

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

3

4 Original Investigation

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6 Jo Corbett¹, Heather C. Massey¹, Joseph T. Costello¹, Michael J. Tipton¹, Rebecca A. Neal²

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8 ¹ Extreme Environments Laboratory, School of Sport, Health and Exercise Science, University
9 of Portsmouth, UK

10 ² Department of Rehabilitation and Sport Sciences, Bournemouth University, UK

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13 Corresponding author: Jo Corbett, University of Portsmouth,

14 School of Sport, Health and Exercise Science

15 Spinnaker Building

16 Cambridge Road

17 Portsmouth

18 Hampshire

19 PO1 2ER

20 jo.corbett@port.ac.uk

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22 Running head: Training in the heat for temperate performance.

23 **Abstract:**

24 We investigated whether an 11-day heat acclimation programme (HA) enhanced endurance
25 performance in a temperate environment, and the mechanisms underpinning any ergogenic
26 effect. Twenty-four males ($\dot{V}O_{2\max}$: $56.7 \pm 7.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) completed either: i) HA consisting
27 of 11 consecutive daily exercise sessions ($60\text{-}90 \text{ minutes} \cdot \text{day}^{-1}$; $n=16$) in a hot environment
28 (40°C , $50\% \text{ RH}$) or; ii) duration and exertion matched exercise in cool conditions (CON; $n=8$
29 [11°C , $60\% \text{ RH}$]). Before and after each programme power at lactate threshold, mechanical
30 efficiency, $\dot{V}O_{2\max}$, peak power output (PPO) and work done during a 30-minute cycle trial
31 (T30) were determined under temperate conditions (22°C , $50\% \text{ RH}$). HA reduced resting (-
32 $0.34 \pm 0.30^\circ\text{C}$) and exercising ($-0.43 \pm 0.30^\circ\text{C}$) rectal temperature, and increased whole-body
33 sweating ($+0.37 \pm 0.31 \text{ L} \cdot \text{hr}^{-1}$) (all $P \leq 0.001$), with no change in CON. Plasma volume increased
34 in HA ($10.1 \pm 7.2\%$, $P < 0.001$) and CON ($7.2 \pm 6.3\%$, $P = 0.015$) with no between-groups
35 difference, whereas exercise heart rate reduced in both groups, but to a greater extent in HA (-
36 $20 \pm 11 \text{ b} \cdot \text{min}^{-1}$) than CON ($-6 \pm 4 \text{ b} \cdot \text{min}^{-1}$). $\dot{V}O_{2\max}$, lactate threshold and mechanical efficiency
37 were unaffected by HA. PPO increased in both groups ($+14 \pm 18 \text{ W}$), but this was not related to
38 alterations in any of the performance or thermal variables, and T30 performance was
39 unchanged in either group (HA: Pre= 417 ± 90 vs. Post= 427 ± 83 kJ; CON: Pre= 418 ± 63 vs.
40 Post= 423 ± 56 kJ). In conclusion, 11-days HA induces thermophysiological adaptations, but
41 does not alter the key determinants of endurance performance. In trained males, the effect of
42 HA on endurance performance in temperate conditions is no greater than that elicited by
43 exertion and duration matched exercise training in cool conditions.

44

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

46

47 Introduction

48 Prolonged (≥ 15 minutes) exercise performance is impaired in a hot environment, relative to
49 cool conditions (Guy *et al.*, 2015). This performance decrement is multi-causal (Nybo *et al.*,
50 2014). Nevertheless, repeated frequent exposure to high ambient heat, either in the laboratory
51 (heat acclimation [HA]), or natural environment (acclimatisation), elicits adaptations that reduce
52 the performance decrement (Keiser *et al.*, 2015; Racinais *et al.*, 2015b). Therefore, HA is a
53 widely advocated intervention for optimising exercise performance in hot environments
54 (Bergeron *et al.*, 2012). However, as early as 1959, following laboratory and field observations
55 on elite distance runners, Bannister and Cotes suggested that ambient temperatures ‘*in the*
56 *range of 15-25°C could become a limiting factor when subjects are performing strenuous*
57 *exercise*’(p. 61). It is now apparent that endurance performance can progressively decline as
58 ambient temperature increases beyond $\sim 10^{\circ}\text{C}$ (Galloway & Maughan 1997; Ely *et al.*, 2007),
59 although this relationship will be influenced by other parameters influencing heat exchange,
60 including exercise mode (Junge *et al.*, 2016) and other climatic factors (Maughan *et al.*, 2012;
61 Otani *et al.*, 2018). Nevertheless, there remains a limited amount of research investigating the
62 effects of HA on endurance performance in these temperate conditions.

