

1 Title of the Article:

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

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19 Heat acclimation for a desert ultra-marathon

20

21 Abstract

22 Purpose

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

28 Methods

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

33 Results

34 STHA improved peak thermal comfort ($-1, P=0.02$), sensation ($-1, P=0.03$) and perceived exertion ($-$
35 $2, P=0.04$). A dissociated relationship between perceptions of fatigue and T_{re} was evident after
36 STHA, with reductions in perceived physical ($-6, P=0.04$) and general ($-2, P=0.04$) fatigue.
37 Exercising T_{re} and HR did not change ($P>0.05$), however, sweat rate increased 14% ($P=0.02$). No
38 changes were found in white blood cell counts or content ($P>0.05$).

39 Conclusions

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

43

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47 Key words

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

49

50 Abbreviations

51	Δ	Change
52	HA	Heat acclimation
53	HR	Heart rate
54	LTHA	Long term heat acclimation
55	MdS	Marathon des Sables
56	MFSI-SF	Multi-dimensional fatigue scale inventory-short form
57	NBM	Nude body mass
58	NUFL	Non-urine fluid loss
59	PV	Plasma volume
60	RPE	Rating of perceived exertion
61	STHA	Short term heat acclimation
62	TC	Thermal comfort
63	TSS	Thermal sensation
64	T_{re}	Rectal Temperature
65	U_{osm}	Urine osmolality
66	U_{sg}	Urine specific gravity
67	WBC	White blood cell

69 Multi-stage, ultra-endurance events within extreme environmental conditions are increasing in
70 popularity (Knoth et al., 2012). The annual Marathon des Sables (MdS), is a ~250 km multi-day
71 race across the Sahara desert in Morocco, where competitors are self-dependent, carrying 5-10 kg
72 of equipment in extreme heat stress (~40°C). Consequently, an array of challenges are experienced
73 including; cumulative fatigue, dehydration, sleep deprivation, energy deficit (Costa et al., 2016),
74 high solar heat loads with limited shade and, prolonged metabolic heat production. Such
75 consequences exacerbate physiological and perceptual strain, augment the likelihood of
76 dermatological injuries and gastrointestinal disorders (Gill et al., 2015; Costa et al., 2016), and
77 increase the risk of exertional heat-related illnesses (EHI) (Coris et al., 2004), which can lead to
78 race-withdrawal and emergency treatment (DeMartini et al., 2014). Therefore, to reduce the
79 deleterious effects of heat stress it is imperative that athletes prepare effectively (Périard et al.,
80 2015; Racinais et al., 2015), however, a paucity of research describing effective and applied
81 preparation strategies, specifically for ultra-marathons in heat stress exists.

82 Heat acclimation (HA) is an intervention comprising 4-21 days of repeated, prolonged exposures to
83 high ambient temperatures (>30°C) and moderate-high relative humidity (>40%) (Racinais et al.,
84 2015; Tyler et al., 2016). The numerous physiological advantages induced by HA have been
85 discussed in detail elsewhere (Sawka et al., 2011; Garrett et al., 2014; Taylor, 2014; Racinais et al.,
86 2015). Short-term HA (STHA) can confer up to 75% of physiological adaptations typically seen
87 following long-term HA (LTHA) (Pandolf, 1998), and appears effective across a range of
88 populations (Costa et al., 2014; Garrett et al., 2014; Gibson et al., 2015a; Mee et al., 2015a; Neal et
89 al., 2015; Willmott et al., 2016). Ultra-endurance athletes may benefit from STHA prior to
90 competition (Costa et al., 2014), particularly when optimal controlled hyperthermia methods
91 (Taylor, 2014; Racinais et al., 2015) are implemented, due to reduced training volume for equal
92 physiological (Gibson et al., 2015a) and cellular adaptations (Gibson et al., 2015b). Beside
93 physiological adaptations, perceived exertion is reduced (Neal et al., 2015), alongside improved
94 thermal comfort (Costa et al., 2014), sensation (Gibson et al., 2015; Neal et al., 2015) and
95 perceived fatigue (Tamm et al., 2015), although such improvements have not been found within
96 ultra-endurance athletes during HA. This is of current interest, as ultra-marathon runners are highly
97 motivated, display mental toughness and attain lower pain perceptions (Hoffman et al., 2014), thus,
98 when fatigued decision making may be compromised leading to injury and, or illness (Maruff et al.,
99 2006).

