assess the effectiveness of STHADe on markers of HA under hot (HST) and temperate conditions (first 20 minutes of GXT at fixed work rate) as well as the efficacy of STHADe on performance (TT) and parameters associated with performance (GXT).

Results

Short-term heat acclimation with permissive dehydration.

During the STHADe a T_{re} ≥38.50 °C was achieved after 25.0(6.3) min and was well maintained at 38.64(0.27) °C for the final hour of exposure, on each occasion ($F_{(2.3,21.1)}$=0.452, *P*=0.673, η_p^2=0.05) (Table 1). Average power was similar on each exposure ($F_{(2.3,20.8)}$=0.219, *P*=0.835, η_p^2=0.02) and heart rate was significantly reduced on days 4 and 5 ($F_{(3.0,27.2)}$=5.245, *P*=0.005, η_p^2=0.37). On average participants lost 3.09(0.64)% bodyweight over the course of each exposure but returned each day with a similar mass ($F_{(3.3, 29.4)}$=1.159, *P*=0.344, η_p^2=0.11) and hydration status ($F_{(2.2, 19.6)}$=0.461, *P*=0.653, η_p^2=0.05).

***************INSERT TABLE 1 NEAR HERE********************************

Heat Acclimation

Ambient conditions were not different between HSTs (pre: 39.5(0.3) °C, 52.2(4.2)% RH; post: 39.8(0.2) °C, 50.4(3.9)% RH, *P*>0.05) and rate of heat production was the same before and after STHADe in the HSTs (pre: 341(45) W·m^{-2}; post: 334(40) W·m^{-2}, $t_{(9)}$=0.707 *P*=0.498, *d*=0.16). The STHADe was effective at inducing HA as evidenced by significant reductions in resting T_{re} ($t_{(9)}$=4.748, *P*=0.001, *d*=1.26) and $\bar{T}_b$ ($t_{(8)}$=3.941, *P*=0.004, *d*=1.13), a lower exercise T_{re} ($t_{(8)}$=2.742, *P*=0.025, *d*=0.46), $\bar{T}_b$ ($t_{(7)}$=2.761, *P*=0.028, *d*=0.44), heart rate ($t_{(9)}$=2.874, *P*=0.018, *d*=0.57) and RPE ($t_{(9)}$=2.751, *P*=0.022, *d*=0.50), oxygen pulse ($t_{(9)}$=2.512, *P*=0.033, *d*=0.29), increased sweating (whole body: $t_{(7)}$=-3.833, *P*=0.006, *d*=0.19; local: $t_{(9)}$=-2.370, *P*=0.042, *d*=0.55) and reduced sweat [Na$^+$] ($t_{(7)}$=2.401, *P*=0.047, *d*=0.98), and a trend towards improved thermal comfort ($t_{(9)}$=-2.215, *P*=0.054, *d*=0.50) (Table 2). We also examined thermal responses under temperate conditions (pre: 22.0(0.3) °C, 68.0(6.9)% RH; post: 22.1(0.1) °C, 62.9(6.1)% RH, *P*>0.05) where reduced thermal (exercise T_{re}: $t_{(9)}$=2.203, *P*=0.055, *d*=0.78; exercise $\bar{T}_b$: $t_{(9)}$=2.428, *P*=0.038, *d*=0.70) and cardiovascular strain (heart rate: $t_{(9)}$=2.874, *P*=0.018, *d*=0.43) were also evident (Table 2).

***************INSERT TABLE 2 NEAR HERE********************************

Graded Exercise Test

There were no significant differences in the pre vs. post STHADe VO$_{2max}$ (4.48(0.46) vs. 4.57(0.43) L·min^{-1}, $t_{(9)}$=-1.255, *P*=0.241, *d*=0.20), f_{Cmax} (184(5) vs. 181(6) beats·min^{-1}, $t_{(9)}$=1.779, *P*=0.109, *d*=0.54), or GME (18.81(1.26) vs. 18.84(1.28)%, $t_{(9)}$=-0.087, *P*=0.934,

d=0.02), but PPO (385(40) vs. 392(39) W, $t_{(9)}$=-3.050, P=0.014, d=0.18) and LT were (253(53) vs. 269(45) W, $t_{(9)}$=-3.180, P=0.011, d=0.28) increased (Figure 2).

