Title of the Article:

Short term heat acclimation prior to a multi-day desert ultra-marathon improves physiological and psychological responses without compromising immune status.

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Heat acclimation for a desert ultra-marathon

Abstract

Purpose

Multi-stage, ultra-endurance events in hot, humid conditions necessitate a-priori thermal adaptation, often achieved through heat acclimation (HA), to improve performance by reducing thermoregulatory strain and perceptions of heat stress. This study investigated the physiological, perceptual and immunological responses to short-term HA (STHA) in athletes preparing for the Marathon des Sables.

Methods

Eight ultra-endurance athletes (age; 42±4 yrs, mass; 81.9±15.0 kg and body fat; 17.6±5.9%) completed 4 days of controlled hyperthermia STHA (60 min·day^{-1}, 45°C and 30% relative humidity). Pre, during and post sessions, physiological and perceptual measures were recorded. Immunological measures were recorded pre-post session 1 and 4.

Results

STHA improved peak thermal comfort (-1,*P*=0.02), sensation (-1,*P*=0.03) and perceived exertion (-2,*P*=0.04). A dissociated relationship between perceptions of fatigue and T_{re} was evident after STHA, with reductions in perceived physical (-6,*P*=0.04) and general (-2,*P*=0.04) fatigue. Exercising T_{re} and HR did not change (*P*>0.05), however, sweat rate increased 14% (*P*=0.02). No changes were found in white blood cell counts or content (*P*>0.05).

Conclusions

Four days of STHA facilitates effective perceptual adaptations and lower feelings of fatigue, without compromising immune status prior to an ultra-endurance race in heat stress. A greater and prolonged physiological strain is required to confer optimal physiological adaptations.

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Key words

Short term heat acclimation; Heat stress; Perceived fatigue; Thermoregulation; Ultra-endurance

Abbreviations

Δ	Change
HA	Heat acclimation
HR	Heart rate
LTHA	Long term heat acclimation
MdS	Marathon des Sables
MFSI-SF	Multi-dimensional fatigue scale inventory-short form
NBM	Nude body mass
NUFL	Non-urine fluid loss
PV	Plasma volume
RPE	Rating of perceived exertion
STHA	Short term heat acclimation
TC	Thermal comfort
TSS	Thermal sensation
T_{re}	Rectal Temperature
U_{osm}	Urine osmolality
U_{sg}	Urine specific gravity
WBC	White blood cell

Introduction

Multi-stage, ultra-endurance events within extreme environmental conditions are increasing in popularity (Knoth et al., 2012). The annual Marathon des Sables (MdS), is a ~250 km multi-day race across the Sahara desert in Morocco, where competitors are self-dependent, carrying 5-10 kg of equipment in extreme heat stress (~40°C). Consequently, an array of challenges are experienced including; cumulative fatigue, dehydration, sleep deprivation, energy deficit (Costa et al., 2016), high solar heat loads with limited shade and, prolonged metabolic heat production. Such consequences exacerbate physiological and perceptual strain, augment the likelihood of dermatological injuries and gastrointestinal disorders (Gill et al., 2015; Costa et al., 2016), and increase the risk of exertional heat-related illnesses (EHI) (Coris et al., 2004), which can lead to race-withdrawal and emergency treatment (DeMartini et al., 2014). Therefore, to reduce the deleterious effects of heat stress it is imperative that athletes prepare effectively (Périard et al., 2015; Racinais et al., 2015), however, a paucity of research describing effective and applied preparation strategies, specifically for ultra-marathons in heat stress exists.

Heat acclimation (HA) is an intervention comprising 4-21 days of repeated, prolonged exposures to high ambient temperatures (>30°C) and moderate-high relative humidity (>40%) (Racinais et al., 2015; Tyler et al., 2016). The numerous physiological advantages induced by HA have been discussed in detail elsewhere (Sawka et al., 2011; Garrett et al., 2014; Taylor, 2014; Racinais et al., 2015). Short-term HA (STHA) can confer up to 75% of physiological adaptations typically seen following long-term HA (LTHA) (Pandolf, 1998), and appears effective across a range of populations (Costa et al., 2014; Garrett et al., 2014; Gibson et al., 2015a; Mee et al., 2015a; Neal et al., 2015; Willmott et al., 2016). Ultra-endurance athletes may benefit from STHA prior to competition (Costa et al., 2014), particularly when optimal controlled hyperthermia methods (Taylor, 2014; Racinais et al., 2015) are implemented, due to reduced training volume for equal physiological (Gibson et al., 2015a) and cellular adaptations (Gibson et al., 2015b). Beside physiological adaptations, perceived exertion is reduced (Neal et al., 2015), alongside improved thermal comfort (Costa et al., 2014), sensation (Gibson et al., 2015; Neal et al., 2015) and perceived fatigue (Tamm et al., 2015), although such improvements have not been found within ultra-endurance athletes during HA. This is of current interest, as ultra-marathon runners are highly motivated, display mental toughness and attain lower pain perceptions (Hoffman et al., 2014), thus, when fatigued decision making may be compromised leading to injury and, or illness (Maruff et al., 2006).