100 Improved perceptions of fatigue and temperature during exercise-heat stress may be beneficial for
101 ultra-marathon performances in the absence of alterations in physiological markers, due to the
102 impact each may have on pacing strategy within individual stages of an event (McCormick et al.,
103 2015). Moreover, when navigation and decision making are necessary, improved perception may
104 be important in ameliorating the combined, and independent effects of heat stress, and exercise on
105 cognitive markers (Taylor et al., 2016). However, minimal evidence regarding athletes preparing
106 for ultra-endurance events exists (Costa et al., 2014) and it is unclear how the relationship between
107 perceptual and physiological markers of heat adaptation change following STHA.

108 While few studies have investigated the effects of HA on immune function, STHA (Guy et al.,
109 2016) and long term passive heat exposure (Kanikowska et al., 2012), report minor challenges to
110 immune, inflammation and endotoxemia status (Walsh et al., 2011). This is in contrast to
111 heightened inflammatory markers (Hailes et al., 2011) and disturbances in immune cell number and
112 function after acute (Mitchell et al., 2002) and chronic (Hailes et al., 2011; Watt et al., 2016)
113 moderate-intensity exercise-heat stress. Consequently, inflammation and leukocytosis occurs with
114 an associated increase in circulating leukocyte cell counts, primarily caused by neutrophilia
115 (Mestre-Alfaro et al., 2012). Such discrepancies are likely due to the exercise prescription, duration
116 and physiological strain experienced as well as intervention efficacy. Predisposing factors
117 including; ineffective preparation (i.e. unacclimated, low training status and high body fat) and a
118 compromised immune status prior to competition (Walsh et al., 2011; Gill et al., 2015) may
119 suppress anti-lipopolysaccharide (LPS) mechanisms, promote inflammatory and pyrogenic
120 activities and increase the susceptibility to EHI (Lim & Mackinnon, 2006; Hailes et al., 2011; Guy
121 et al., 2016). This will compromise the ecological validity of HA, should individuals experience
122 immune dysfunction or other related illnesses (Costa et al., 2016), which have negative impacts
123 upon health, heat tolerance and imminent exercise performance (Pyne et al., 2005). Therefore,
124 monitoring biomarkers associated with immune function and overtraining, alongside tracking heat
125 load, perceptual feedback and training intensity during intervention protocols is required (Guy et
126 al., 2016). Consequently, the aim of this study was to investigate the physiological and perceptual
127 markers of heat adaptation, and immune responses to STHA within a group of athletes preparing
128 for a multi-day desert ultra-marathon. It was hypothesised STHA would induce heat adaptation
129 without evidence of immune dysfunction.

130 Methods

131 Athletes

132 Eight un-acclimated, male ultra-endurance runners (>150 km weekly) (age; 42 ± 4 years, body
133 mass; 81.9 ± 15.0 kg, stature; 178 ± 8 cm, sum of 4 skin fold; 39.0 ± 14.7 mm and body fat; $17.6 \pm$
134 5.9%) volunteered and provided written informed consent for the study, which was conducted in
135 accordance with the Institution's ethics and governance committee and Declaration of Helsinki
136 (2013). Athletes had not experienced hot conditions (> 25°C) for >3 months and abstained from
137 caffeine, alcohol and strenuous activity for 24hrs prior to each session. Athletes also restricted food
138 intake 2hrs prior to exercise, but maintained normal diet during the intervention. Athletes were
139 instructed to arrive euhydrated (urine osmolality [U_{osm}] <700 mOsm $\cdot\text{kg}^{-1}$ and specific gravity [U_{sg}]
140 <1.030) (Sawka et al., 2007), or consumed 500mL of water over 30 mins before confirmation of
141 hydration status prior to commencing exercise (n=1, session 1 and 4).