63

64 It has been hypothesised that HA might attenuate any ‘thermal’ decrement in performance that
65 is evident in temperate conditions, in a manner similar to that evident under hotter conditions
66 (Shvartz *et al.*, 1977; Corbett *et al.*, 2014), although experimental data are lacking.
67 Alternatively, HA could be ergogenic through ‘non-thermal’ mechanisms related to
68 haematological (Lorenzo *et al.*, 2010; Oberholzer *et al.*, 2019), cardiovascular (Coyle *et al.*,
69 1990; Lorenzo *et al.*, 2010) and skeletal muscle adaptations (Kodesh & Horowitz, 2010; Goto
70 *et al.*, 2011), and their effects on $\text{VO}_{2\text{max}}$ (Lorenzo *et al.*, 2010; Waldron *et al.*, 2019), lactate
71 threshold (Lorenzo *et al.*, 2010) and mechanical efficiency (Shvartz *et al.*, 1977; Sawka *et al.*,
72 1983). These parameters are the key physiological determinants of endurance performance
73 (Joyner & Coyle, 2008) and any improvement should translate to a performance benefit.
74 However, the ergogenic potential of heat under cooler conditions is contentious (Minson &
75 Cotter, 2016; Nybo & Lundby, 2016) with some studies providing evidence for an ergogenic
76 effect (Sawka *et al.*, 1985; Lorenzo *et al.* 2010; Racinais *et al.*, 2014; McCleave *et al.*, 2017;) and
77 others reporting no effect (Karlsen *et al.*, 2015; Keiser *et al.*, 2015; Mikkelsen *et al.*, 2019).

78

79 It has been suggested that the discrepant findings between studies are due to variations in study
80 design including methodological limitations such as absence of a control group, limited

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

98
99 Accordingly, the aims of the present study were, twofold. Firstly, to determine whether a
100 medium-term HA intervention would enhance endurance performance under temperate
101 conditions compared to a control group undertaking an exertion matched exercise programme
102 in a cool environment. We utilised temperate rather than cool conditions to enable the
103 evaluation of potential ergogenic effects of HA arising from both ‘non-thermal’ and ‘thermal’
104 mechanisms and because these conditions are common during athletic competition, but have
105 received limited attention in the scientific literature. A medium-term HA was selected to ensure
106 near-complete cardiovascular and sudomotor adaptation to heat (Racinais *et al.*, 2015a) and for
107 consistency with previous research demonstrating an ergogenic benefit of HA under cool
108 conditions (Lorenzo *et al.*, 2010). We utilised a perception based control group to address
109 concerns over appropriately matching the physiological strain of the intervention and control
110 groups in environmental-stressor research (Mikkelsen *et al.*, 2019). Secondly, we sought to
111 provide insight into the mechanism(s) underpinning any ergogenic effect. Our hypotheses were
112 that HA would: 1) improve thermoregulation; 2) improve the key physiological determinants
113 of endurance performance (efficiency, lactate threshold, $\text{VO}_{2\text{max}}$, and; 3) increase endurance
114 performance in a temperate environment.

115 **Method**

116 **Participants**

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

124

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

126

127 **Experimental design**

128 The data presented in this study were from a programme of work investigating the ergogenic
129 potential of HA in a temperate environment, and include data pooled from our published work
130 (Neal *et al.*, 2016; Rendell *et al.*, 2017) as well as previously unpublished data. The study
131 employed a between-groups design and the study design is summarised in Figure 1. An
132 experimental group (HA; $n=16$) completed a medium-term HA programme consisting of daily
133 exercise in a hot environment (40°C, 50% RH). Thereafter, a separate control group (CON,
134 $n=8$) completed an exertion and duration matched programme in a cool environment (11°C,
135 60% RH). Before the intervention, all participants completed a temperate graded cycling
136 exercise test (GXT: 22°C, 50% RH); 16 participants (eight from each group) also completed
137 (on a separate day) an additional temperate 30 minute performance trial (T30: 22°C, 50% RH).
138 All participants undertook a heat stress test (HST: 40°C, 50% RH) at the start (day 1) and end
139 (day 11) of the intervention period. The HA intervention consisted of eight isothermal heat
140 strain sessions (ISO) with a HST on day 6. Participants in CON completed exertion and
141 duration matched exercise in a cool environment (11°C, 60% RH). After the intervention
142 participants repeated the temperate GXT and T30.