Improved perceptions of fatigue and temperature during exercise-heat stress may be beneficial for ultra-marathon performances in the absence of alterations in physiological markers, due to the impact each may have on pacing strategy within individual stages of an event (McCormick et al., 2015). Moreover, when navigation and decision making are necessary, improved perception may be important in ameliorating the combined, and independent effects of heat stress, and exercise on cognitive markers (Taylor et al., 2016). However, minimal evidence regarding athletes preparing for ultra-endurance events exists (Costa et al., 2014) and it is unclear how the relationship between perceptual and physiological markers of heat adaptation change following STHA.

While few studies have investigated the effects of HA on immune function, STHA (Guy et al., 2016) and long term passive heat exposure (Kanikowska et al., 2012), report minor challenges to immune, inflammation and endotoxemia status (Walsh et al., 2011). This is in contrast to heightened inflammatory markers (Hailes et al., 2011) and disturbances in immune cell number and function after acute (Mitchell et al., 2002) and chronic (Hailes et al., 2011; Watt et al., 2016) moderate-intensity exercise-heat stress. Consequently, inflammation and leukocytosis occurs with an associated increase in circulating leukocyte cell counts, primarily caused by neutrophilia (Mestre-Alfaro et al., 2012). Such discrepancies are likely due to the exercise prescription, duration and physiological strain experienced as well as intervention efficacy. Predisposing factors including; ineffective preparation (i.e. unacclimated, low training status and high body fat) and a compromised immune status prior to competition (Walsh et al., 2011; Gill et al., 2015) may suppress anti-lipopolysaccharide (LPS) mechanisms, promote inflammatory and pyrogenic activities and increase the susceptibility to EHI (Lim & Mackinnon, 2006; Hailes et al., 2011; Guy et al., 2016). This will compromise the ecological validity of HA, should individuals experience immune dysfunction or other related illnesses (Costa et al., 2016), which have negative impacts upon health, heat tolerance and imminent exercise performance (Pyne et al., 2005). Therefore, monitoring biomarkers associated with immune function and overtraining, alongside tracking heat load, perceptual feedback and training intensity during intervention protocols is required (Guy et al., 2016). Consequently, the aim of this study was to investigate the physiological and perceptual markers of heat adaptation, and immune responses to STHA within a group of athletes preparing for a multi-day desert ultra-marathon. It was hypothesised STHA would induce heat adaptation without evidence of immune dysfunction.

Methods

Athletes

Eight un-acclimated, male ultra-endurance runners (>150 km weekly) (age; 42 ± 4 years, body mass; 81.9 ± 15.0 kg, stature; 178 ± 8 cm, sum of 4 skin fold; 39.0 ± 14.7 mm and body fat; 17.6 ± 5.9%) volunteered and provided written informed consent for the study, which was conducted in accordance with the Institution's ethics and governance committee and Declaration of Helsinki (2013). Athletes had not experienced hot conditions (>25°C) for >3 months and abstained from caffeine, alcohol and strenuous activity for 24hrs prior to each session. Athletes also restricted food intake 2hrs prior to exercise, but maintained normal diet during the intervention. Athletes were instructed to arrive euhydrated (urine osmolality [U_{osm}] <700 mOsm·kg^{-1} and specific gravity [U_{sg}] <1.030) (Sawka et al., 2007), or consumed 500mL of water over 30 mins before confirmation of hydration status prior to commencing exercise (n=1, session 1 and 4).

Experimental design

Each athlete visited the laboratory for 4 HA sessions 72hrs prior to starting the MdS. Prior to and post session 1 and 4, physiological and perceptual measures were recorded, blood samples were collected and the multidimensional fatigue symptom inventory-short form (MFSI-SF) questionnaire completed.

