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1 **Heat acclimation by post-exercise hot water immersion in**  
2 **the morning reduces thermal strain during morning and**  
3 **afternoon exercise-heat-stress**

4

5 **Submission type:**

6

7 Original investigation

8

9 **Authors:**

10

11 Michael J. Zurawlew, Jessica A. Mee and Neil P. Walsh

12

13 **Affiliations:**

14

15 College of Health and Behavioural Sciences, Bangor

16 University, Bangor, Gwynedd, LL57 2PZ, UK.

17

18 **Corresponding Author:**

19

20 Dr Jessica Mee

21

22 College of Health and Behavioural Sciences,

23 Bangor University,

24 Bangor,

25 LL57 2PZ,

26 UK.

27 Email: j.a.mee@bangor.ac.uk

28 Telephone: + 44 1248 388309

29

30 **Preferred Running Head:**

31 Hot water immersion heat acclimation

32 **Abstract**

33

34 **Purpose:** Recommendations state that to acquire the greatest  
35 benefit from heat acclimation the clock-time of heat  
36 acclimation sessions should match the clock-time of expected  
37 exercise-heat stress. It remains unknown if adaptations by post-  
38 exercise hot water immersion (HWI) demonstrate time of day  
39 dependent adaptations. Thus, we examined whether adaptations  
40 following post-exercise HWI completed in the morning were  
41 present during morning and afternoon exercise-heat stress.

42

43 **Methods:** Ten males completed an exercise-heat stress test  
44 commencing in the morning (0945-h: AM) and afternoon  
45 (1445-h: PM; 40 min; 65%  $\dot{V}O_{2\max}$  treadmill run) before (PRE)  
46 and after (POST) heat acclimation. The 6-day heat acclimation  
47 intervention involved a daily, 40 min treadmill-run (65%  
48  $\dot{V}O_{2\max}$ ) in temperate conditions followed by  $\leq$  40 min HWI  
49 (40°C; 0630–1100-h).

50

51 **Results:** Adaptations by 6-day post-exercise HWI in the  
52 morning were similar in the morning and afternoon. Reductions  
53 in resting rectal temperature ( $T_{re}$ ; AM;  $-0.34 \pm 0.24^\circ\text{C}$ , PM;  $-$   
54  $0.27 \pm 0.23^\circ\text{C}$ ;  $P = 0.002$ ),  $T_{re}$  at sweating onset (AM;  $-0.34 \pm$   
55  $0.24^\circ\text{C}$ , PM;  $-0.31 \pm 0.25^\circ\text{C}$ ;  $P = 0.001$ ), and end-exercise  $T_{re}$   
56 (AM;  $-0.47 \pm 0.33^\circ\text{C}$ , PM;  $-0.43 \pm 0.29^\circ\text{C}$ ;  $P = 0.001$ ), heart  
57 rate (AM;  $-14 \pm 7$  beats $\cdot\text{min}^{-1}$ , PM;  $-13 \pm 6$  beats $\cdot\text{min}^{-1}$ ;  $P <$   
58  $0.01$ ), rating of perceived exertion ( $P = 0.01$ ), and thermal  
59 sensation ( $P = 0.005$ ) were not different in the morning  
60 compared to the afternoon.

61

62 **Conclusion:** Morning heat acclimation by post-exercise hot  
63 water immersion induced adaptations at rest and during exercise-  
64 heat stress in the morning and mid-afternoon.

65

66 **Key Words:** Thermoregulation; hot bath; heat acclimation;  
67 acclimatisation; circadian rhythm.

68 **Introduction**

69 Prior to exercise-heat stress, athletes and military personnel are  
70 advised to complete a period of heat acclimation to alleviate  
71 heat strain and improve exercise capacity in the heat.<sup>1</sup> The  
72 adaptive responses that improve exercise capacity in the heat  
73 include an earlier onset and an increase in sweating rate, a  
74 reduction in cardiovascular strain and improved thermal  
75 comfort.<sup>2-4</sup> Despite practical limitations, heat acclimation  
76 recommendations state that individuals should exercise in the  
77 heat on 5–14 occasions, maintaining a specific degree of  
78 hyperthermia (rectal temperature ( $T_{re}$ );  $\geq 38.5^{\circ}\text{C}$ ) for  $\geq 60$  min.<sup>5</sup>  
79 To acquire the greatest benefit, consensus recommendations  
80 state that heat acclimation sessions should be scheduled at the  
81 anticipated time of day of future exercise-heat stress.<sup>1,5-9</sup> The  
82 underpinning evidence for this recommendation stems from the  
83 observations that heat acclimation adaptations are clock-time  
84 dependent; albeit, this was shown in a passive model of heat  
85 stress.<sup>10</sup> It remains to be shown whether clock-time dependent  
86 adaptations extend to an exercise model of heat stress. From a  
87 practical standpoint, adhering to this recommendation without  
88 disturbing training or sleep patterns is problematic, since  
89 athletes and military personnel often move between time zones.  
90 Moreover, military personnel may not have pre-warning  
91 regarding the time of day when exertional-heat strain may  
92 occur, or they may be exposed to heat strain throughout the  
93 day.