143

144 *****Figure 1 near here*****

145

146 **Experimental procedures**

147 *Isothermal strain sessions*

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

155

156 *Control sessions*

157 Participants in the CON group cycled in a cool environment (11°C, 60% RH, air velocity $\sim 2\text{-}$
158 $3\text{ m}\cdot\text{s}^{-1}$) and were instructed to adjust their work rate to elicit the same (group mean) RPE as
159 reported by participants in the HA group at the corresponding time (5-minute intervals), on the
160 equivalent intervention day. Participants were blinded to all feedback including power output
161 and heart rate and were able to freely adjust the resistance provided by the cycle ergometer. On
162 days 2-5 and 7-10 the sessions lasted for a total of 90 minutes (paralleling the equivalent ISO
163 sessions in the HA group). On day 6 the session lasted 60 minutes (paralleling the HST
164 undertaken on day 6 by the HA group).

165

166 *Heat stress test*

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

170

171 *Graded exercise tests*

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

176

177 *30-minute performance trial*

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

181

182 **General procedures**

183 Participants abstained from alcohol throughout the experimental period. Before the HSTs,
184 GXTs and T30s participants abstained from caffeine for 12 hours and consumed a similar diet
185 before each test. Compliance with experimental controls was verbally verified on each
186 laboratory attendance. Nude body mass (dry) was measured pre- and post- laboratory sessions
187 (Electronic Weight Indicator I10, Ohaus Corporation, Parsippany, NJ, USA) to determine
188 whole-body sweat rate, adjusted for fluid ingested; participants were provided with a 3.6%
189 carbohydrate-electrolyte fluid to provide energy and minimise dehydration (Science in Sport,
190 Nelson, UK) during HA (1.75 L in 0.25 L boluses at 15 minute intervals) and HST sessions
191 (1.25 L in 0.25 L boluses at 15 minute intervals). GXTs and T30 trials were undertaken on a
192 Lode Excalibur cycle ergometer (Lode B.V., Groningen, the Netherlands); all other exercise
193 sessions were undertaken on a calibrated Computrainer cycle ergometer, (RacerMate Inc.,
194 Seattle, WA, USA). Ambient conditions were measured by a WBGT logger (Squirrel 1000,
195 Grant Instruments, Cambridge, UK), T_{rec} by a thermistor (Grant Instruments, Cambridge, UK)
196 self-inserted 15 cm beyond the anal sphincter and heart rate by short range telemetry (Polar
197 RS800, Polar Electro, Kempele, Finland). Skin temperature (T_{sk}) was measured using
198 thermistors on the chest, biceps, thigh and calf (Grant Instruments, Cambridge, UK). VO_2 was
199 measured using an online metabolic cart (Quark B2, COSMED, Rome, Italy). Blood lactate
200 concentration [Lac] was determined from fingertip capillary blood samples (Biosen C-line, EKF
201 Diagnostic, Cardiff, UK). Venous blood samples (forearm antecubital vein) were obtained
202 before the pre- and post-intervention HST (K2 EDTA blood collection tubes, Beckton Dickson
203 & Company, Plymouth, UK) following 10 minutes of seated rest for measurement of
204 haemoglobin concentration [Hb] (201+ HemoCue, Sweden) and haematocrit (Hct) (Hawksley,
205 Lancing, UK) in triplicate.

206

207 **Data analyses**

208 Data obtained during the GXTs were used to calculate: i) power output at [Lac] of 2 mmol. L⁻¹
209 and 4 mmol·L⁻¹; ii) gross mechanical efficiency (GME); iii) VO_{2max} ; iv) peak power output
210 (PPO). Power at a given fixed blood [Lac] was calculated by interpolation of the power vs.
211 [Lac] relationship. Gross mechanical efficiency was calculated from the respiratory data
212 measured over the final 45 s of the stage at a power output of 185 W, with the exception of two
213 participants where this was in excess their lactate threshold and the data from a lower power
214 output was used. VO_{2max} was defined as the highest 15 s average VO_2 , with PPO defined as the

215 power achieved at volitional exhaustion. Plasma volume changes were calculated using the
216 method of Dill & Costill (1974).