Short term heat acclimation protocol

HA was completed within hot, dry conditions (44.6 ± 1.4°C and 30 ± 6% relative humidity, wet-bulb globe temperature [WBGT] 34°C), for 60 mins·day^{-1} for 4 days inside a purpose-built environmental chamber (WatFlow, TISS, UK), without fans or direct heat stimuli. HA session 1 and 4 were prescribed and adjusted according to Gibson et al. (2016) for the use of a controlled hyperthermia method. Athletes cycled at 2 W·kg^{-1} to achieve a target T_{re} of 38.5ºC, where they then rested and, or cycled to maintain target temperature for the remainder of the session. Cycling was chosen as it is non-weight-bearing and practical, thus reducing injury risk during tapering and enabling 4-6 athletes to train simultaneously (Willmott et al., 2016). During sessions 2 and 3, athletes either cycled at 2 W·kg^{-1} or ran on the treadmill at a self-selected pace (6-10 km·hr^{-1}) to reach and maintain target T_{re}. Treadmill exercise was prescribed to enable athletes to educate themselves on predicted race-pace, heart rate (HR) zones and estimated fluid losses, which was considered vital for their safe and successful preparations. Fluid ingestion was restricted during

session 1 and 4 to estimate non-urine fluid loss (NUFL) accurately, while during sessions 2 and 3, athletes were permitted to practice drinking *ad libitum* to prepare for race conditions.

Physiological measures and equipment

On arrival to session 1, skinfold thickness was calculated using skinfold calipers (Harpenden, Baty International, UK) across four standard sites, which estimated body fat percentage (Durnin & Womersley, 1974). Stature and nude body mass (NBM) were measured using physician (Detecto Scale Company, USA) and weighing scales (Adam Equipment Co Ltd., UK), respectively. Urine samples determined hydration indices of U_{osm} (Pocket Pal-Osmo, Vitech Scientific, Ltd) and U_{sg} (Atago Co., Refractometer, Japan). T_{re} was assessed using a single-use rectal probe (449H, Henleys Medical, UK), placed 10 cm past the anal sphincter, while HR was measured using monitors (Polar, Finland) affixed to the chest. T_{re} and HR were recorded at rest then at 5 min intervals during each session. Cycle ergometers (Monark 620 Ergomedic, Sweden) and a motorised treadmill (Woodway ELG2 GmbH) were used during exercise. NUFL was estimated by the difference in towel-dried NBM pre and post exercise, corrected for fluid intake and urine output.

Perceptual measures

Thermal comfort (TC, Zhang et al., 2004) from 0 (comfortable) to 5 (very uncomfortable), thermal sensation (TSS, Toner et al., 1986) from 0 (unbearably cold) to 8 (unbearably hot) and ratings of perceived exertion (RPE, Borg, 1982) from 6 (no exertion) to 20 (maximal exertion), were recorded at 5 min intervals. Perceptions of fatigue were measured using the MFSI-SF questionnaire (Stein et al., 2004) from 0 (not at all) to 4 (extremely), prior to and post sessions 1 and 4. These items load equally onto five fatigue subscales (General, Physical, Emotional, Mental, Vigour) and an overall Total Fatigue scale.

Immunological measures

Capillary blood samples were collected in 300 µl Lithium Heparin microvettes 10 mins pre and post HA sessions, while participants were sat upright. Whole blood samples were assessed using an automated haematology analyser (XT200i, Sysmex, UK). White blood cells (WBC) and WBC content (neutrophils, eosinophils, basophils, lymphocytes and monocytes) were measured and corrected for change in plasma volume (ΔPV), which was estimated from haemoglobin and haematocrit (Dill & Costill, 1974). Capillary blood sample collection was chosen due to athlete preference, non-invasiveness, reduced discomfort and convenience, and itis a reliable and accurate method (Ponampalam et al., 2012).

