The effect of medium-term heat acclimation on endurance performance in a temperate environment.

Original Investigation

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Running head: Training in the heat for temperate performance.

Abstract:

We investigated whether an 11-day heat acclimation programme (HA) enhanced endurance performance in a temperate environment, and the mechanisms underpinning any ergogenic effect. Twenty-four males ($\dot{V}O_{2max}$: 56.7±7.5 mL·kg^{-1}·min^{-1}) completed either: i) HA consisting of 11 consecutive daily exercise sessions (60-90 minutes·day^{-1}; *n*=16) in a hot environment (40°C, 50% RH) or; ii) duration and exertion matched exercise in cool conditions (CON; *n*=8 [11°C, 60% RH]). Before and after each programme power at lactate threshold, mechanical efficiency, VO_{2max}, peak power output (PPO) and work done during a 30-minute cycle trial (T30) were determined under temperate conditions (22°C, 50% RH). HA reduced resting (-0.34±0.30°C) and exercising (-0.43±0.30°C) rectal temperature, and increased whole-body sweating (+0.37±0.31 L·hr^{-1}) (all P≤0.001), with no change in CON. Plasma volume increased in HA (10.1±7.2%, P<0.001) and CON (7.2±6.3%, P=0.015) with no between-groups difference, whereas exercise heart rate reduced in both groups, but to a greater extent in HA (-20±11 b·min^{-1}) than CON (-6±4 b·min^{-1}). VO_{2max}, lactate threshold and mechanical efficiency were unaffected by HA. PPO increased in both groups (+14±18W), but this was not related to alterations in any of the performance or thermal variables, and T30 performance was unchanged in either group (HA: Pre=417±90 *vs*. Post=427±83 kJ; CON: Pre=418±63 vs. Post=423±56 kJ). In conclusion, 11-days HA induces thermophysiological adaptations, but does not alter the key determinants of endurance performance. In trained males, the effect of HA on endurance performance in temperate conditions is no greater than that elicited by exertion and duration matched exercise training in cool conditions.

Key words: Acclimatisation; thermal; hot; training; temperature.

Introduction

Prolonged (≥15 minutes) exercise performance is impaired in a hot environment, relative to cool conditions (Guy *et al*., 2015). This performance decrement is multi-causal (Nybo *et al*., 2014). Nevertheless, repeated frequent exposure to high ambient heat, either in the laboratory (heat acclimation [HA]), or natural environment (acclimatisation), elicits adaptions that reduce the performance decrement (Keiser *et al*., 2015; Racinais *et al*., 2015b). Therefore, HA is a widely advocated intervention for optimising exercise performance in hot environments (Bergeron *et al*., 2012). However, as early as 1959, following laboratory and field observations on elite distance runners, Bannister and Cotes suggested that ambient temperatures '*in the range of 15-25°C could become a limiting factor when subjects are performing strenuous exercise*'(p. 61). It is now apparent that endurance performance can progressively decline as ambient temperature increases beyond ~10°C (Galloway & Maughan 1997; Ely *et al*., 2007), although this relationship will be influenced by other parameters influencing heat exchange, including exercise mode (Junge *et al*., 2016) and other climatic factors (Maughan *et al*., 2012; Otani *et al*., 2018). Nevertheless, there remains a limited amount of research investigating the effects of HA on endurance performance in these temperate conditions.

It has been hypothesised that HA might attenuate any 'thermal' decrement in performance that is evident in temperate conditions, in a manner similar to that evident under hotter conditions (Shvartz *et al*., 1977; Corbett *et al*., 2014), although experimental data are lacking. Alternatively, HA could be ergogenic through 'non-thermal' mechanisms related to haematological (Lorenzo *et al*., 2010; Oberholzer *et al*., 2019), cardiovascular (Coyle *et al*., 1990; Lorenzo *et al*., 2010) and skeletal muscle adaptations (Kodesh & Horowitz, 2010; Goto *et al*., 2011), and their effects on VO_{2max} (Lorenzo *et al*., 2010; Waldron *et al*., 2019), lactate threshold (Lorenzo *et al*., 2010) and mechanical efficiency (Shvartz *et al*.,1977; Sawka *et al*., 1983). These parameters are the key physiological determinants of endurance performance (Joyner & Coyle, 2008) and any improvement should translate to a performance benefit. However, the ergogenic potential of heat under cooler conditions is contentious (Minson & Cotter, 2016; Nybo & Lundby, 2016) with some studies providing evidence for an ergogenic effect (Sawka *et al*., 1985; Lorenzo *et al*. 2010; Racinais et al., 2014; McCleave *et al*., 2017;) and others reporting no effect (Karlsen *et al*., 2015; Keiser *et al*., 2015; Mikkelsen *et al*., 2019).

It has been suggested that the discrepant findings between studies are due to variations in study design including methodological limitations such as absence of a control group, limited evidence of adaption, or environmental conditions imposing a negligible thermal burden on endurance performance (Corbett *et al*., 2014). Indeed, the majority of studies to date have examined cool, rather than temperate, conditions (e.g. Lorenzo *et al*. 2010; Karlsen *et al.*, 2015; Keiser *et al*., 2015; Racinais *et al*., 2015b; Mikkelsen *et al*., 2019; Oberholzer *et al*., 2019), despite the fact that temperate conditions are common during athletic competition. Moreover, whilst some investigations have included a control group, selecting an appropriate 'matching parameter' to isolate the effect of thermal adaption is challenging due to the multifaceted nature of physiological stress, as has recently been highlighted (Mikkelsen *et al*., 2019). For example, the use of percentage VO_{2max} (Lorenzo *et al*., 2010) or 'usual training' Karlsen *et al*. (2015) would elicit a greater relative exercise intensity and training stimulus in the heat compared to a cooler environment. Conversely, matching cardiovascular strain (Keiser *et al*., 2015) would typically elicit a lower absolute work rate in the heat. However, the rating of perceived exertion (RPE [Borg, 1982]) is generated by multiple afferent signals including heart rate, metabolic and ventilatory parameters, as well as muscular strain (Hampson *et al*., 2001), and appears to be the mediator used by athletes to regulate their exercise intensity (Tucker, 2009). As such, RPE provides an integrated index of the whole-body training-stimulus and represents an appropriate 'matching parameter' with strong ecological validity.