***************INSERT FIGURE 2 NEAR HERE*****************************

Time Trial

One participant had to be stopped early during the pre-STHADe TT as they attained the T_{re} withdrawal criterion despite the temperate exercise conditions, but they reached 3.26 km (21.2%) further in the post-STHADe TT before reaching the withdrawal criteria, whilst sustaining identical power (324 W) over the matched pre-STHADe distance. Mean performance times for the nine participants completing the 20-km TTs were 33:18.1(1:24.2) min:s and 33:06.8(1:42.8) min:s for the pre- and post-STHADe tests respectively, and were not significantly different ($t_{(8)}$=0.940, P=0.375, d=0.12), although there was a trend for a higher mean power output (249(27) W vs. 254(32) W for pre- and post-STHADe, respectively, $t_{(8)}$=-2.228, P=0.056, d=0.17). Analysis of the pacing data (figure 3) indicated no significant main effect for test (pre vs. post STHADe), but a significant main effect for distance, and significant interaction effect (test × distance). *Post-hoc* analysis of the interaction effect ($F_{(9,72)}$=4.990, P=0.000, η_p^2=0.38) indicated significant differences in the pre- vs. post- power output for the first and the final 10% of the TT.

Discussion

The primary aim of this study was to examine the effect of STHADe on cycling performance in a temperate environment among a cohort of trained (performance level 3) cyclists, and to provide insight into the mechanisms underpinning any beneficial effects. The main findings of our study support our first two hypotheses (1) STHADe would be effective at inducing heat acclimation; 2) STHADe would favourable influence thermoregulation under temperate conditions), but not our final hypothesis (STHADe would be ergogenic under temperate conditions). Our data indicate that: 1) STHADe was effective at inducing HA, as evidenced by the reduced thermal and cardiovascular strain whilst exercising in the heat; 2) the STHADe also reduced thermal and cardiovascular strain under temperate exercise conditions; 3) LT and PPO achieved in the temperate GXT were improved; 4) temperate TT performance was not significantly affected.

Although most HA regimens typically employ 7-21 daily heat and exercise exposures (Taylor, 2014), it has recently been suggested that STHADe is an effective and time efficient means of inducing HA, particularly for trained athletes (Garrett *et al*., 2012). Our data indicated that the STHADe was effective, with a reduced resting T_{re} and $\bar{T}_b$, a lower exercise T_{re}, $\bar{T}_b$, heart rate and RPE, increased sweating and reduced sweat [Na^+], and a trend towards improved thermal comfort. These findings are largely in keeping with other research employing similar short term HA regimens, although our sudomotor adaptations tended to be more pronounced given that others have not detected differences in whole body sweat rates (Garrett *et al*., 2009; Garrett *et al*., 2012; Garrett *et al*., 2014). More importantly given our primary aim, thermal strain was also reduced under temperate conditions, as evidenced by the reduced $\bar{T}_b$, $\bar{T}_{sk}$, heart rate and a trend towards a lower exercise T_{re} during temperate exercise at a fixed work rate. Although others have previously shown that HA can reduce thermal strain under cooler conditions, these studies have typically used longer HA regimens (Shvartz *et al*., 1977; Kobayashi *et al*., 1980), a substantially hotter 'temperate' condition than the present study (Kobayashi *et al*., 1980), or a fixed workload protocol (Lorenzo *et al.,* 2010), making it difficult to establish the basis of these changes. Gibson *et al.,* (2015) have suggested that isothermic HA protocols elicit a maintained thermal strain in comparison to the diminishing thermal strain of fixed work-rate protocols, although both methods provide a sufficient stimulus for increased heat shock protein-72 gene expression; a recent review on the topic suggests it is important to maintain the adaptation impulse to facilitate plasma volume expansion (Taylor, 2014). The reduced thermal strain in temperate conditions following STHADe is a novel finding with practical relevance for athletes preparing to compete under environmental conditions posing a moderate, yet still potentially limiting (Galloway & Maughan, 1997; Ely *et al*., 2007), thermal burden.