94  
95 The scheduling of passive heat acclimation on core temperature  
96 circadian rhythm and thermoregulatory responses was  
97 examined in a series of investigations in rats<sup>11,12</sup> and then in  
98 humans.<sup>10</sup> Six adult men and women heat acclimated via 9-10  
99 daily, 4-h passive heat exposures commencing in the afternoon  
100 ( $46^{\circ}\text{C}$  and 20% relative humidity), achieved a reduced resting  
101  $T_{re}$  and sweating onset (latency and core temperature threshold)  
102 during subsequent hot water immersion of the legs ( $42^{\circ}\text{C}$ ). The  
103 relatively modest adaptations (e.g. reduction in resting  $T_{re}$   
104  $\sim 0.2^{\circ}\text{C}$ ) were only present at the clock-time of daily heat  
105 exposures (1500 – 1700-h), but not in the morning (0900 –  
106 1100-h). The authors suggested that the clock-time dependent  
107 adaptations were due to circadian pattern changes in core  
108 temperature, associated with altered autonomic  
109 thermoregulatory function, and coined the term ‘time memory’  
110 to describe their observations. Others support this concept,  
111 whereby the suprachiasmatic nucleus within the hypothalamus  
112 is thought to retain the clock-time of previous heat exposures,  
113 establishing a new core temperature circadian pattern.<sup>6,13</sup> These  
114 findings inform the current recommendation that exercise-heat  
115 acclimation sessions should be scheduled at the anticipated  
116 clock-time of future exercise-heat stress.<sup>1,5-9</sup> However,  
117 evidence challenging this notion demonstrates that exercise-

118 heat acclimation, performed in the afternoon (1500 to 1700 h),  
119 initiates reductions in thermal strain ( $T_{re}$ ;  $-0.3^{\circ}\text{C}$ ) and  
120 cardiovascular strain (heart rate (HR);  $-13 \text{ beats}\cdot\text{min}^{-1}$ ) during  
121 exercise-heat stress tests performed in the morning (0900 to  
122 1200 h).<sup>14</sup>

123

124 Post-exercise hot water immersion (HWI) completed on 6  
125 consecutive days represents a practical, economical, and  
126 effective heat acclimation strategy<sup>15</sup> which elicits adaptations  
127 that compare favourably to exercise heat acclimation  
128 strategies.<sup>16</sup> However, it remains to be shown whether post-  
129 exercise HWI heat acclimation adaptations are present at a  
130 different clock-time to when the daily intervention occurs.  
131 Thus, the aim of the current study was to assess whether  
132 adaptations following 6-day post-exercise HWI performed in  
133 the morning are observed during both morning and mid-  
134 afternoon exercise-heat stress.

135 **Methods**

136

137 **Participants**

138 Ten recreationally active males (mean  $\pm$  SD, age:  $23 \pm 4$  years;  
139 body mass:  $72.8 \pm 7.8$  kg;  $\dot{V}O_{2\max}$   $58.2 \pm 8.4$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ )  
140 provided written informed consent to participate in the current  
141 study. All participants, were healthy, non-smokers, free from  
142 any known cardiovascular or metabolic diseases, were not  
143 taking any medication, and had not been exposed to hot  
144 environmental conditions in the 3 months prior to commencing  
145 testing. The study received local ethical approval and was  
146 conducted in accordance with the Declaration of Helsinki  
147 (2013).

148

149 **Study design**

150 To assess whether morning heat acclimation improves  
151 thermoregulatory responses during morning (0945 h; AM) and  
152 mid-afternoon (1445 h; PM) exercise-heat stress, participants  
153 performed two experimental trials on the same day, before  
154 (PRE) and after (POST) heat acclimation. The times selected  
155 for the experimental trials align with previous research showing  
156 the clock-time dependency for heat acclimation adaptations,<sup>10</sup>  
157 where there is a meaningful difference in resting core  
158 temperature ( $\sim 0.3$ – $0.4^\circ\text{C}$  between AM and PM).<sup>17</sup> Heat  
159 acclimation involved six consecutive daily post-exercise HWI  
160 in the morning between 0630-h and 1100-h, as described  
161 previously.<sup>15</sup> To control for any training and/or hydrostatic  
162 effects Zurawlew et al.<sup>15</sup> demonstrated that six consecutive  
163 daily post-exercise ( $18^\circ\text{C}$ ) thermoneutral water immersion  
164 ( $34^\circ\text{C}$ ) resulted in no effect on subsequent thermoregulatory  
165 measures at rest and during exercise-heat stress in seven males  
166 ( $\dot{V}O_{2\max}$   $60.1 \pm 8.9$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ).