Accordingly, the aims of the present study were, twofold. Firstly, to determine whether a medium-term HA intervention would enhance endurance performance under temperate conditions compared to a control group undertaking an exertion matched exercise programme in a cool environment. We utilised temperate rather than cool conditions to enable the evaluation of potential ergogenic effects of HA arising from both 'non-thermal' and 'thermal' mechanisms and because these conditions are common during athletic competition, but have received limited attention in the scientific literature. A medium-term HA was selected to ensure near-complete cardiovascular and sudomotor adaptation to heat (Racinais *et al*., 2015a) and for consistency with previous research demonstrating an ergogenic benefit of HA under cool conditions (Lorenzo *et al*., 2010). We utilised a perception based control group to address concerns over appropriately matching the physiological strain of the intervention and control groups in environmental-stressor research (Mikkelsen *et al*., 2019). Secondly, we sought to provide insight into the mechanism(s) underpinning any ergogenic effect. Our hypotheses were that HA would: 1) improve thermoregulation; 2) improve the key physiological determinants of endurance performance (efficiency, lactate threshold, VO_{2max}, and; 3) increase endurance performance in a temperate environment.

Method

Participants

A convenience sample of 24 trained (Performance Level 2 and 3 [De Pauw *et al.*, 2013]) males provided written informed consent before participating in this study (Table 1). Based upon previously reported data for the improvement in VO_{2max} following heat acclimation (Lorenzo et al., 2010) a power calculation indicated that a sample size of 18 would enable detection of a between-groups difference with a 0.5 enrolment ratio, β of 0.80, and α of 0.05 (clincalc.com). The study was approved by the University's Science Faculty Research Ethics Committee and conformed to the Declaration of Helsinki, except for registration in a database.

******************************Table 1 near here*****************************

Experimental design

The data presented in this study were from a programme of work investigating the ergogenic potential of HA in a temperate environment, and include data pooled from our published work (Neal *et al.*, 2016; Rendell *et al.*, 2017) as well as previously unpublished data. The study employed a between-groups design and the study design is summarised in Figure 1. An experimental group (HA; *n*=16) completed a medium-term HA programme consisting of daily exercise in a hot environment (40°C, 50% RH). Thereafter, a separate control group (CON, *n*=8) completed an exertion and duration matched programme in a cool environment (11°C, 60% RH). Before the intervention, all participants completed a temperate graded cycling exercise test (GXT: 22°C, 50% RH); 16 participants (eight from each group) also completed (on a separate day) an additional temperate 30 minute performance trial (T30: 22°C, 50% RH). All participants undertook a heat stress test (HST: 40°C, 50% RH) at the start (day 1) and end (day 11) of the intervention period. The HA intervention consisted of eight isothermal heat strain sessions (ISO) with a HST on day 6. Participants in CON completed exertion and duration matched exercise in a cool environment (11°C, 60% RH). After the intervention participants repeated the temperate GXT and T30.

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Experimental procedures

Isothermal strain sessions

To acclimate participants in the HA group we employed the ISO method, as described previously (Neal *et al.*, 2016; Rendell *et al.*, 2017). Briefly, participants were instructed to cycle in a hot environment (40°C, 50% RH) at a work rate eliciting an RPE of 15 (measured at 5 minute intervals throughout [Borg, 1982]) until rectal temperature (T_{rec}) reached 38.5°C. Thereafter, external power output was adjusted as appropriate to maintain the target T_{rec} (±0.2°C) and a small amount of convective cooling (air velocity ~2-3 $m \cdot s^{-1}$) was provided to facilitate the exercise component, for a total session duration of 90 minutes.

Control sessions

Participants in the CON group cycled in a cool environment (11°C, 60% RH, air velocity ~2-3 $m \cdot s^{-1}$) and were instructed to adjust their work rate to elicit the same (group mean) RPE as reported by participants in the HA group at the corresponding time (5-minute intervals), on the equivalent intervention day. Participants were blinded to all feedback including power output and heart rate and were able to freely adjust the resistance provided by the cycle ergometer. On days 2-5 and 7-10 the sessions lasted for a total of 90 minutes (paralleling the equivalent ISO sessions in the HA group). On day 6 the session lasted 60 minutes (paralleling the HST undertaken on day 6 by the HA group).

Heat stress test

Participants cycled in a hot environment (40°C, 50% RH, air velocity 3.5 $m \cdot s^{-1}$) at a self-selected fixed cadence for 60 minutes at a work rate equivalent to 35% of the peak power output (PPO) reached in the initial GXT (see below).

Graded exercise tests

GXTs were completed in a temperate environment (22°C, 50% RH air velocity 3.5 $m.s^{-1}$) as described previously (Neal *et al.*, 2016; Rendell *et al.*, 2017). These tests were used to determine the key endurance performance parameters (VO_{2max}, lactate threshold, mechanical efficiency) and to determine the external work rate for the HST based upon the PPO achieved.

30-minute performance trial

After a standardized warm up participants commenced a 30-minute ‘all-out’ cycle ergometer performance trial in a temperate environment (22°C, 50% RH, air velocity 3.5 $m \cdot s^{-1}$). ‘Performance’ was defined as the total work completed within the designated time (kJ).