Statistical analyses

All data are reported as mean ± standard deviation (SD), and were assessed for normality and sphericity prior to further statistical analyses using SPSS (IBM version 22.0). All physiological data pre-to-post session and between session 1 and 4 were analysed using dependent samples *t-tests*. While all perceptual data were analysed using Wilcoxon signed rank tests. Peak measures were recorded at the end of each session, in addition to calculating the change in T_{re} (ΔT_{re}) and time to target T_{re}. Relationships between perceptual and physiological measures were examined using Spearman's Rho correlation coefficient (r_s). Effect sizes were estimated and meaningful differences evaluated using Cohen's *d* (Cohen, 1988). Statistical significance was accepted as $P<0.05$. A *priori* meaningful limits for physiological adaptations were ΔT_{re} >0.20°C, ΔHR >5 beats·min^{-1}, ΔPV >5% and ΔNUFL >0.20 L·hr^{-1} (Willmott et al., 2015), >1 in scale scores for perceptual measures and fatigue scales, and >10% for immunological markers.

Results

Physiological measures

There were no differences (P>0.05) in resting measures for hydration status, NBM, HR or T_{re} (Table 1). A meaningful reduction in HR_{peak} (-7 beats·min^{-1}, t=1.72, P=0.13, d=0.8) was observed during session 4, alongside a significantly larger NUFL (+197 mL, t=3.22, P=0.01, d=0.7) and subsequent sweat rate (+0.2 L·hr^{-1}, t=3.22 P=0.01, d=0.7) compared to session 1 (Table 1). Resting PV increased 3.5%, although exercising HR and T_{re} did not differ (P>0.05) between session 1 and 4. Athletes were required to exercise for an additional ~4 min to reach target T_{re} during session 4.

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

Perceptual measures

A significantly (P<0.05) lower exercising mean and peak RPE, TSS and TC were observed during session 4 compared to session 1 (Table 2). General and Physical fatigue scales significantly increased pre to post session 1 (Z=2.03 and P=0.04, Z=2.05 and P=0.04, respectively), but not session 4 (Z=0.27 and P=0.89, Z=0.81 and P=0.41, respectively). No differences were observed in the other fatigue scales (P>0.05) (Table 2).

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

Immunological measures

Significant (P<0.05) pre-to-post changes in WBC, neutrophil, lymphocyte, eosinophil and basophil were observed during session 1 and 4 (Table 3). However, there were no differences in any immunological measures pre and post HA, between session 1 and 4.

*****INSERT TABLE 3 HERE*****

Marathon des Sables performance

Seven out of the eight athletes completed the MdS in a mean time of 44:04:34 ± 9:58:42 hr:min:sec. Finishing times ranged from 33:55:00 to 59:55:00 hr:min:sec, with three athlete's final race positions in the top 8%. One athlete withdrew during stage 3 due to medical conditions (dermatological injury), yet none experienced EHI. Individual stage times (distance) were 6:32:06 ± 1:56:34 (34 km) 7:12:30 ± 1:53:32 (41.3 km), 6:42:46 ± 1:34:00 (37.5 km), 18:00:27 ± 4:29:42 (84.3 km) and 6:30:26 ± 1:27:59 hr:min:sec (42.2 km). There were no correlations between total performance time, nor for each stage of the MdS and change in heat adaptation after STHA.

Correlations

Session 1

Of the MFSI-SF scales where pre to post differences were observed, ΔGeneral fatigue was found to correlate with T_{repeak} (r_s=0.81, P=0.02) and RPE (r_s=0.77, P=0.02). ΔPhysical fatigue correlated with T_{repeak} (r_s=0.84, P=0.01), ΔT_{re} (r_s=0.72, P=0.05), body fat (r_s=0.72, P=0.05) and RPE (r_s=0.71, P=0.05). Following up on the significant differences in RPE, correlations were found between RPE and T_{repeak} (r_s=0.85, P=0.01), and TSS (r_s=0.77, P=0.03). RPE_{peak} also correlated with TSS_{peak} (r_s=0.74, P=0.04), and between TSS and T_{repeak} (r_s=0.72, P=0.04).

Session 4

The significant correlations found for ΔGeneral and ΔPhysical fatigue in session 1 were no longer significant, nor were additional correlations found in these scales. However, correlations were shown between RPE and T_{re} (r_s=0.72, P=0.05), and between RPE_{peak} and NUFL (r_s=0.85, P=0.01), TSS (r_s=0.78, P=0.02) and TSS_{peak} (r_s=0.86, P=0.01).