142 Experimental design

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

147 Short term heat acclimation protocol

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

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

162 Physiological measures and equipment

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

174 Perceptual measures

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

182 Immunological measures

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

191 Statistical analyses

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

203 Results

204 Physiological measures

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

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

212 Perceptual measures

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

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

219 Immunological measures

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

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

224 Marathon des Sables performance

225 Seven out of the eight athletes completed the MdS in a mean time of $44:04:34 \pm 9:58:42$
226 hr:min:sec. Finishing times ranged from 33:55:00 to 59:55:00 hr:min:sec, with three athlete's final
227 race positions in the top 8%. One athlete withdrew during stage 3 due to medical conditions
228 (dermatological injury), yet none experienced EHI. Individual stage times (distance) were $6:32:06$
229 $\pm 1:56:34$ (34 km) $7:12:30 \pm 1:53:32$ (41.3 km), $6:42:46 \pm 1:34:00$ (37.5 km), $18:00:27 \pm 4:29:42$
230 (84.3 km) and $6:30:26 \pm 1:27:59$ hr:min:sec (42.2 km). There were no correlations between total
231 performance time, nor for each stage of the MdS and change in heat adaptation after STHA.

232 Correlations

233 Session 1

234 Of the MFSI-SF scales where pre to post differences were observed, Δ General fatigue was found to
235 correlate with T_{repeak} ($r_s=0.81$, $P=0.02$) and RPE ($r_s=0.77$, $P=0.02$). Δ Physical fatigue correlated
236 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 ($r_s=0.71$,
237 $P=0.05$). Following up on the significant differences in RPE, correlations were found between RPE
238 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}
239 ($r_s=0.74$, $P=0.04$), and between TSS and T_{repeak} ($r_s=0.72$, $P=0.04$).

240 Session 4

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

245

246 Discussion

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

256 Perceptual responses

257 During session 4, improved peak perceptual scores of TC (25%), TSS (14%), and RPE (17%) (all
258 $P < 0.05$ and $d > 1.0$) were observed compared to session 1. Of notable interest, the improvements in
259 exercising TC and TSS appear without concurrent reductions in T_{re} over the course of STHA.
260 Positive relationships expectedly appeared between RPE and T_{re} , and TSS during session 1 and 4.
261 However, during session 4, only RPE was correlated with fluid loss. Therefore, the reduction in
262 RPE at the same fixed exercise intensity during session 4 is likely related to the improved comfort
263 levels, contributed by superior sweat rate and expected lower skin temperature, as opposed to a
264 reduced physiological strain (Flouris & Schlader, 2015). This is due to the prescribed
265 environmental conditions during STHA, which were purposely uncomfortable and perceptually
266 stressing in an attempt to improve perceptual sensitivity during the MdS. The differentiation in
267 thermal perception during heat stress, where TSS which represents the relative intensity of the
268 temperature being sensed (Attia, 1984) varies to TC, which reflects the subjective indifference with
269 the environment (Mercer, 2001). These perceptual adaptations likely represent a reduced tendency
270 to lower self-selected exercise intensity in the heat, and may sustain decision making and cognitive
271 tasks during the race (Taylor et al., 2016). Therefore, highlighting the importance of behavioural
272 thermoregulation during endurance performances in heat stress (Flouris & Schlader, 2015).

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

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

292 Immunological responses

293 Immunological results remained within normal clinical levels throughout STHA and are in
294 accordance with previous acute exercise-heat stress literature (Mitchell et al., 2002; McFarlin and
295 Mitchell, 2003). Increased transient responses in WBC (25%), neutrophil (30%) and lymphocyte
296 (18%) counts, were observed following session 1, which typically return to baseline within 24hrs
297 (Kakanis et al., 2010). No differences were found compared to session 4, which displayed similar
298 responses for WBC (17%), neutrophil (16%) and lymphocyte (22%) counts, due to maintenance of
299 T_{re} during controlled hyperthermia. Nor were changes observed in resting measures over the course
300 of STHA, suggesting a maintained immune status and no detrimental effect of repeated exercise-
301 heat stress prior to departing for the MdS. However, it is acknowledged that a more comprehensive
302 overview of immune biomarkers should be assessed for clinical significance (Albers et al., 2005).
303 The findings of this study are in line with Guy et al. (2016) who reported no effects on
304 inflammatory markers, LPS or evidence of endotoxemia after STHA. Whereas, our results are in
305 contrast to Hailes et al. (2011), who reported increased pro- and anti-inflammatory markers at rest
306 after consecutive exercise-heat stress and a reduced response to a subsequent bout of heat-stress,
307 thus suggesting unacclimated or lower trained individuals may be at an increased susceptibility to
308 EHI if ineffectively prepared. Both the current study and Guy et al. (2016) are in accordance with
309 the preparation recommendations by Pyne et al. (2014), which attempts to enhance exercise
310 performance, while also improving thermotolerance and reducing the likelihood of endotoxin