General procedures

Participants abstained from alcohol throughout the experimental period. Before the HSTs, GXTs and T30s participants abstained from caffeine for 12 hours and consumed a similar diet before each test. Compliance with experimental controls was verbally verified on each laboratory attendance. Nude body mass (dry) was measured pre- and post- laboratory sessions (Electronic Weight Indicator I10, Ohaus Corporation, Parsippany, NJ, USA) to determine whole-body sweat rate, adjusted for fluid ingested; participants were provided with a 3.6% carbohydrate-electrolyte fluid to provide energy and minimise dehydration (Science in Sport, Nelson, UK) during HA (1.75 L in 0.25 L boluses at 15 minute intervals) and HST sessions (1.25 L in 0.25 L boluses at 15 minute intervals). GXTs and T30 trials were undertaken on a Lode Excalibur cycle ergometer (Lode B.V., Groningen, the Netherlands); all other exercise sessions were undertaken on a calibrated Computrainer cycle ergrometer, (RacerMate Inc., Seattle, WA, USA). Ambient conditions were measured by a WBGT logger (Squirrel 1000, Grant Instruments, Cambridge, UK), T_{rec} by a thermistor (Grant Instruments, Cambridge, UK) self-inserted 15 cm beyond the anal sphincter and heart rate by short range telemetry (Polar RS800, Polar Electro, Kempele, Finland). Skin temperature (T_{sk}) was measured using thermistors on the chest, biceps, thigh and calf (Grant Instruments, Cambridge, UK). VO_2 was measured using an online metabolic cart (Quark B2, COSMED, Rome, Italy). Blood lactate concertation [Lac] was determined from fingertip capillary blood samples (Biosen C-line, EKF Diagnostic, Cardiff, UK). Venous blood samples (forearm antecubital vein) were obtained before the pre- and post-intervention HST (K2 EDTA blood collection tubes, Beckton Dickson & Company, Plymouth, UK) following 10 minutes of seated rest for measurement of haemoglobin concentration [Hb] (201+ HemoCue, Sweden) and haematocrit (Hct) (Hawksley, Lancing, UK) in triplicate.

Data analyses

Data obtained during the GXTs were used to calculate: i) power output at [Lac] of 2 mmol. L^{-1} and 4 mmol·L^{-1}; ii) gross mechanical efficiency (GME); iii) VO_{2max} ; iv) peak power output (PPO). Power at a given fixed blood [Lac] was calculated by interpolation of the power *vs.* [Lac] relationship. Gross mechanical efficiency was calculated from the respiratory data measured over the final 45 s of the stage at a power output of 185 W, with the exception of two participants where this was in excess their lactate threshold and the data from a lower power output was used. VO_{2max} was defined as the highest 15 s average VO_2, with PPO defined as the power achieved at volitional exhaustion. Plasma volume changes were calculated using the method of Dill & Costill (1974).

Statistical analyses were undertaken using SPSS Version 25 (IBM, New York, USA). Data are presented mean±SD, unless otherwise stated, and significance was set *a-priori* at $P \leq 0.05$. Independent samples *t*-tests were used to assess the between-group differences in participant characteristics and average physiological responses during the HA and CON intervention sessions. Mixed model two-way ANOVAs were used to assess the condition, time, and interaction (condition × time) effects of the interventions on physiological responses and performance over time. Student's *t*-tests were employed for *post-hoc* analysis of significant condition, time and interaction effects, with Independent-samples analysis performed for between-groups (condition) comparisons and paired-samples analysis performed for within-groups comparison (time). Where a significant improvement in temperate exercise performance was evident Pearson's correlation was undertaken to investigate whether this was related to changes in any endurance performance parameters (power output at 2 $mmol \cdot L^{-1}$ and 4 $mmol \cdot L^{-1}$ [Lac]; gross mechanical efficiency; VO_{2max}) or thermal adaptations (T_{rec}, heart rate, sweat rate, plasma volume expansion). Inter-individual variation in the adaptation to heat was expressed as the standard deviation of the true individual response (SD_R), according to Atkinson and Batterham (2015).

Results

Intervention period

In both groups, there was high adherence to the intervention, with 100% of the prescribed session completed in the HA group and 97% of the prescribed sessions completed in the CON group. Ambient temperature was higher in HA than CON during the intervention period (39.4±0.4°C, 55.2±4.5% RH *vs*. 10.2±0.6°C, 66.5±3.0% RH, $P<0.001$ for T_{air}). As a consequence, compared to participants in CON undertaking duration and exertion-matched exercise, those in HA were hotter (HA=38.46±0.11°C *vs.* CON=38.08±0.28°C , P<0.001), with a higher sweat production (HA=1.40±0.33 L·hr^{-1} *vs.* CON=0.46±0.20 L·hr^{-1}, P<0.001) and greater cardiovascular strain (HA=138±8 beats·minute^{-1} *vs*. CON=130±9 beats·minute^{-1}, P=0.044), but a lower external work rate (HA=103±16 W *vs*. CON=137±29 W, P=0.001).

Thermophysiological adaptations

There were no significant effects of condition (*i.e.* HA *vs*. CON) on resting T_{rec}, average T_{rec}, whole body sweat rate, or average heart rate, as assessed during the HST, although in each instance the main effect of time (*i.e.* Pre *vs*. Post) and the condition × time interaction were significant. Post-hoc analysis of these significant effects showed that neither resting, nor average T_{rec} were significantly different following CON, but both resting T_{rec} (-0.34±0.30°C, P=0.001, SD_R±0.25°C) and exercise T_{rec} (-0.43±0.30°C, P<0.001, SD_R±0.11°C) were reduced following HA. Similarly, whole body sweat rate was increased after HA (+0.37±0.31 L·hr^{-1}, P<0.001, SD_R±0.19 L·hr^{-1}), but remained unchanged in CON (Figures 2a-c). Heart rate was significantly reduced following both HA (-20±11 b·min^{-1}, P<0.001, SD_R±11 b·min^{-1}) and CON (-6±4 b·min^{-1}, P=0.003), with a between-groups difference also evident post-intervention (P<0.001; figure 2d). Plasma volume increased in HA (10.1±7.2%, P<0.001, SD_R±3.5%) and CON (7.2±6.3%, P=0.015) with no between-groups difference.