Discussion

The aim of the current study was to investigate the physiological, perceptual and immunological responses of a group of athletes completing STHA in preparation for the MdS. At a physiological level a lack of differences were found in typical markers of STHA (HR or T_{re}), yet sweat rate significantly increased and plasma volume expansion was observed. During STHA, significant improvements in perceptual markers of thermal comfort, sensation and perceived exertion towards exercise-heat stress were found. Moreover, a significant attenuation in perceived fatigue, in addition to a dissociation between perceptions of fatigue and $T_{re,}$ were observed after STHA. No greater changes were observed in WBC count or content across the duration of STHA, thus suggesting maintained immune status and no detrimental effect of repeated exercise-heat stress.

Perceptual responses

During session 4, improved peak perceptual scores of TC (25%), TSS (14%), and RPE (17%) (all $P<0.05$ and $d>1.0$) were observed compared to session 1. Of notable interest, the improvements in exercising TC and TSS appear without concurrent reductions in T_{re} over the course of STHA. Positive relationships expectedly appeared between RPE and T_{re}, and TSS during session 1 and 4. However, during session 4, only RPE was correlated with fluid loss. Therefore, the reduction in RPE at the same fixed exercise intensity during session 4 is likely related to the improved comfort levels, contributed by superior sweat rate and expected lower skin temperature, as opposed to a reduced physiological strain (Flouris & Schlader, 2015). This is due to the prescribed environmental conditions during STHA, which were purposely uncomfortable and perceptually stressing in an attempt to improve perceptual sensitivity during the MdS. The differentiation in thermal perception during heat stress, where TSS which represents the relative intensity of the temperature being sensed (Attia, 1984) varies to TC, which reflects the subjective indifference with the environment (Mercer, 2001). These perceptual adaptations likely represent a reduced tendency to lower self-selected exercise intensity in the heat, and may sustain decision making and cognitive tasks during the race (Taylor et al., 2016). Therefore, highlighting the importance of behavioural thermoregulation during endurance performances in heat stress (Flouris & Schlader, 2015).

Pre to post differences in General and Physical fatigue scales after session 1 (6 ± 7 and 3 ± 3, respectively) but not session 4 (0 ± 2 and 1 ± 2, respectively), indicate STHA was effective in reducing the degree of perceived fatigue in these dimensions. Additionally, the negative relationship between General and Physical fatigue, and T_{re} was no longer present after session 4. This result suggests STHA changes the way athletes' perceive their physiological signals from T_{re},

as after repeated heat exposures T_{re} was no longer an indicator of perceived fatigue. This was in accordance with findings after LTHA (Tamm et al., 2015), indicating lowered feelings of fatigue and exertion, which are less effected by temperature modulation when individuals are heat acclimated. The positive relationship between RPE and fluid loss showed the opposite effect, whereby, during session 4 a positive relationship between RPE_{peak} and NUFL was found, in contrast to session 1. A novel finding of the current study and an intriguing interpretation of these data, is that there is a possible disassociation of signals from T_{re} with perception of General and Physical fatigue and an association of NUFL with RPE after STHA. This result is consistent with the sensory association hypothesis suggested by Watt et al. (2016), who showed a sensory association of T_{re} with chronic repeated heat exposure. However, this study extends their results by demonstrating for the first time, that heat exposure can result in sensory disassociation, possibly due to exercise-heat stress experience (Tamm et al., 2015), which can benefit athletes during their tapering for such ultra-endurance events, although, further research is required to confirm a HA or training effect.

Immunological responses

Immunological results remained within normal clinical levels throughout STHA and are in accordance with previous acute exercise-heat stress literature (Mitchell et al., 2002; McFarlin and Mitchell, 2003). Increased transient responses in WBC (25%), neutrophil (30%) and lymphocyte (18%) counts, were observed following session 1, which typically return to baseline within 24hrs (Kakanis et al., 2010). No differences were found compared to session 4, which displayed similar responses for WBC (17%), neutrophil (16%) and lymphocyte (22%) counts, due to maintenance of T_{re} during controlled hyperthermia. Nor were changes observed in resting measures over the course of STHA, suggesting a maintained immune status and no detrimental effect of repeated exercise-heat stress prior to departing for the MdS. However, it is acknowledged that a more comprehensive overview of immune biomarkers should be assessed for clinical significance (Albers et al., 2005). The findings of this study are in line with Guy et al. (2016) who reported no effects on inflammatory markers, LPS or evidence of endotoxemia after STHA. Whereas, our results are in contrast to Hailes et al. (2011), who reported increased pro- and anti-inflammatory markers at rest after consecutive exercise-heat stress and a reduced response to a subsequent bout of heat-stress, thus suggesting unacclimated or lower trained individuals may be at an increased susceptibility to EHI if ineffectively prepared. Both the current study and Guy et al. (2016) are in accordance with the preparation recommendations by Pyne et al. (2014), which attempts to enhance exercise performance, while also improving thermotolerance and reducing the likelihood of endotoxin