It has been suggested that dehydration acts as an independent stimulus for HA by augmenting plasma volume expansion, which confers improved cardiovascular stability and a reduced T_{re}, in comparison to maintaining euhydration (Garrett *et al*., 2011; Garrett *et al*., 2014). However, despite the reduced thermal and cardiovascular strain following the STHADe, plasma volume was unchanged. Reduced deep body and/or skin temperature, reduced sympathetic nervous activity, or increased venous tone, could account for the reduction in heart rate (Périard *et al*., 2015) and resulting improvement in oxygen pulse, and others have noted dissociation between cardiovascular and plasma volume changes with HA (Garrett *et al*., 2009). Moreover, a lack of plasma volume expansion with short-term HA has been

previously reported by others (Garrett *et al*., 2009) and the capacity for plasma volume expansion may be reduced in trained athletes, such as our participants, who may already be hypervolemic (Heinicke *et al*., 2001). With these individuals dehydration will decrease plasma volume until sufficient fluids are consumed (Costill & Fink, 1974) and failure to adequately replace water, amino acids, electrolytes, and carbohydrates following exercise might impair hypervolemic adaptations (Ozaki *et al*., 2009). Furthermore, the level of dehydration experienced by our participants (~3.1% bodyweight) during each heat exposure was greater than in previous studies documenting plasma volume expansion with STHADe (~1.8-2.1% bodyweight [Garrett *et al*., 2012; Garrett *et al*., 2014]). It has been suggested that trained athletes might require a greater dehydration stimulus than lesser trained athletes when employing STHADe strategies (Garrett *et al*., 2014); our data do not support this, particularly given that the participants maintained their daily body mass and presented with a similar urine osmolality on each day. An alternative possibility is that plasma volume expansion was masked by concomitant changes in red cell volume, which appear to be more pronounced with permissive dehydration (Garrett *et al*., 2014) and could have impacted on that ability to detect plasma volume changes using the Dill and Costill (1974) method. Future studies employing STHADe should consider other techniques for assessing plasma volume changes such as CO rebreathing or Evans-blue dye.

Given the lack of measureable change in plasma volume it is, perhaps, unsurprising that we did not identify any changes in VO_{2max}; it has been suggested that for VO_{2max} to increase with HA, plasma volume must expand sufficiently to increase cardiac output via a Frank-Starling effect, and this must offset any haemodilution (Coyle *et al*., 1990). Moreover, in those studies in which VO_{2max} was improved with HA, the intervention duration was at least 9 days (Sawka *et al*., 1985; Lorenzo *et al*., 2010). Similarly, GME was not altered by the STHADe, although improved economy following HA is not a ubiquitous finding, being confined to a limited number of studies that employed more prolonged HA, participants of lower fitness (Shvartz *et al*., 1977; Sawka *et al*., 1983), and no control group (Sawka *et al*., 1983). A transition from fast myosin to the more efficient slow myosin form has been demonstrated following HA in rat cardiac muscle (Horowitz *et al*., 1986), but the time course for these adaptions (21 days) is in excess of the STHADe employed in the present study and the relevance for human skeletal muscle is not clear. In contrast, our data have for the first time shown that STHADe can significantly improve LT, as has been shown previously following a longer HA regimen (Lorenzo *et al*., 2010). The mechanisms underpinning this effect are unclear. Although

reduced carbohydrate metabolism has been shown following HA (Young *et al.*, 1985), our respiratory exchange ratio data suggest that this was not the case in the present study and haemodilution is implausible given the lack of change in plasma volume. Corbett *et al.* (2014) have highlighted a number of adaptions to heat which could explain an improved LT, such as a transition from fast myosin to the more efficient slow myosin form, increased strength, or altered lactate kinetics and better preservation of splanchnic lactate removal as a consequence of improved O_2 supply. However, the relevance of these mechanisms for the short duration intervention used in the present study is unclear and some of these adaptations might also have been expected to enhance economy and/or VO_{2max}.