217

218 Statistical analyses were undertaken using SPSS Version 25 (IBM, New York, USA). Data are
219 presented mean±SD, unless otherwise stated, and significance was set *a-priori* at $P\leq 0.05$.
220 Independent samples *t*-tests were used to assess the between-group differences in participant
221 characteristics and average physiological responses during the HA and CON intervention
222 sessions. Mixed model two-way ANOVAs were used to assess the condition, time, and
223 interaction (condition × time) effects of the interventions on physiological responses and
224 performance over time. Student's *t*-tests were employed for *post-hoc* analysis of significant
225 condition, time and interaction effects, with Independent-samples analysis performed for
226 between-groups (condition) comparisons and paired-samples analysis performed for within-
227 groups comparison (time). Where a significant improvement in temperate exercise
228 performance was evident Pearson's correlation was undertaken to investigate whether this was
229 related to changes in any endurance performance parameters (power output at 2 mmol·L⁻¹ and
230 4 mmol·L⁻¹ [Lac]; gross mechanical efficiency; VO_{2max}) or thermal adaptations (*T*_{rec}, heart rate,
231 sweat rate, plasma volume expansion). Inter-individual variation in the adaptation to heat was
232 expressed as the standard deviation of the true individual response (SD_R), according to
233 Atkinson and Batterham (2015).

234 **Results**

235 ***Intervention period***

236 In both groups, there was high adherence to the intervention, with 100% of the prescribed
237 session completed in the HA group and 97% of the prescribed sessions completed in the CON
238 group. Ambient temperature was higher in HA than CON during the intervention period
239 ($39.4\pm 0.4^{\circ}\text{C}$, $55.2\pm 4.5\%$ RH vs. $10.2\pm 0.6^{\circ}\text{C}$, $66.5\pm 3.0\%$ RH, $P<0.001$ for T_{air}). As a
240 consequence, compared to participants in CON undertaking duration and exertion-matched
241 exercise, those in HA were hotter (HA= $38.46\pm 0.11^{\circ}\text{C}$ vs. CON= $38.08\pm 0.28^{\circ}\text{C}$, $P<0.001$), with
242 a higher sweat production (HA= $1.40\pm 0.33\text{ L}\cdot\text{hr}^{-1}$ vs. CON= $0.46\pm 0.20\text{ L}\cdot\text{hr}^{-1}$, $P<0.001$) and
243 greater cardiovascular strain (HA= $138\pm 8\text{ beats}\cdot\text{minute}^{-1}$ vs. CON= $130\pm 9\text{ beats}\cdot\text{minute}^{-1}$,
244 $P=0.044$), but a lower external work rate (HA= $103\pm 16\text{ W}$ vs. CON= $137\pm 29\text{ W}$, $P=0.001$).

245

246 ***Thermophysiological adaptations***

247 There were no significant effects of condition (*i.e.* HA vs. CON) on resting T_{rec} , average T_{rec} ,
248 whole body sweat rate, or average heart rate, as assessed during the HST, although in each
249 instance the main effect of time (*i.e.* Pre vs. Post) and the condition \times time interaction were
250 significant. Post-hoc analysis of these significant effects showed that neither resting, nor
251 average T_{rec} were significantly different following CON, but both resting T_{rec} ($-0.34\pm 0.30^{\circ}\text{C}$,
252 $P=0.001$, $\text{SD}_R\pm 0.25^{\circ}\text{C}$) and exercise T_{rec} ($-0.43\pm 0.30^{\circ}\text{C}$, $P<0.001$, $\text{SD}_R\pm 0.11^{\circ}\text{C}$) were reduced
253 following HA. Similarly, whole body sweat rate was increased after HA ($+0.37\pm 0.31\text{ L}\cdot\text{hr}^{-1}$,
254 $P<0.001$, $\text{SD}_R\pm 0.19\text{ L}\cdot\text{hr}^{-1}$), but remained unchanged in CON (Figures 2a-c). Heart rate was
255 significantly reduced following both HA ($-20\pm 11\text{ b}\cdot\text{min}^{-1}$, $P<0.001$, $\text{SD}_R\pm 11\text{ b}\cdot\text{min}^{-1}$) and CON
256 ($-6\pm 4\text{ b}\cdot\text{min}^{-1}$, $P=0.003$), with a between-groups difference also evident post-intervention
257 ($P<0.001$; figure 2d). Plasma volume increased in HA ($10.1\pm 7.2\%$, $P<0.001$, $\text{SD}_R\pm 3.5\%$) and
258 CON ($7.2\pm 6.3\%$, $P=0.015$) with no between-groups difference.