meditated EHI. Consequently, more emphasis on an athlete's immune status is warranted, as increased physiological strain and possible insufficient recovery during LTHA may compromise athletes' health and incur minor illnesses (Lim et al., 2009; Walsh et al., 2011), thus reducing training quality and impairing exercise performance (Tyler et al., 2016).

Physiological responses

Sweat rate significantly improved (+0.2 $L \cdot hr^{-1}$ [+14%]) after STHA, in line with similar studies (Gibson et al., 2015a; Mee et al., 2015a; Neal et al., 2015). While superior responses are expected after LTHA (Racinais et al., 2015), peripheral sudomotor adaptation observed in this study is contributed by hypervolemia (3.5%) and the magnitude of heat stress during our STHA protocol, as strong relationships are reported between sweat rate and environmental conditions (Tyler et al., 2016). Moreover, as central adaptations (i.e. lower sweat setpoint) typically occur after LTHA and are concurrent with T_{re} reductions, a likely mechanism is the peripheral modulation of sweat gland output, which are associated with local skin temperature (Shibasaki et al., 2006). Therefore, the magnitude of heat stress during STHA provoked a greater sweat gland activity and output for effective fluid loss within the athletes, as opposed to the optimal elevated T_{re} required for central adaptations. This finding suggests athletes tapering for competition in hot conditions should either amplify ambient temperature or restrict heat evaporative loss during STHA, when preparation time for typical sudomotor adaptations is limited.

Meaningful reductions in HR_{peak} were evident (-7 $beats \cdot min^{-1}$, d=0.8), although these did not significantly differ pre to post HA. However, evidence of improved acclimation state appeared as athletes were required to exercise for longer until reaching the target T_{re} during session 4 (+4 mins), as found within other STHA studies (Garrett et al., 2012; Gibson et al., 2015a; Mee et al., 2015a). A limited time (20-25 mins per session) was spent above 38.5°C, which may explain the lower mean ΔPV (+3.5%) and relatively limited cardiovascular and thermoregulatory adaptations (Sawka et al., 2011; Racinais et al., 2015), compared to other studies (Garrett et al., 2012; Tyler et al., 2016).

Limitations and future direction

Regrettably, we were unable to perform pre or post heat acclimation state (Willmott et al., 2015), heat stress or maximal oxygen uptake tests, due to time constraints and athlete availability. Moreover, as a vast range of split times were observed for each stage of the event, tailored HA determined by prior aerobic capacity and heat acclimation state tests may be required for individuals or teams of similar physical characteristics competing in future multi-day endurance

events in extreme conditions. Future applied research is required to investigate HA efficacy in trained athletes in order to confer total heat adaptation, as they appear partially acclimated and may require a longer and, or more intense HA protocol. However, if time is restricted and, or environmental chambers are inaccessible while preparing for athletic events in hot conditions, coaches may seek alternate methods that increase the magnitude of physiological strain to confer optimal adaptations. Such strategies include; higher intensity exercise prescription (Houmard et al., 1990; Wingo, 2015), pre/post warm water immersion (Zurawlew et al., 2015; Ruddock et al., 2016), larger magnitudes of heat stress and, or combined restrictive evaporative heat loss (i.e. sauna suits) (Mee et al., 2015b). It is also suggested consecutive or intermittent twice daily HA protocols (Willmott et al., 2016) may suit the athlete's training commitments to sustain the quality of tapering. However, further investigations are required to fully assess the efficacy of these alternate methods of HA for optimal heat adaptation.