It would not be unreasonable to hypothesise that some of the adaptations observed following STHADe in the present study might favourably impact upon exercise performance. For instance, the work output at the LT has previously been shown to be a powerful predictor of performance in endurance event (Coyle *et al.*, 1991) and is sensitive to changes in performance (Jones, 1998). Likewise, it has been suggested that improved thermoregulatory capability might be expected to favourably affect temperate performance in a similar manner to that observed when exercising in hot conditions, albeit to a lesser degree (Corbett *et al.*, 2014). Nonetheless, temperate TT performance was not significantly improved following STHADe, although there was a more blunted start and slightly faster finish in the post-STHADe TT; greater variability in the start- and end-TT power has been reported previously (Thomas *et al.*, 2012), but these differences in power were numerically small. However, it should be noted that there was some evidence for a developing ergogenic effect as indicated by the increased PPO achieved in the GXT and tendency for higher power output during the TT. A higher PPO output in a GXT has been noted previously following longer-term HA (Sawka *et al.*, 1985), but the differences in mean TT power were numerically small, and given that the power output is related to cycling velocity with an exponent of between 2.6 and 3 (Atkinson *et al.*, 2003) this effect was insufficient to significantly influence temperate TT performance. Interestingly, the power at LT explained a large amount of the variation in average TT power pre- (r^2=0.75, *P*=0.001) and post STHADe (r^2=0.80, *P*<0.001), but the ΔLT following STHADe did not share a significant amount of variance with the ΔTT power (r^2=0.11, *P*=0.927); this could indicate glycogen depletion which would manifest in lower lactate production and higher power output for a given [Lac], but impaired performance (Hughes *et al.*, 1982), although we had strong experimental controls in place to negate this possibility. Moreover, association does not equate with causality and the LT does not directly

influence performance *per se*, but rather is typically used as a surrogate of sustainable percentage of VO_{2max} (Joyner, 1991). Finally, the heat related performance decrement observed in temperate conditions is small relative to exercise under hotter conditions and the improved thermoregulatory capability may have been insufficient to meaningfully impact upon this. A recent study has shown that wearing a cooling vest did not significantly impact on exercise performance in temperate conditions (25 °C, 55% RH), despite reductions in heart rate, skin temperature and improved thermal comfort (Eijsvogels *et al*., 2014). It should be noted that whilst STHADe has been shown to be ergogenic under hot conditions (Garrett *et al*., 2012), ergogenic effects under temperate conditions have not been demonstrated and it may be that a longer HA programme is necessary. Moreover, those studies demonstrating ergogenic effects following longer-term HA in temperate conditions have typically used time to exhaustion trials (Sawka *et al*., 1985; Scoon *et al*., 2007; Lorenzo *et al*., 2010; Buchheit *et al*., 2011; Buchheit *et al*., 2013; Racinais *et al*., 2014) and the equivalent effect on performance in a time-trial of a similar duration will be less. Before dismissing the STHADe model, future studies should consider examining a variety of performance tests, with increased sample sizes to improve statistical power; an observed power of $\beta=0.15$ was calculated for TT time in the present study. Longer HA protocols should also be considered, as evidence suggests that a continued forcing function (controlled hyperthermia) can maintain the adaptive stimulus for up to three weeks, thereby augmenting the PV expansion and sudomotor effects (Patterson *et al*., 2004), whereas the beneficial effects of permissive dehydration require further confirmation. Finally, evidence is emerging to suggest that HA may attenuate physiological stress in hypoxia (Heled *et al*., 2012); the efficacy of HA for temperate, high-altitude environments, warrants investigation.

A possible criticism of the present study is the lack of a control group, but we feel that is was justified given the lack of performance effect in the temperate TT, which was the primary focus of the present study. However, TT performance has been shown to be extremely robust when participants are blinded to time feedback and are unable to 'goal-set' (Corbett *et al*., 2009) and given the very high habitual training of our participants, any performance improvement observed within a five–day training intervention would have practical merit. The high sweat-losses of our participants during their daily heat exposures is consistent with their high–initial training status, which would confer them partial heat adaptation (Shvartz *et al*., 1977), but this may have meant that the fluid replacement strategies in place may have been insufficient for enabling the participants to replace daily water, amino acids,

electrolytes, and carbohydrates, thereby impacting on the adaptation to heat. Although this may represent a relevant practical scenario with good ecological relevance for trained athletes undergoing HA, future studies will need to carefully the magnitude of dehydration stimulus during HA and associated fluid replacement strategies.

In conclusion, STHADe was effective at inducing HA, with evidence of improved thermoeffector responses and reduced thermal and cardiovascular strain under both hot and temperate exercise conditions. However, in contrast to our hypothesis, the permissive dehydration stimulus did not induce hypervolemia, with no change in blood and plasma volume evident following the STHADe. VO_{2max} and GME were unaffected by the STHADe, but LT was significantly improved. There was some evidence for a developing ergogenic effect, as indicated by a trend towards a small increase in TT mean power, and an increased PPO at the end of a GXT, but the mechanisms underpinning this effect were unclear. Future studies need to elucidate the role of permissive dehydration in HA and determine the optimal level of dehydration for maximising the hypervolemic responses to short–term HA, the minimum time course for meaningful ergogenic effects of HA, and the mechanisms underpinning any effects.