****************************Figure 2 near here********************************

Temperate exercise performance

Power at 2 mmol.L^{-1} [Lac] was not significantly increased in either the HA (Pre=179±38 W *vs*. Post=187±46 W [n=13]) or CON groups (Pre=180±26 W *vs*. Post=178±37 W). This was also the case for the power at 4 mmol.L^{-1} [Lac] (HA: Pre=228±41 W *vs*. Post=233±42 W; CON: Pre=227±34 W *vs*. Post=231±34 W [figure 3a]). Likewise, VO_{2max} was not significantly increased in either HA (Pre=57.7±8.2 mL·kg^{-1}·min^{-1} *vs*. Post=58.9±7.8 mL·kg^{-1}·min^{-1}) or CON

(Pre= 54.8±5.8 mL·kg^{-1}·min^{-1} *vs*. 52.2±7.9 mL·kg^{-1}·min^{-1}) (figure 3b). However, there was a significant main effect of time on gross mechanical efficiency, with post-hoc analysis identifying that gross mechanical efficiency was unchanged in HA (Pre=18.2±1.6 % *vs*. Post=18.5±1.2 % P=0.321), but was significantly increased in CON (Pre=18.4±0.7 % *vs*. Post=19.5±1.1 %, P=0.006) (figure 3c).

There was a significant main effect of time on PPO, with post-hoc analysis identifying that PPO was significantly increased after both HA (Pre=344±43 W *vs*. Post=355±39 W, P=0.043) and CON (Pre=340±36 W *vs*. Post=360±34 W, P=0.002) (figure 3d); the effects of condition and condition × time interaction were not significant. Correlation analysis indicated that the change in PPO was not related to the change in any individual endurance performance (r values between -0.04 and 0.33, P>0.05) or thermal adaptation parameter (r values between -0.15 and 0.05, P>0.05). Moreover, total work done in the T30 was not significantly increased in either group (HA: Pre=417±90 KJ *vs*. Post=427±83 KJ; CON: Pre=418±63 KJ *vs*. Post=423±56 KJ) (figure 3e).

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Discussion

There has been ongoing debate regarding the ergogenic potential of HA for exercise under cooler conditions (Minson & Cotter, 2016; Nybo & Lundby, 2016). It has been suggested that HA induces a range of adaptation that can improve endurance exercise performance in cooler conditions (Lorenzo *et al*., 2010; Corbett *et al*., 2014; Minson & Cotter, 2016). It is hypothesised that these effects may be particularly advantageous in temperate environments, where performance may also be impaired by the thermal strain posed by the environment, albeit to a lesser extent than in hot environments, and both 'thermal' and 'non-thermal' adaptations associated with HA may be beneficial (Corbett *et al*., 2014). However, the present study demonstrates that whilst medium-term HA induced significant thermophysiological adaptations, it did not alter the key determinants of endurance performance in a temperate environment. Therefore, we accept our first hypothesis and reject our second hypothesis. Moreover, in a cohort of trained males, the effect of medium-term HA on 30-minute endurance performance in a temperate environment was no greater than that elicited by exertion and duration matched exercise training in cool conditions; we also, therefore, reject our third hypothesis.

The medium term HA programme effectively induced a range of 'thermal' adaptations, consistent with the heat acclimated phenotype, including reductions in exercise T_{rec} (-0.43°C) and heart rate (-20 $b \cdot min^{-1}$), and increases in whole body sweating rate (+0.37 $L \cdot hr^{-1}$) and plasma volume (+10.1%). These adaptions were of a magnitude consistent with, or even slightly in excess of, previous HA research (Tyler *et al*., 2016). However, these thermal adaptations did not translate into a significant ergogenic effect. The environmental conditions in the present study approximated a Wet Bulb Globe Temperature (WBGT) of 18°C which has been reported to result in a 3.3% reduction in marathon running performance (Ely *et al*., 2007), although the impairment might be lessened with the shorter exposure-duration in the present study. Moreover, it has been argued that WBGT is not a good predictor of the effect of ambient conditions on exercise performance and that the integrated index is superior (Junge *et al*., 2016). The conditions in the present study elicited an integrated index of ~590, which according to Junge *et al*. (2016) should have caused a significant thermally mediated performance impairment. Nevertheless, even in hot conditions HA does not fully restore the reduction in work rate elicited by the environment (Racinais *et al*., 2015b), whereas the lower wind speeds in the present study reduced the ability to dissipate heat to the environment through convection and forced evaporation (Saunders *et al*., 2005) which might lessen the effectiveness of HA. In addition, a recent meta-analysis suggests that ergogenic effects of HA are more evident in ‘time to exhaustion’ performance models than the ‘total work’ model used in the present study (Benjamin *et al*., 2019), although time to exhaustion models may exaggerate the true ergogenic effect (Hopkins *et al.,* 1999). Therefore, the modest thermal burden imposed by the environment, combined with a potentially small-magnitude performance effect related to improved thermoregulation in this environment, may have been less than the sensitivity of our performance model. Alternatively, there is evidence of a temporal delay in the ergogenic benefits of HA (McCleave *et al*., 2017), which may only manifest >72 hours after the intervention (Waldron *et al*., 2019), although we are cautious in this interpretation given that PPO in the GXT was increased when assessed ~48-hours after the HA. This effect was evident in both groups, indicating that HA was no more effective than CON in improving PPO, and was unrelated to any of the thermal adaptations or changes in any of the determinants of endurance. However, we cannot exclude a training effect on anaerobic metabolism, whereas motivation or learning effects (Hopkins, 2000) could also have contributed to the increased PPO.