Practical application

The STHA protocol prescribed during this study, which was designed to maximise the magnitude of heat stress (34°C WBGT) to maintain target T_{re} (38.5°C) (Taylor, 2014) and reduce exercise intensity (Gibson et al., 2015a), is applicable to coaches and their athletes while tapering for endurance events within hot conditions. While accommodating the athlete's time and duration restrictions, meaningful adaptations above predefined limits in sweat rate (14%) and plasma volume (3.5%) were observed within the 4 days prior to departure, without any decrement to health or expected HA decay as athletes began the MdS 72hrs later. Although STHA prepares individuals for competing in heat stress (Taylor, 2014), due to the multi-day ultra-endurance event, such physiological adaptations may not solely influence the likelihood of attaining an EHI or improve performance *per se*, due to the longevity of the race and numerous exogenous factors, unlike temperate, single-day events (i.e. marathon). Therefore, coaches and athletes from cooler conditions who cannot complete LTHA to optimally adapt to heat stress, may still benefit from this rapid STHA protocol to improve perceptual responses towards the heightened magnitude of heat stress, while also benefitting from key factors such as; educational awareness of T_{re}, pacing strategies, individual HR zones and sweat rates, equipment checks and improving confidence levels prior to departure.

Conclusion

In conclusion, this is the first study to adopt a controlled hyperthermia STHA protocol with athletes preparing for a multi-day desert marathon. The STHA protocol induced favourable improvements

in perceptual adaptations of thermal comfort, thermal sensation and perceived exertional measures, as well as reducing the perceptions of fatigue. Although STHA did not confer full physiological heat adaptation, likely due to a sub-optimal strain, sweat rate was significantly improved owing to the high level of prescribed heat stress. Lastly, immune status was unaffected by repeated exercise-heat stress, suggesting athletes remained in good health prior to departing for the multi-day ultra-endurance event, while recognising their individual time constraints.

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Conflict of interest

The authors confirm there are no conflicts of interest

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	Session 1	Session 4	Δ 1 to 4	*P* (*d*)

Tables

Table 1. Mean ± SD physiological responses at rest, during and post short term heat acclimation

		Rest		
NBM (kg)	81.6 ± 14.7	81.7 ± 15.2	0.1 ± 0.6	0.32 (0.0)
HR ($b \cdot min^{-1}$)	59 ± 9	58 ± 4	-1 ± 7	0.29 (0.1)
T_{re} (°C)	37.00 ± 0.37	36.98 ± 0.24	-0.02 ± 0.21	0.42 (0.1)
U_{osm} ($mOsm \cdot kg^{-1}$)	580 ± 448	581 ± 303	1 ± 468	0.50 (0.0)
U_{sg}	1.022 ± 0.015	1.019 ± 0.009	-0.002 ± 0.015	0.39 (0.2)
∆PV (%)			3.5 ± 2.8	
		Exercise		
HR ($b \cdot min^{-1}$)	131 ± 9	127 ± 9	-4 ± 8	0.14 (0.4)
HR_{peak} ($b \cdot min^{-1}$)	158 ± 17	151 ± 20	-7 ± 13	0.13† (0.8)
T_{re} (°C)	38.32 ± 0.29	38.23 ± 0.23	-0.09 ± 0.31	0.22 (0.4)
T_{repeak} (°C)	39.02 ± 0.36	38.92 ± 0.24	-0.10 ± 0.42	0.27 (0.3)
ΔT_{re} (°C)	2.02 ± 0.41	1.94 ± 0.29	-0.08 ± 0.41	0.30 (0.2)
Time to 38.5°C (min:sec)	34:11 ± 7:43	38:11 ± 12:44	4:00 ± 11:44	0.18 (0.4)
NUFL (mL)	1411 ± 594	1608 ± 626	197 ± 204	0.01* (0.7)
Sweat rate ($L \cdot hr^{-1}$)	1.41 ± 0.59	1.61 ± 0.63	0.20 ± 0.20	0.01*† (0.7)
		Pre to post session		
Pre-post ∆PV (%)	-1.8 ± 4.9	-2.4 ± 5.1		0.80 (0.1)

*represents a significant difference ($P \leq 0.05$) and † a meaningful change between session 1 and 4. ∆ = change.