167

168 **Preliminary measurements**

169  $\dot{V}O_{2\max}$  was assessed using a continuous incremental exercise  
170 test on a motorised treadmill (HP Cosmos Mercury 4.0,  
171 Nussdorf-Traunstein, Germany) in temperate laboratory  
172 conditions ( $20^\circ\text{C}$ ) as described previously.<sup>18</sup> Using the  
173 interpolation of the running speed –  $\dot{V}O_2$  relationship, a running  
174 speed that elicited 65%  $\dot{V}O_{2\max}$  was determined. This speed  
175 was verified with a 60 s expired gas sample collected by  
176 Douglas bag method, 30 min after the  $\dot{V}O_{2\max}$  test. This  
177 individualised running speed was used for the PRE and POST  
178 experimental trials and the daily exercise prior to HWI.

179

180 **Experimental trials**

181 Participants completed a food diary 24-h prior to the PRE  
182 experimental trial and were instructed to replicate this diet 24-h  
183 prior to the POST experimental trial. The food diary verified  
184 that no alcohol, diuretics, or caffeine were consumed. Twenty-

185 four hours prior to, and on the day of the experimental trials  
186 participants were also instructed to refrain from any additional  
187 exercise. As sleeping patterns can influence thermoregulation,<sup>1</sup>  
188 participants were instructed to sleep between 2200-h and 0700-  
189 h to ensure a similar circadian pattern prior to each  
190 experimental trial. This was confirmed by monitoring sleep,  
191 using an Actigraph worn on the non-dominant arm with epoch  
192 length set to 1 min (Actigraph GT3X Version 4.4.0, Actigraph,  
193 Pensacola, USA). Data was subsequently analysed for sleep  
194 efficiency (number of sleep min, divided by total number of  
195 min in bed, multiplied by 100 to convert to percentage) and  
196 sleep duration using Actilife+Sleep Version 6 (Actigraph,  
197 Pensacola, USA).

198

199 On the day of each experimental trial, participants arrived at the  
200 laboratory at 0730 h. On arrival, they were provided with a  
201 standardised breakfast ( $0.03 \text{ MJ}\cdot\text{kg}^{-1}$ ) and a bolus of water ( $7$   
202  $\text{mL}\cdot\text{kg}^{-1}$  body mass) as previously described.<sup>15</sup> At 0800-h  
203 dressed in a t-shirt, running shorts, socks and trainers  
204 participants rested for 20 min in temperate laboratory  
205 conditions ( $20^\circ\text{C}$ ). A venous blood sample was taken without  
206 stasis and assessed for haemoglobin concentration and  
207 haematocrit percentage to determine changes in plasma volume.  
208 A mid-flow urine sample was analysed for urine specific  
209 gravity using a handheld refractometer (Atago Uricon-Ne  
210 refractometer, NSG Precision cells, New York, USA) to  
211 confirm euhydration (urine specific gravity  $< 1.030$ ).<sup>19</sup> A rectal  
212 thermistor was fitted and  $T_{\text{re}}$  was recorded continuously  
213 between 0900-h and 1540-h. A pre-exercise nude body mass  
214 was recorded using digital platform scales (Model 705; Seca,  
215 Hamburg, Germany) and the participants were instrumented for  
216 the exercise protocol. To establish baseline measures  
217 participants rested for a further 30 min in temperate laboratory  
218 conditions ( $20^\circ\text{C}$ ).