A second aim of this study was to examine the effect of HA on the key determinants of endurance performance, namely, VO_{2max}, efficiency and lactate threshold (Joyner & Coyle, 2008). An increase in VO_{2max} has been proposed as central to any ergogenic effect of HA, with an increase in cardiac output due to hypervolemia and the Frank-Starling law proposed as a putative mechanism (Lorenzo *et al*., 2010; Corbett *et al*., 2014). However, in keeping with recent studies (Karlsen *et al*., 2015; Keiser *et al.*, 2015; Mikkelsen *et al*., 2019), we did not detect any change in VO_{2max} following HA, despite a significantly increased PV (10.1[7.2]%). Indeed, it has been suggested that any benefit of PV expansion on cardiac output, will be balanced by a haemodilution effect resulting in no change in VO_{2max} (Coyle *et al*., 1990). Likewise, neither the power at lactate threshold nor gross mechanical efficiency were significantly improved by HA, which is contrast to some (Shvartz *et al.*, 1977: Sawka *et al*., 1983; Lorenzo *et al*., 2010), but not all (Karlsen *et al*., 2015; Keiser *et al.*, 2015; Mikkelsen *et al*., 2019) previous research. The lack of change in the key determinants of endurance was consistent with our null effect on endurance performance (T30), and we speculate that the apparently equivocal findings between studies in this area likely stems from important methodological differences, which we have sought to address.

Some research reporting an ergogenic effect of HA has lacked a control group (Sawka *et al*; 1985; Racinais *et al*., 2014), meaning that it is not possible to isolate the effect to heat from confounding factors, including the daily exercise undertaken within the HA *i.e.* a training effect. To isolate the independent effect of heat from training, other investigations have employed a control group undertaking some form of 'matched' exercise in an environment limiting the thermal strain *i.e.* cool conditions. However, as recently recognised by Mikkelsen *et al*. (2019), appropriate matching of groups with superimposed environmental stress presents difficulties due to the multifaceted nature of training stress. For example, approaches prescribing external work rate based upon performance in cool conditions (Lorenzo *et al.,* 2010) do not take into account the effect of elevated ambient temperature on cardiovascular strain (Wingo *et al*., 2012). Conversely, matching groups based upon cardiovascular strain will likely result in differences in the mechanical work component (Keiser *et al*., 2015). Others have instructed participants to continue their normal training (Karlsen *et al.*, 2015; Mikkelsen *et al*., 2019), but the additional physiological strain associated with HA can compromise the ability to maintain habitual training (Mikkelsen *et al*., 2019). Acknowledging the multifaceted nature of training stress, we prescribed intensity on the basis of RPE, which is generated as a result of multiple afferent signals including heart rate, metabolic and ventilatory parameters (Hampson et al., 2001), and appears to be the key mediator that athletes use to regulate exercise intensity (Tucker, 2009). This method resulted in slightly higher cardiovascular strain in HA compared to CON, despite a slightly lower external work rate, but this is to be expected given the between-groups differences in environmental conditions and subsequent effects on thermal strain. Importantly, this novel approach better reflects the integration of multiple afferents and the multifaceted nature of training stress than matching approaches that utilise a single physiological parameter, as well as possessing good ecological validity and practical relevance for athletes attempting to undertake the same training in a hot or cold environment.

The present study was not without limitation and we acknowledge that a within-participant crossover design is typically stronger than a between-groups design. However, in the context of the present study, a between-groups design has some advantages over the within-participant approach because the time-course of decay in heat acclimation can be prolonged (Weller *et al*., 2007) and there is some evidence to support a heat acclimation 'memory' (Tetievsky *et al*., 2014). This necessitates that within-participant designs include long washout periods which can increase the confounding influences of seasonal changes in acclimatisation status (Inoue *et al*., 1995) as well as changes in other factors known to influence thermoregulation including training status, fitness status, health and anthropometric factors (Havenith & van Middendorp, 1990). Moreover, our large sample size was adequately powered to detect between-groups differences in our key outcome measures and we also employed appropriate statistical techniques for between-groups analysis.

In summary, although a number of previous studies have investigated the effect of HA on performance in cool conditions, in many of these the potential for training effects or confounding effects arising from the control measures employed cannot be discounted. Likewise, few studies have examined temperate conditions, where performance may still be limited by 'thermal' factors. The present study, employing an exertion and duration matched control group, has demonstrated that a medium term HA programme was effective at inducing a range of significant thermophysiological adaptions, whereas these parameters were unchanged by CON. However, the HA programme did not improve any of the key physiological determinants of endurance performance, including the power at lactate threshold, gross mechanical efficiency, or VO_{2max}. Despite the improved thermoregulatory capability, the effect of a medium-term HA programme on 30-minute endurance performance in a temperate environment amongst a group of trained men was no greater than that elicited by exertion and duration matched exercise training undertaken in cool conditions.

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References

Atkinson G, Batterham AM. (2015). True and false inter-individual differences in the physiological response to an intervention. Exp Physiol. 100(6):577-588.

Bannister RG, Cotes JE, (1959). The effect of changes in environmental temperature upon body temperature and performance during strenuous exercise. J Physiol. 147(3):60-62.

Benjamin CL, Sekiguchi Y, Fry LA, et al. (2019). Performance changes following heat acclimation and the factors that influence these changes: meta-analysis and meta-regression. Front Physiol, 10, 1448.

Bergeron MF, Bahr R, Bärtsch P, et al. (2012) International Olympic Committee consensus statement on thermoregulatory and altitude challenges for high-level athletes. Br J Sports Med. 46(11):770-779.

Borg GA. (1982). Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 14:377–381.

Corbett J, Neal RA, Lunt HC, et al. (2014). Adaptation to heat and exercise performance under cooler conditions: a new hot topic. Sports Med. 44(10):1323-1331.

Coyle EF, Hopper MK, Coggan AR. (1990). Maximal oxygen uptake relative to plasma volume expansion. Int J Sports Med. 11(2):116-119.

De Pauw K, Roelands B, Cheung SS, et al. (2013). Guidelines to classify subject groups in sport-science research. Int J Sports Physiol Perform. 8(2):111-122.

Dill DB, Costill DL. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol. 37, 247-248.

Ely MR, Cheuvront SN, Roberts WO, et al. (2007). Impact of weather on marathon-running performance. Med Sci Sports Exerc. 39(3):487-493.

Galloway SD, Maughan RJ. (1997). Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. Med Sci Sports Exerc. 29(9):1240-1249.