Table 2. Mean ± SD perceptual responses at rest, during and after short term heat acclimation sessions

1

	Session 1	Session 4	Δ 1 to 4	*P* (*d*)
		Rest		
TSS	5.5 ± 0.4	5.1 ± 0.6	-0.4 ± 0.6	0.11 (0.8)
TC	3 ± 1	2 ± 1*	-1 ± 1	0.05 (1.0)
		Exercise		
RPE	12 ± 2	10 ± 2*	-2 ± 1	0.01 (1.0)
RPE_{peak}	15 ± 1	13 ± 2*	-2 ± 2	0.04 (1.3)
TSS	5.7 ± 0.4	5.1 ± 0.6*	-0.6 ± 0.5	0.04 (1.2)
TSS_{peak}	6.5 ± 0.6	5.6 ± 1.0*	-0.9 ± 0.7	0.03 (1.1)
TC	3 ± 1	2 ± 1*	-1 ± 1	0.02 (1.0)
TC_{peak}	4 ± 1	3 ± 1*	-1 ± 1	0.02 (1.0)
		Pre to post session		
MFSI-SF	**ΔSession 1 (*P*)**		**ΔSession 4 (*P*)**	
General	6 ± 7† (0.04)		0 ± 2* (0.89)	
Physical	3 ± 3† (0.04)		1 ± 2* (0.41)	
Emotional	0 ± 1 (0.74)		-1 ± 1 (0.11)	
Mental	1 ± 1 (0.11)		0 ± 1 (1.00)	
Vigor	-4 ± 6 (0.12)		1 ± 5 (0.85)	
Total	13 ± 15 (0.08)		0 ± 3 (0.85)	

*represents a significant difference ($P \leq 0.05$) between session 1 and 4, and † between ($P \leq 0.05$) pre and post session 1. Δ = change, MFSI-SF = multidimensional fatigue symptom inventory-short form.

2 Table 3. Mean ± SD immunological markers pre and post short term heat acclimation sessions.

	Session 1			Session 4			Δ 1 and 4		
	Pre	**Post**	**Δ (*P*, *d*)**	**Pre**	**Post**	**Δ (*P*, *d*)**	**ΔPre (p, *d*)**	**ΔPost (*P*, *d*)**	**Δ *P***
WBC ($10^9 \cdot L^{-1}$)	5.82 ± 1.84	6.98 ± 1.63*	1.15 ± 1.16 (0.04, 0.7)	5.56 ± 1.93	6.63 ± 2.20*	1.07 ± 0.85 (0.01, 0.6)	-0.26 ± 1.69 (0.70, 0.1)	-0.27 ± 2.01 (0.76, 0.1)	0.98
Neutrophil ($10^9 \cdot L^{-1}$)	3.58 ± 1.66	4.43 ± 1.72*	0.85 ± 0.85 (0.00, 0.5)	3.48 ± 1.51	4.11 ± 1.55*	0.63 ± 0.53 (0.00, 0.4)	-0.11 ± 1.31 (0.57, 0.1)	-0.32 ± 1.42 (0.58, 0.2)	0.89
Lymphocytes ($10^9 \cdot L^{-1}$)	1.59 ± 0.39	1.87 ± 0.50*	0.28 ± 0.30 (0.05, 0.6)	1.49 ± 0.42	1.97 ± 0.63 *	0.47 ± 0.33 (0.01, 0.9)	-0.10 ± 0.37 (0.51, 0.2)	0.10 ± 0.66 (0.71, 0.2)	0.34
Monocytes ($10^9 \cdot L^{-1}$)	0.46 ± 0.13	0.51 ± 0.14	0.05 ± 0.17 (0.45, 0.4)	0.43 ± 0.16	0.47 ± 0.13	0.06 ± 0.10 (0.16, 0.3)	-0.02 ± 0.17 (0.80, 0.2)	-0.01 ± 0.25 (0.92, 0.3)	0.93
Eosinophil ($10^9 \cdot L^{-1}$)	0.17 ± 0.07	0.13 ± 0.05*	-0.04 ± 0.04 (0.04, 0.7)	0.13 ± 0.04	0.11 ± 0.05	-0.01 ± 0.05 (0.55, 0.4)	-0.04 ± 0.05 (0.05†, 0.7)	0.02 ± 0.03 (0.18, 0.4)	0.27
Basophil ($10^9 \cdot L^{-1}$)	0.027 ± 0.013	0.034 ± 0.011*	0.007 ± 0.008 (0.05, 0.0)	0.024 ± 0.013	0.031 ± 0.015	0.007 ± 0.010 (0.09, 0.6)	-0.003 ± 0.018 (0.69, 1.0)	-0.003 ± 0.011 (0.52, 0.0)	1.00

*represents a significant difference ($P \leq 0.05$) between pre and post, † between pre session 1 and 4. Δ = change.

3

4