259

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

261

262 ***Temperate exercise performance***

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

268 (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
269 significant main effect of time on gross mechanical efficiency, with post-hoc analysis
270 identifying that gross mechanical efficiency was unchanged in HA (Pre=18.2±1.6 % vs.
271 Post=18.5±1.2 % P=0.321), but was significantly increased in CON (Pre=18.4±0.7 % vs.
272 Post=19.5±1.1 %, P=0.006) (figure 3c).

273

274 There was a significant main effect of time on PPO, with post-hoc analysis identifying that
275 PPO was significantly increased after both HA (Pre=344±43 W vs. Post=355±39 W, P=0.043)
276 and CON (Pre=340±36 W vs. Post=360±34 W, P=0.002) (figure 3d); the effects of condition
277 and condition × time interaction were not significant. Correlation analysis indicated that the
278 change in PPO was not related to the change in any individual endurance performance (r values
279 between -0.04 and 0.33, P>0.05) or thermal adaptation parameter (r values between -0.15 and
280 0.05, P>0.05). Moreover, total work done in the T30 was not significantly increased in either
281 group (HA: Pre=417±90 KJ vs. Post=427±83 KJ; CON: Pre=418±63 KJ vs. Post=423±56 KJ)
282 (figure 3e).

283

284 *****Figure 3 near here*****

285 Discussion

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

301
302 The medium term HA programme effectively induced a range of ‘thermal’ adaptations,
303 consistent with the heat acclimated phenotype, including reductions in exercise T_{rec} (-0.43°C)
304 and heart rate ($-20 \text{ b}\cdot\text{min}^{-1}$), and increases in whole body sweating rate ($+0.37 \text{ L}\cdot\text{hr}^{-1}$) and
305 plasma volume ($+10.1\%$). These adaptations were of a magnitude consistent with, or even
306 slightly in excess of, previous HA research (Tyler *et al.*, 2016). However, these thermal
307 adaptations did not translate into a significant ergogenic effect. The environmental conditions
308 in the present study approximated a Wet Bulb Globe Temperature (WBGT) of 18°C which has
309 been reported to result in a 3.3% reduction in marathon running performance (Ely *et al.*, 2007),
310 although the impairment might be lessened with the shorter exposure-duration in the present
311 study. Moreover, it has been argued that WBGT is not a good predictor of the effect of ambient
312 conditions on exercise performance and that the integrated index is superior (Junge *et al.*,
313 2016). The conditions in the present study elicited an integrated index of ~ 590 , which according
314 to Junge *et al.* (2016) should have caused a significant thermally mediated performance
315 impairment. Nevertheless, even in hot conditions HA does not fully restore the reduction in
316 work rate elicited by the environment (Racinais *et al.*, 2015b), whereas the lower wind speeds
317 in the present study reduced the ability to dissipate heat to the environment through convection
318 and forced evaporation (Saunders *et al.*, 2005) which might lessen the effectiveness of HA. In

319 addition, a recent meta-analysis suggests that ergogenic effects of HA are more evident in ‘time
320 to exhaustion’ performance models than the ‘total work’ model used in the present study
321 (Benjamin *et al.*, 2019), although time to exhaustion models may exaggerate the true ergogenic
322 effect (Hopkins *et al.*, 1999). Therefore, the modest thermal burden imposed by the
323 environment, combined with a potentially small-magnitude performance effect related to
324 improved thermoregulation in this environment, may have been less than the sensitivity of our
325 performance model. Alternatively, there is evidence of a temporal delay in the ergogenic
326 benefits of HA (McCleave *et al.*, 2017), which may only manifest >72 hours after the
327 intervention (Waldron *et al.*, 2019), although we are cautious in this interpretation given that
328 PPO in the GXT was increased when assessed ~48-hours after the HA. This effect was evident
329 in both groups, indicating that HA was no more effective than CON in improving PPO, and
330 was unrelated to any of the thermal adaptations or changes in any of the determinants of
331 endurance. However, we cannot exclude a training effect on anaerobic metabolism, whereas
332 motivation or learning effects (Hopkins, 2000) could also have contributed to the increased
333 PPO.