Goto K, Oda H, Kondo H, et al. (2011). Responses of muscle mass, strength and gene transcripts to long-term heat stress in healthy human subjects. Eur J Appl Physiol. 111:17-27

Guy JH, Deakin GB, Edwards AM, et al. (2015). Adaptation to hot environmental conditions: an exploration of the performance basis, procedures and future directions to optimise opportunities for elite athletes. Sports Med. 45(3):303–311.

Hampson DB, St Clair Gibson A, Lambert MI, et al. (2001). The influence of sensory cues on the perception of exertion during exercise and central regulation of exercise performance. Sports Med. 31(13):935-952.

Havenith G, & van Middendorp H. (1990). The relative influence of physical fitness, acclimatization state, anthropometric measures and gender on individual reactions to heat stress. Eur J Appl Physiol Occup Physiol. 61(5-6):419-427.

Hopkins WG. (2000). Measures of reliability in sports medicine and science. Sports Med. 30(1):1-15.

Hopkins WG, Hawley JA, Burke LM. (1999). Design and analysis of research on sport performance enhancement. Med Sci Sports Exerc. 31(3):472-485.

Inoue Y, Nakao M, Okudaira S, et al. (1995). Seasonal variation in sweating responses of older and younger men. Eur J Appl Physiol Occup Physiol. 70(1):6-12.

Joyner MJ, Coyle EF. (2008). Endurance exercise performance: the physiology of champions. J Physiol. 586(1):35-44.

Junge N, Jørgensen R, Flouris AD, et al. (2016). Prolonged self-paced exercise in the heat - environmental factors affecting performance. Temperature (Austin). 3(4):539-548.

Karlsen A, Racinais S, Jensen MV, et al. (2015). Heat acclimatization does not improve VO2max or cycling performance in a cool climate in trained cyclists. Scand J Med Sci Sports. 25 Suppl 1:269-276.

Keiser S, Fluck D, Huppin F, et al. (2015). Heat training increases exercise capacity in hot but not in temperate conditions: a mechanistic counter-balanced cross-over study. Am J Physiol Heart Circ Physiol. 309:H750-761

Kodesh E, Horowitz M. (2010). Soleus adaptation to combined exercise and heat acclimation: physiogenomic aspects. Med Sci Sports Exerc. 42:943-952

Lorenzo S, Halliwill JR, Sawka MN, et al. (2010). Heat acclimation improves exercise performance. J Appl Physiol. 109(4):1140-1147.

Maughan RJ, Otani H, Watson P. (2012). Influence of relative humidity on prolonged exercise capacity in a warm environment. Eur J Appl Physiol. 112(6):2313–2321.

McCleave EL, Slattery KM, Duffield R, et al. (2017). Temperate performance benefits after heat, but not combined heat and hypoxic training. Med Sci Sports Exerc. 49(3):509-517.

Mikkelsen CJ, Junge N, Piil JF, et al. (2019). Prolonged heat acclimation and aerobic performance in endurance trained athletes. Front Physiol. 1;10:1372.

Minson CT, Cotter JD. (2016) CrossTalk proposal: Heat acclimatization in a cool condition. J Physiol. 594(2): 241-243.

Neal RA, Massey HC, Tipton MJ, et al. (2016). Effect of permissive dehydration on induction and decay of heat acclimation, and temperate exercise performance. Front Physiol. 7:564.

Nybo L, Rasmussen P, & Sawka MN. (2014). Performance in the heat-physiological factors of importance for hyperthermia-induced fatigue. Comp Physiol, 4(2), 657–689.

Nybo L, Lundby C. (2016). CrossTalk opposing view: Heat acclimatization does not improve performance in a cool condition. J Physiol. 594(2): 245-247.

Oberholzer L, Siebenmann C, Mikkelsen CJ, et al. (2019). Hematological adaptations to prolonged heat acclimation in endurance-trained males. Front Physiol. 10:1379.

Otani H, Kaya M, Tamaki A, et al. (2018). Air velocity influences thermoregulation and endurance exercise capacity in the heat. Appl Physiol Nutr Metab. 43(2):131-138.

Racinais S, Alonso JM, Coutts AJ, et al. (2015a). Consensus recommendations on training and competing in the heat. Br J Sports Med. 49(18):1164-73.

Racinais S, Buchheit M, Bilsborough J, et al. (2014). Physiological and performance responses to a training camp in the heat in professional Australian football players. Int J Sports Physiol Perform. 9(4):598-603.

Racinais S, Périard JD, Karlsen A, (2015b). Effect of heat and heat acclimatization on cycling time trial performance and pacing. Med Sci Sports Exerc. 2015;47(3):601-606.

Rendell RA, Prout J, Costello JT, et al. (2017). Effects of 10 days of separate heat and hypoxic exposure on heat acclimation and temperate exercise performance. Am J Physiol Regul Integr Comp Physiol. 313(3):R191-R201.

Saunders AG, Dugas JP, Tucker R, et al. (2005). The effects of different air velocities on heat storage and body temperature in humans cycling in a hot, humid environment. Acta Physiol Scand. 183(3):241-255.

Sawka MN, Pandolf KB, Avellini BA, et al. (1983). Does heat acclimation lower the rate of metabolism elicited by muscular exercise? Aviat Space Environ Med. 54:27-31.

Sawka MN, Young AJ, Cadarette BS, et al. (1985). Influence of heat stress and acclimation on maximal aerobic power. Eur J Appl Physiol Occup Physiol. 53(4):294–298.

Shvartz E, Shapiro Y, Magazanik A, et al. (1977). Heat acclimation, physical fitness, and responses to exercise in temperate and hot environments. J Appl Physiol Respir Environ Exerc Physiol. 43(4):678-683.

Tetievsky A, Assayag M, Ben-Hamo R, et al. (2014) Heat acclimation memory: do the kinetics of the deacclimated transcriptome predispose to rapid reacclimation and cytoprotection? J Appl Physiol (1985). 117(11):1262-77.