Perspective

The present study examined the effect of STHADe on HA and temperate exercise performance. STHADe induced HA, with improved thermoeffector responses and reduced thermal and cardiovascular strain evident under both hot and temperate exercise conditions, but there was no evidence of hypervolemia. VO_{2max} and efficiency were unaffected by the STHADe, but LT was improved and there was evidence for a developing ergogenic effect, with a trend towards a small increase in time trial mean power, and an increased peak power at the end of a graded exercise test, but the mechanisms underpinning this effect were unclear.

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Tables

Table 1: Mean(SD) daily responses during short term heat acclimation with permissive dehydration. [*] denotes significant difference from day 1 ($P \leq 0.05$). [a] denotes $n=9$ as one participant was unable to achieve $T_{re} > 38.5°C$ on every day.

	Day 1	Day 2	Day 3	Day 4	Day 5	P value
Time to T_{re} of 38.5 °C (min)[a]	22.9(5.0)	26.9(7.1)	26.6(8.0)	24.4(6.1)	24.3(5.6)	0.377
Average session T_{re} (final 60 min [°C])	38.62(0.25)	38.65(0.23)	38.62(0.19)	38.67(0.22)	38.64(0.28)	0.770
Average session fc (b·min^{-1})	144(17)	141(16)	138(17)	138(16)*	135(19)*	0.004
Average power (W)	104(18)	104(21)	100(17)	104(19)	102(17)	0.926
Pre-exercise mass (kg)	71.12(7.25)	71.14(7.12)	71.22(7.05)	71.44(7.33)	71.37(7.49)	0.344
Session body water loss (L·hr^{-1})	1.46(0.43)	1.47(0.47)	1.45(0.37)	1.56(0.33)	1.43(0.30)	0.514
Urine osmolality (mOsmo·kg^{-1})	386(282)	514(299)	420(285)	443(340)	446(363)	0.653

Table 2: Mean(SD) physiological responses during HST and during a standard 20 min exercise task conducted in temperate conditions before and after 5-days short term heat acclimation with permissive dehydration (Data refer to average over exercise period, unless otherwise stated). * denotes significant difference pre vs. post short term heat acclimation with permissive dehydration ($P \leq 0.05$); (*) denotes trend for difference ($P \leq 0.10$). $n=10$, except [a] denotes $n=9$, [b] denotes $n=8$, due to technical error. During the HST, after STHADe, two participants briefly (8 mins) exercised at the incorrect (reduced) power output; data are included up to that time point for these two participants.

	Hot conditions		Temperate conditions	
	Before	**After**	**Before**	**After**
Thermal				
Resting T_{re} (°C)	37.29 ± 0.18	37.05 ± 0.20*	37.47 ± 0.22	37.27 ± 0.29*
Exercise T_{re} (°C)	37.95 ± 0.40	37.77 ± 0.39*[a]	37.57 ± 0.25	37.41 ± 0.32(*)
Resting $\bar{T}_{sk}$ (°C)	36.22 ± 0.23	36.16 ± 0.18[a]	32.34 ± 0.35	31.83 ± 0.70(*)
Exercise $\bar{T}_{sk}$ (°C)	37.30 ± 0.56	37.19 ± 0.46	30.09 ± 0.46	29.65 ± 0.47*
Resting $\bar{T}_{b}$ (°C)	37.15 ± 0.17	36.94 ± 0.20*[a]	36.96 ± 0.22	36.72 ± 0.30*
Exercise $\bar{T}_{b}$ (°C)	37.93 ± 0.43	37.75 ± 0.38*[b]	36.83 ± 0.26	36.63 ± 0.31*
Thermoregulatory				
Body water loss (L·hr^{-1})	1.84 ± 0.66	1.97 ± 0.71*[b]	-	-
Upper-back sweat rate (L·m^2·h^{-1})	0.75 ± 0.16	0.84 ± 0.17*	0.12 ± 0.04	0.14 ± 0.07
Sweat sodium (mmol·L^{-1})	43 ± 12	33 ± 8*[b]	-	-
Skin blood flow (AU)	306 ± 74	316 ± 64	97.56 ± 59.59	106.60 ± 57.22
f_c (beats·min^{-1})	141 ± 15	134 ± 9*	113 ± 13	108 ± 10*
Δ Plasma volume Pre-Post STHADe (%)	-	1.18 ± 8.01	-	-
Δ Blood volume Pre-Post STHADe (%)	-	0.75 ± 4.88	-	-
Metabolic				
VO$_2$ (L·min^{-1})	2.24 ± 0.35	2.23 ± 0.30	1.96 ± 0.23	1.98 ± 0.19
RER	0.89 ± 0.04	0.87 ± 0.05	0.88 ± 0.06	0.90 ± 0.04
Metabolic heat production (W·m^{-2})	341 ± 45	334 ± 40		
O$_2$ pulse (mL·beat^{-1})	16.00 ± 2.24	16.64 ± 2.25*	17.40 ± 2.09	18.47 ± 2.13*
Cardiac output (L·min^{-1})	20.92 ± 2.49	21.05 ± 2.35	19.36 ± 1.84	19.61 ± 1.52
Perceptual				
RPE	11 ± 2	10 ± 2*	-	-
Thermal comfort (cm)	9 ± 4	11 ± 4(*)	-	-
Thermal sensation (cm)	16 ± 2	14 ± 2	-	-