334

335 A second aim of this study was to examine the effect of HA on the key determinants of
336 endurance performance, namely, VO_{2max} , efficiency and lactate threshold (Joyner & Coyle,
337 2008). An increase in VO_{2max} has been proposed as central to any ergogenic effect of HA, with
338 an increase in cardiac output due to hypervolemia and the Frank-Starling law proposed as a
339 putative mechanism (Lorenzo *et al.*, 2010; Corbett *et al.*, 2014). However, in keeping with
340 recent studies (Karlsen *et al.*, 2015; Keiser *et al.*, 2015; Mikkelsen *et al.*, 2019), we did not
341 detect any change in VO_{2max} following HA, despite a significantly increased PV (10.1[7.2]%).
342 Indeed, it has been suggested that any benefit of PV expansion on cardiac output, will be
343 balanced by a haemodilution effect resulting in no change in VO_{2max} (Coyle *et al.*, 1990).
344 Likewise, neither the power at lactate threshold nor gross mechanical efficiency were
345 significantly improved by HA, which is contrast to some (Shvartz *et al.*, 1977; Sawka *et al.*,
346 1983; Lorenzo *et al.*, 2010), but not all (Karlsen *et al.*, 2015; Keiser *et al.*, 2015; Mikkelsen *et*
347 *al.*, 2019) previous research. The lack of change in the key determinants of endurance was
348 consistent with our null effect on endurance performance (T30), and we speculate that the
349 apparently equivocal findings between studies in this area likely stems from important
350 methodological differences, which we have sought to address.

351

352 Some research reporting an ergogenic effect of HA has lacked a control group (Sawka *et al.*;
353 1985; Racinais *et al.*, 2014), meaning that it is not possible to isolate the effect to heat from
354 confounding factors, including the daily exercise undertaken within the HA *i.e.* a training
355 effect. To isolate the independent effect of heat from training, other investigations have
356 employed a control group undertaking some form of ‘matched’ exercise in an environment
357 limiting the thermal strain *i.e.* cool conditions. However, as recently recognised by Mikkelsen
358 *et al.* (2019), appropriate matching of groups with superimposed environmental stress presents
359 difficulties due to the multifaceted nature of training stress. For example, approaches
360 prescribing external work rate based upon performance in cool conditions (Lorenzo *et al.*,
361 2010) do not take into account the effect of elevated ambient temperature on cardiovascular
362 strain (Wingo *et al.*, 2012). Conversely, matching groups based upon cardiovascular strain will
363 likely result in differences in the mechanical work component (Keiser *et al.*, 2015). Others have
364 instructed participants to continue their normal training (Karlsen *et al.*, 2015; Mikkelsen *et al.*,
365 2019), but the additional physiological strain associated with HA can compromise the ability
366 to maintain habitual training (Mikkelsen *et al.*, 2019). Acknowledging the multifaceted nature
367 of training stress, we prescribed intensity on the basis of RPE, which is generated as a result of
368 multiple afferent signals including heart rate, metabolic and ventilatory parameters (Hampson
369 *et al.*, 2001), and appears to be the key mediator that athletes use to regulate exercise intensity
370 (Tucker, 2009). This method resulted in slightly higher cardiovascular strain in HA compared
371 to CON, despite a slightly lower external work rate, but this is to be expected given the
372 between-groups differences in environmental conditions and subsequent effects on thermal
373 strain. Importantly, this novel approach better reflects the integration of multiple afferents and
374 the multifaceted nature of training stress than matching approaches that utilise a single
375 physiological parameter, as well as possessing good ecological validity and practical relevance
376 for athletes attempting to undertake the same training in a hot or cold environment.

377

378 The present study was not without limitation and we acknowledge that a within-participant
379 crossover design is typically stronger than a between-groups design. However, in the context
380 of the present study, a between-groups design has some advantages over the within-participant
381 approach because the time-course of decay in heat acclimation can be prolonged (Weller *et al.*,
382 2007) and there is some evidence to support a heat acclimation ‘memory’ (Tetievsky *et al.*,
383 2014). This necessitates that within-participant designs include long washout periods which
384 can increase the confounding influences of seasonal changes in acclimatisation status (Inoue *et*
385 *al.*, 1995) as well as changes in other factors known to influence thermoregulation including

386 training status, fitness status, health and anthropometric factors (Havenith & van Middendorp,
387 1990). Moreover, our large sample size was adequately powered to detect between-groups
388 differences in our key outcome measures and we also employed appropriate statistical
389 techniques for between-groups analysis.