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

315 Physiological responses

316 Sweat rate significantly improved ($+0.2 \text{ L}\cdot\text{hr}^{-1}$ [+14%]) after STHA, in line with similar studies
317 (Gibson et al., 2015a; Mee et al., 2015a; Neal et al., 2015). While superior responses are expected
318 after LTHA (Racinais et al., 2015), peripheral sudomotor adaptation observed in this study is
319 contributed by hypervolemia (3.5%) and the magnitude of heat stress during our STHA protocol, as
320 strong relationships are reported between sweat rate and environmental conditions (Tyler et al.,
321 2016). Moreover, as central adaptations (i.e. lower sweat setpoint) typically occur after LTHA and
322 are concurrent with T_{re} reductions, a likely mechanism is the peripheral modulation of sweat gland
323 output, which are associated with local skin temperature (Shibasaki et al., 2006). Therefore, the
324 magnitude of heat stress during STHA provoked a greater sweat gland activity and output for
325 effective fluid loss within the athletes, as opposed to the optimal elevated T_{re} required for central
326 adaptations. This finding suggests athletes tapering for competition in hot conditions should either
327 amplify ambient temperature or restrict heat evaporative loss during STHA, when preparation time
328 for typical sudomotor adaptations is limited.

329 Meaningful reductions in HR_{peak} were evident ($-7 \text{ beats}\cdot\text{min}^{-1}$, $d=0.8$), although these did not
330 significantly differ pre to post HA. However, evidence of improved acclimation state appeared as
331 athletes were required to exercise for longer until reaching the target T_{re} during session 4 (+4 mins),
332 as found within other STHA studies (Garrett et al., 2012; Gibson et al., 2015a; Mee et al., 2015a).
333 A limited time (20-25 mins per session) was spent above 38.5°C , which may explain the lower
334 mean ΔPV (+3.5%) and relatively limited cardiovascular and thermoregulatory adaptations (Sawka
335 et al., 2011; Racinais et al., 2015), compared to other studies (Garrett et al., 2012; Tyler et al.,
336 2016).

337 Limitations and future direction

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

343 events in extreme conditions. Future applied research is required to investigate HA efficacy in
344 trained athletes in order to confer total heat adaptation, as they appear partially acclimated and may
345 require a longer and, or more intense HA protocol. However, if time is restricted and, or
346 environmental chambers are inaccessible while preparing for athletic events in hot conditions,
347 coaches may seek alternate methods that increase the magnitude of physiological strain to confer
348 optimal adaptations. Such strategies include; higher intensity exercise prescription (Houmard et al.,
349 1990; Wingo, 2015), pre/post warm water immersion (Zurawlew et al., 2015; Ruddock et al.,
350 2016), larger magnitudes of heat stress and, or combined restrictive evaporative heat loss (i.e. sauna
351 suits) (Mee et al., 2015b). It is also suggested consecutive or intermittent twice daily HA protocols
352 (Willmott et al., 2016) may suit the athlete's training commitments to sustain the quality of
353 tapering. However, further investigations are required to fully assess the efficacy of these alternate
354 methods of HA for optimal heat adaptation.

355 Practical application

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

372 Conclusion

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

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

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383 Conflict of interest

384 The authors confirm there are no conflicts of interest

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Session 1	Session 4	Δ 1 to 4	<i>P</i> (<i>d</i>)
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Tables

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

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Rest				
NBM (kg)	81.6 ± 14.7	81.7 ± 15.2	0.1 ± 0.6	0.32 (0.0)
HR (b·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·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)
ΔPV (%)			3.5 ± 2.8	
Exercise				
HR (b·min⁻¹)	131 ± 9	127 ± 9	-4 ± 8	0.14 (0.4)
HR_{peak} (b·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·hr⁻¹)	1.41 ± 0.59	1.61 ± 0.63	0.20 ± 0.20	0.01*† (0.7)
Pre to post session				
Pre-post ΔPV (%)	-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. Δ = change.

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

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

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

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

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