219

220 At 0945-h dressed in running shorts, socks and trainers,  
221 participants entered the environmental chamber ( $33^\circ\text{C}$ , 40%  
222 relative humidity; Delta Environmental Systems, Chester, UK)  
223 to complete the AM trial which involved running for 40 min at  
224  $65\% \dot{V}\text{O}_{2\text{max}}$  (1% gradient) as previously described.<sup>15</sup> During  
225 this time, no fluids were consumed.  $T_{\text{re}}$ , mean skin temperature  
226 ( $T_{\text{sk}}$ ), and HR were monitored continuously and rating of  
227 perceived exertion (RPE)<sup>20</sup> and thermal sensation<sup>21</sup> were  
228 recorded every 10 min. Local forearm sweating rate was  
229 measured every 20 s for the first 15 min of exercise to assess  
230 the onset of sweating as previously described.<sup>15</sup> Oxygen uptake  
231 ( $\dot{V}\text{O}_2$ ), and respiratory exchange ratio (RER) were assessed  
232 from 60 s expired gas samples collected by Douglas bag  
233 method immediately prior to 10<sup>th</sup>, 20<sup>th</sup>, 30<sup>th</sup> and 40<sup>th</sup> min of  
234 exercise. On completion of the AM trial, participants exited the

235 environmental chamber. A nude body mass was taken 15 min  
236 following the cessation of exercise to estimate whole body  
237 sweating rate (WBSR). Participants then rested in temperate  
238 laboratory conditions (20°C) dressed in t-shirt, running shorts,  
239 socks and trainers during which fluid intake matched body  
240 mass losses during the AM trial. At 1230 h, participants were  
241 provided with a standardised lunch (0.03 MJ·kg<sup>-1</sup>) and a bolus  
242 of water (7 mL·kg<sup>-1</sup> body mass). At 1330 h, participants were  
243 prepared for the PM experimental trial. At 1445 h, participants  
244 entered the environmental chamber to complete the PM trial,  
245 adopting identical procedures to the AM experimental trial.

246

### 247 **Post-exercise HWI heat acclimation**

248 The post-exercise HWI heat acclimation intervention was  
249 performed on 6 consecutive days as previously described.<sup>3</sup>  
250 During the intervention, participants were instructed to reduce  
251 their normal training by the volume of endurance exercise  
252 completed during the intervention in the laboratory and  
253 consume their normal diet and fluid intake, including caffeine  
254 and alcohol ( $\leq 3$  units per day). Participants arrived at the  
255 laboratory between 0630-h and 0830-h. Prior to exercise a nude  
256 body mass was taken and participants were fitted with a rectal  
257 thermistor and HR monitor.  $T_{re}$  and HR were continually  
258 monitored throughout the exercise and HWI. Participants ran  
259 for 40 min at 65%  $\dot{V}O_{2max}$  (1% gradient) on a motorised  
260 treadmill in temperate laboratory conditions (20°C) dressed in  
261 shorts, socks, and trainers as previously described.<sup>15</sup> In the first  
262 20 min of exercise, a bolus of water (5 mL·kg<sup>-1</sup> of body mass)  
263 was consumed. At the cessation of exercise, participants were  
264 transferred to the hot water bath (2–3 min transition)  
265 submerged to the neck dressed in shorts as previously  
266 described.<sup>15</sup> The water was maintained at 40°C for the duration  
267 of the immersion. Immersion ended after 40 min unless the  
268 participants removed themselves due to discomfort or  $T_{re}$   
269 exceeded 39.9°C. Upon removal from the hot water bath,  
270 participants rested in a seated position for 15 min without fluid  
271 following which a nude body mass was recorded and adjusted  
272 for fluid intake as a measure of WBSR. Participants were then  
273 free to leave the laboratory when  $T_{re} \leq 38.5^\circ\text{C}$ .

274

### 275 **Measurement and instrumentation**

276 *Body temperatures:*  $T_{re}$  was measured using a flexible, sterile,  
277 disposable thermistor (Henleys Medical Supplies Ltd., Herts,  
278 UK) and recorded using a data logger (YSI model 4000A, YSI,  
279 Dayton, USA). Prior to insertion, a bead was fixed to the rectal  
280 thermistor 10 cm from the inserted end; this ensured the  
281 thermistor remained inserted to the same depth throughout the  
282 trial. To assess cumulative hyperthermia, an area under the  
283 curve analysis (time  $T_{re}$  was  $\geq 38.5^\circ\text{C}$ ) was performed on the  
284 daily  $T_{re}$  during the intervention as previously described.<sup>22</sup> Skin

285 thermistors (Grant EUS-U, Cambridge, UK) were attached to  
286 the right side of the body (on the chest at a midpoint between  
287 the acromion process and the nipple, the lateral mid-bicep, the  
288 anterior mid-thigh, and lateral calf) and recorded using a  
289 portable data logger (Grant SQ2020, Cambridge, UK). Mean  
290  $T_{sk}$  was calculated using a four-site weighted equation.<sup>23</sup>

291

292 *Sweating responses:* Local forearm sweat rate was measured by  
293 dew point hygrometry during all experimental trials as  
294 previously described.<sup>18</sup> Sweating threshold was calculated by  
295 plotting individual relationships between local forearm sweat  
296 rate and  $T_{re}$ , as previously described.<sup>24</sup> Changes in dry nude  
297 body mass were used to estimate WBSR during all intervention  
298 days and experimental trials.