390

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

404

405

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575 **Table legends**

576

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

579

580

581 **Figure legends**

582

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

588

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

595

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

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	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·kg ⁻¹ ·min ⁻¹)	56.7±7.5	57.7±8.2	54.8±5.8

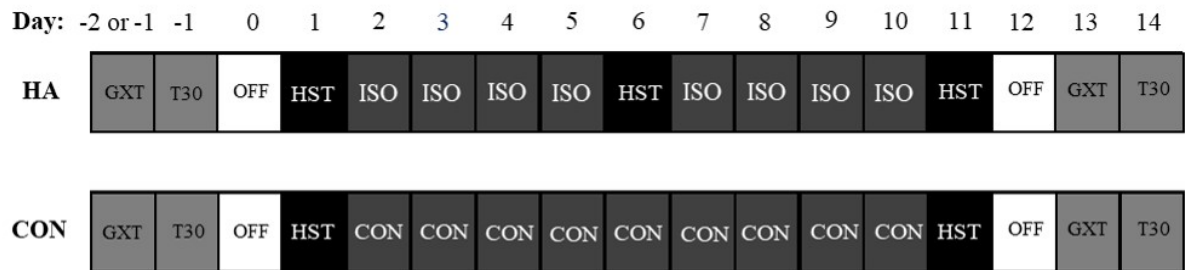
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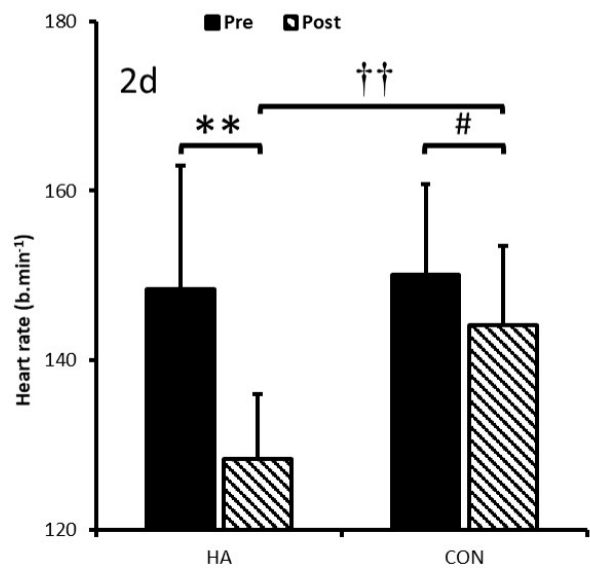
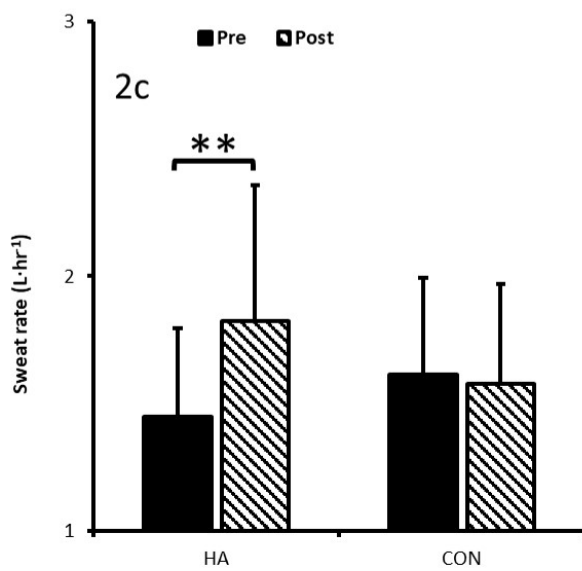
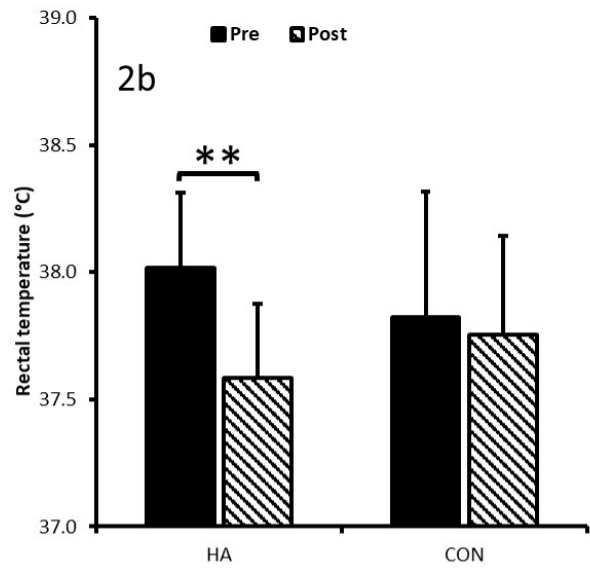
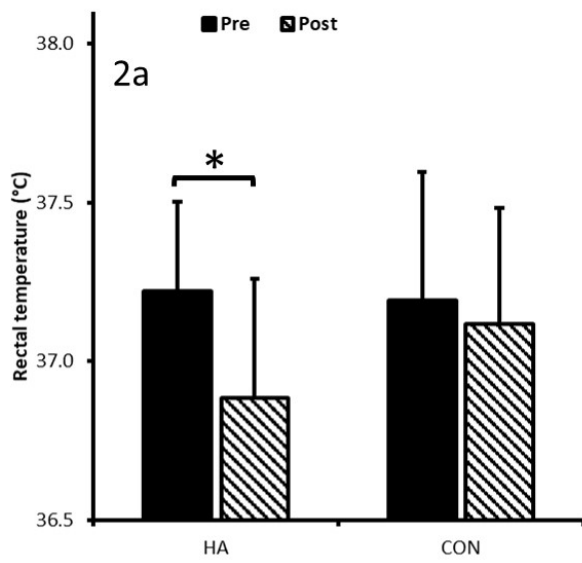
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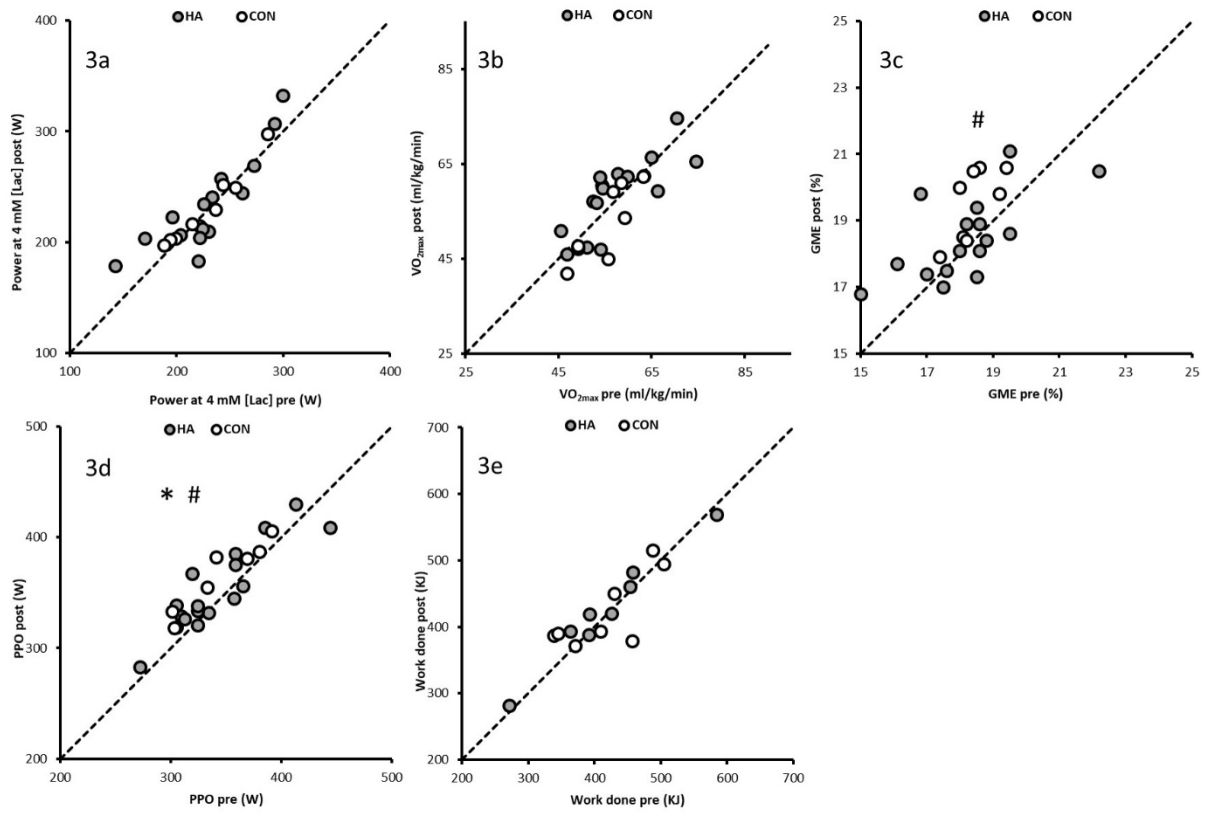


Table I. Overall and sub-group (HA = heat acclimation; CON = Control) participant characteristics. Data presented as mean \pm SD. (Table view)

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