Figures

Day	1	2	3	4	5	6	7	8	9	10	11	12	13
Test	GXT	TT		HST	CH	CH	CH	CH	CH	HST		GXT	TT
Phase	Pre				STHADe					Post			

Figure 1: Schematic of experimental protocol undertaken by participants. All participants undertook a short term heat acclimation programme with permissive dehydration (STHADe) consisting of five consecutive days of exercise (90 minutes·day^{-1}) in the heat (~40 °C, 50% RH) using the controlled hyperthermia (CH) technique. Pre- and post-STHADe participants undertook: 1) a Graded Exercise Test (GXT) in a temperate environment (~22 °C, 65% RH); 2) a 20 km Time Trial (TT) in a temperate environment; 3) a Heat Stress Test (HST) in the hot environment.
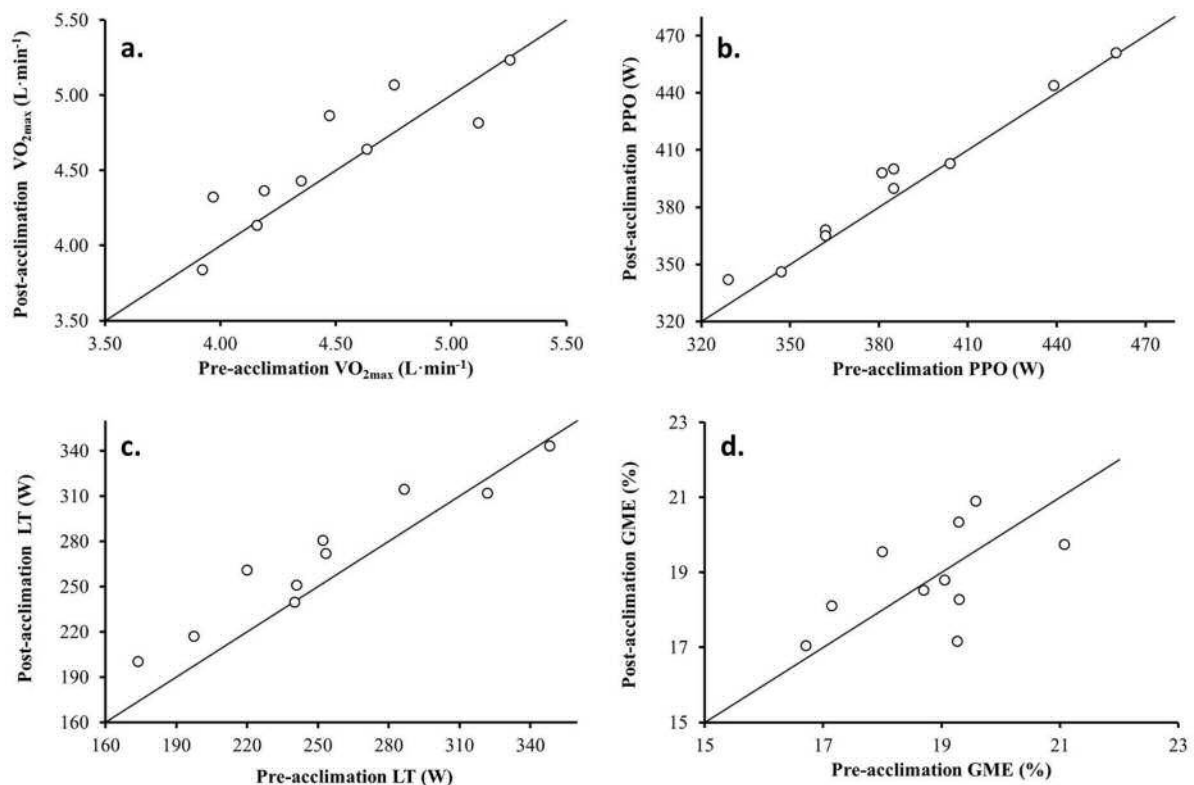