Tyler CJ, Reeve T, Hodges GJ, et al. (2016). the effects of heat adaptation on physiology, perception and exercise performance in the heat: a meta-analysis. Sports Med. 46(11):1699-1724.

Tucker R. (2009). The anticipatory regulation of performance: the physiological basis for pacing strategies and the development of a perception-based model for exercise performance. Br J Sports Med. 43(6):392-400.

Waldron, M., Jeffries, O., Tallent, J. et al. (2019). The time course of adaptations in thermoneutral maximal oxygen consumption following heat acclimation. Eur J Appl Physiol. 119, 2391-2399.

Weller AS, Linnane DM, Jonkman AG, et al. (2007). Quantification of the decay and re-induction of heat acclimation in dry-heat following 12 and 26 days without exposure to heat stress. Eur J Appl Physiol. 102(1):57-66.

Wingo JE, Ganio MS, Cureton KJ. (2012). Cardiovascular drift during heat stress: implications for exercise prescription. Exerc Sport Sci Rev. 40(2):88-94.

Table legends

Table 1: Overall and sub-group (HA= heat acclimation; CON = Control) participant characteristics. Data presented as mean±SD

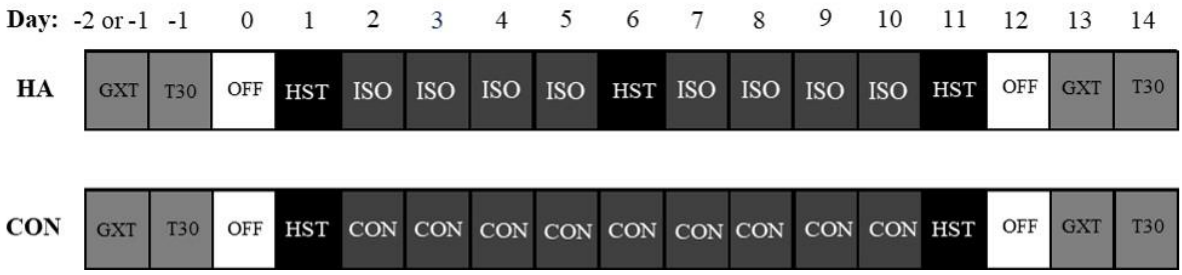
Figure legends

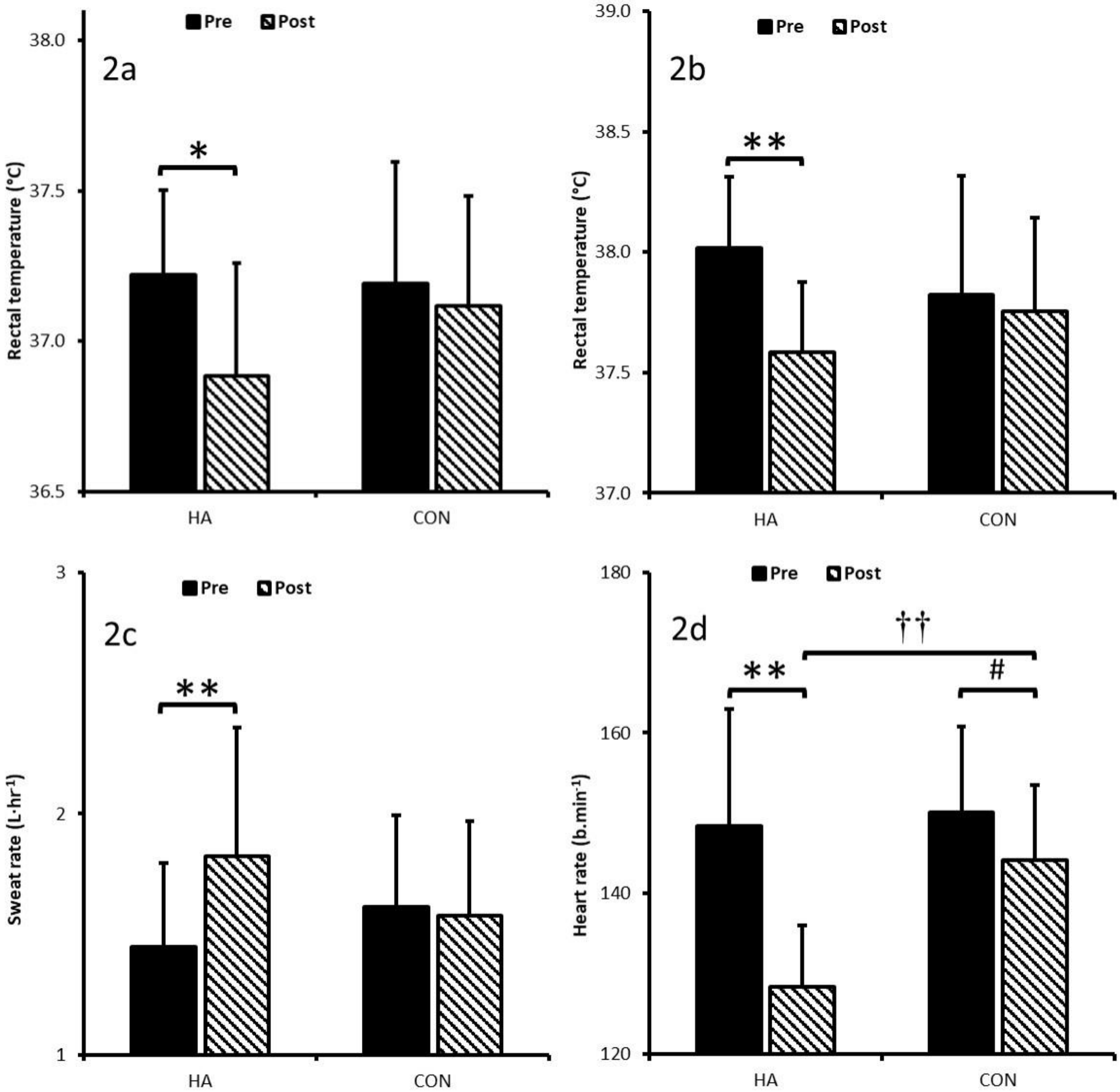
Figure 1. Experimental protocols for the heat acclimation (HA) group (40°C, 50% RH) using the isothermal strain approach (ISO), and the control group (CON) undertaking duration and exertion matched exercise in a cool environment (11°C, 50% RH)). GXT = graded exercise test (22°C, 50% RH); T30 = 30-minute performance trial (22°C, 50% RH); HST = heat stress test (40°C, 50% RH).