Figure 2: Individual results from temperate graded exercise test performed pre and post short term heat acclimation with permissive dehydration. Figure 2a: Maximal oxygen uptake (VO$_{2max}$). Figure 2b: Peak power output (PPO). Figure 2c: Lactate threshold (LT). Figure 2d: Gross mechanical efficiency (GME).
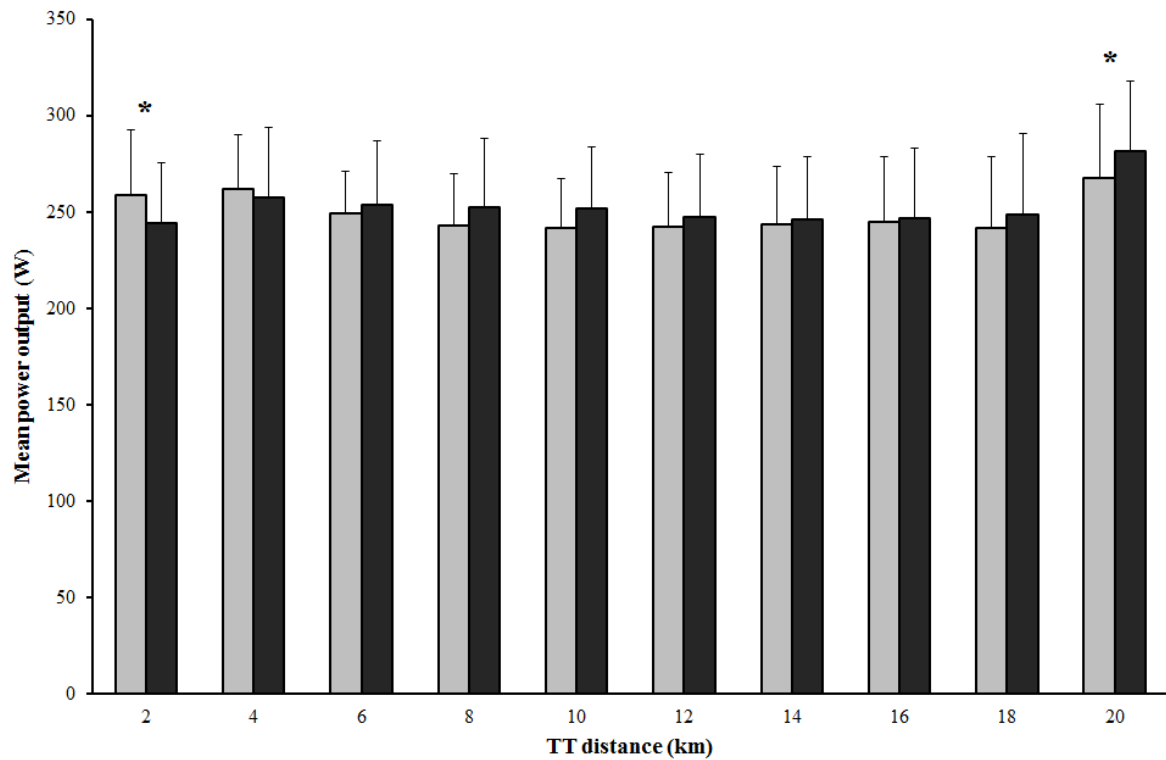
Figure 3: Pacing strategies pre-(grey bars) and post- (filled bars) for 2 km power output bins (mean±SD) over a 20 km cycle time trial in a temperate environment (*n*=9). * denotes a significant difference between pre- and post-tests (*P*≤0.05).