Figure 2: Mean±SD thermophysiological responses during a heat stress test (40°C, 50% RH) before and after a heat acclimation programme (HA: *n*=16) or exertion and duration matched cool exercise programme (CON: *n*=8). 2a: Resting rectal temperature; 2b: Mean exercise rectal temperature; 2c: Whole-body sweat rate; 2d: Mean exercise heart rate: *significant effect of HA, P<0.05; **significant effect of HA, <0.001; #significant effect of CON, P<0.05; ††significant difference between HA and CON, P<0.001.

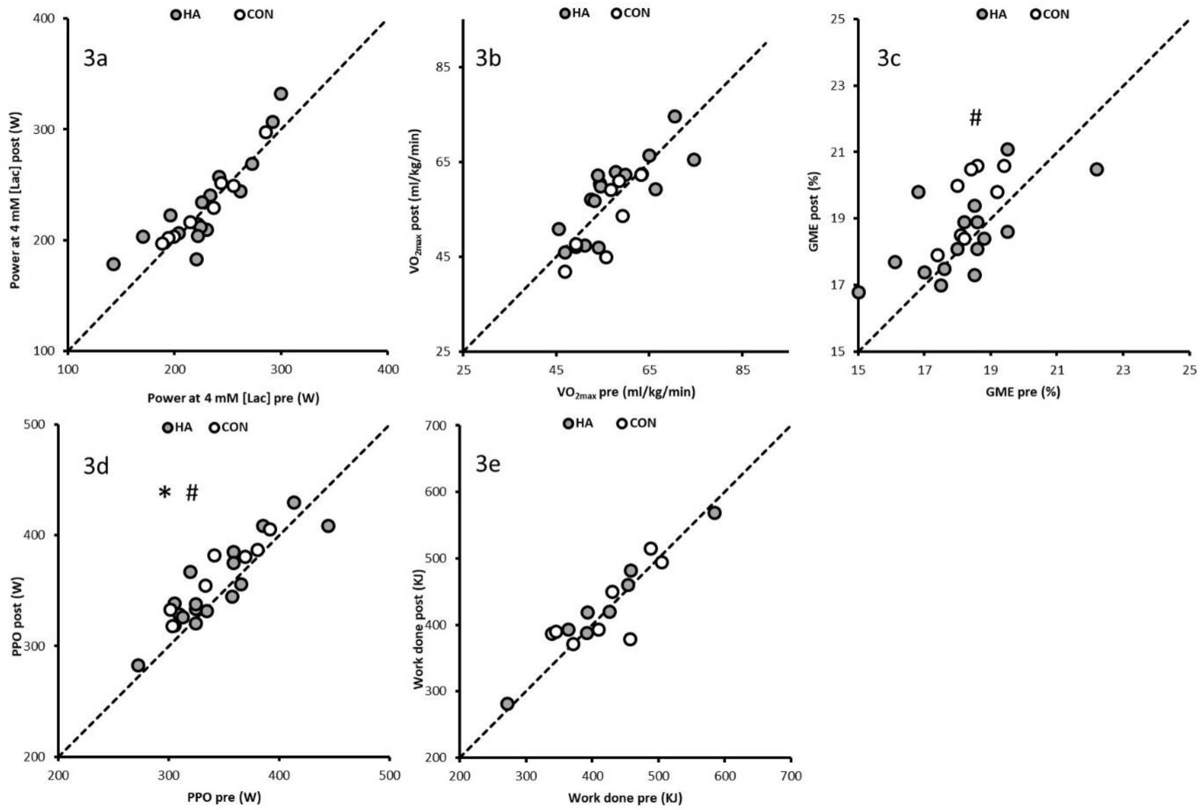
Figure 3: Individual data showing temperate (22°C, 50% RH) endurance performance parameters pre and post a heat acclimation programme (HA) or an exertion and duration matched cool exercise programme (CON). Black dashed line represents line of identity 3a: Power at 4 mmol.L-1 blood lactate concentration; 3b: Maximal oxygen uptake (VO2max); 3c: Gross mechanical efficiency (GME); 3d: Graded exercise test peak power output (PPO); 3e: Total work done in a 30 minute performance trial. *=significant effect of HA; #=Significant effect of CON, P<0.05.

	Overall (n=24)	HA (n=16)	CON (n=8)
Age (yrs)	22±4	23±5	22±3
Height (m)	1.81±0.04	1.81±0.05	1.80±0.03
Mass (kg)	75.3±8.3	74.5±6.5	77.0±11.5
BSA (m^2)	1.95±0.11	1.94±0.10	1.96±0.14
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	56.7±7.5	57.7±8.2	54.8±5.8





2a
Pre
Post
Rectal temperature (°C)
38.0
37.5
37.0
36.5
*
HA
CON
2b
Pre
Post
Rectal temperature (°C)
39.0
38.5
38.0
37.5
37.0
**
HA
CON
2c
Pre
Post
Sweat rate (L·hr-1)
3
2
1
**
HA
CON
2d
Pre
Post
Heart rate (b.min-1)
180
160
140
120
**
††
#
HA
CON


HA
CON
3a
Power at 4 mM [Lac] post (W)
Power at 4 mM [Lac] pre (W)
3b
VO2max post (ml/kg/min)
VO2max pre (ml/kg/min)
3c
#
GME post (%)
GME pre (%)
3d
* #
PPO post (W)
PPO pre (W)
3e
Work done post (KJ)
Work done pre (KJ)