299

300 *Blood sample collection and analysis:* Prior to the PRE and  
301 POST, AM experimental trial venous blood samples were  
302 collected from an antecubital vein without stasis into a 6 mL  
303 EDTA vacutainer (BD, Oxford, UK). Aliquots of whole blood  
304 were used for the immediate determination of haemoglobin  
305 concentration (g·dL) in duplicate (201+ Hemocue, Sheffield,  
306 UK) and haematocrit percentage in triplicate (capillary tube  
307 method). The change in plasma volume was estimated as  
308 previously described.<sup>25</sup>

309

### 310 **Statistical analysis**

311 Using previous data<sup>15</sup>, a sample size estimation (G\*Power  
312 3.1.2) with an alpha level of 0.05 and power of 0.95,  
313 determined that eight participants were required to detect a  
314 significant difference in resting  $T_{re}$  (-0.27°C) and end-exercise  
315  $T_{re}$  (-0.36°C) following post-exercise HWI heat acclimation. To  
316 ensure adequate power and allowing for dropout, 10  
317 participants were recruited. Data is presented as mean  $\pm$   
318 standard deviation (SD) and statistical significance was  
319 accepted at  $P < 0.05$ . All data were checked for normality and  
320 sphericity. Paired sample t-tests were used to assess the  
321 differences between the heat acclimation status (changes from  
322 PRE to POST) in the morning and afternoon (AM and PM).  
323 Two-way repeated measures analysis of variance (ANOVA)  
324 with Greenhouse Geisser correction to the degrees of freedom  
325 (where necessary) were used to assess differences between the  
326 heat acclimation status (changes from PRE and POST) and the  
327 time of day (AM and PM). Friedman test was used to assess  
328 differences between the PRE and POST, AM and PM trials for  
329 measures of RPE and thermal sensation. When statistical  
330 significance was found, Wilcoxon Signed Rank tests were used  
331 to identify where the difference occurred. Partial  $\eta^2$  ( $\eta p^2$ ) were  
332 reported to analyse the magnitude of the effects. Cohen<sup>26</sup> has  
333 provided benchmarks to define small ( $\eta p^2 = 0.01$ ), medium ( $\eta p^2$   
334 = 0.06), and large ( $\eta p^2 = 0.14$ ) effects. All data was analysed

335 using SPSS version 20 (IBM Corporation, NY, USA), or  
336 GraphPad Prism Version 5.02 (GraphPad Software Inc. La  
337 Jolla, USA).  
338

339 **Results**

340

341 **Post-exercise HWI heat acclimation**

342 All participants completed a 40 min treadmill run at 65%  
343  $\dot{V}O_{2max}$ , followed by HWI ( $\leq 40$  min) on six consecutive days.  
344 HWI time increased from  $30 \pm 6$  min on day 1 to  $40 \pm 0$  min on  
345 day 6 (Table 1). Daily end  $T_{re}$  averaged  $39.34 \pm 0.29^\circ\text{C}$  and  
346 daily area under the curve averaged  $27 \pm 13^\circ\text{C}\cdot\text{min}^{-1}$ . No  
347 differences were observed for change in  $T_{re}$  or the area under  
348 the curve between the daily sessions, demonstrating a constant  
349 endogenous stimulus for adaptation during the 6-day  
350 intervention (Table 1:  $P > 0.05$ ).

351

352 **Experimental trials**

353 There were no differences in sleep efficiency nor sleep duration  
354 the night before the experimental trials ( $P > 0.05$ ). Heat  
355 acclimation adaptations were not influenced by the time of day,  
356 evidenced by no interaction effects for measures of: resting  $T_{re}$ ;  
357  $T_{re}$  at sweating onset; end-exercise  $T_{re}$ ; HR; RPE; thermal  
358 sensation;  $T_{sk}$ ;  $\dot{V}O_2$ ; RER and WBSR ( $P > 0.05$ ). Main effects  
359 for the time of day (AM vs. PM) were observed, with higher  
360 values in the afternoon compared to the morning for measures  
361 of: resting  $T_{re}$  ( $P = 0.008$ ,  $np^2 = 0.56$ );  $T_{re}$  at sweating onset ( $P =$   
362  $0.002$ ,  $np^2 = 0.69$ ); end-exercise HR ( $P = 0.008$ ,  $np^2 = 0.56$ )  
363 and mean RER ( $P = 0.001$ ,  $np^2 = 0.72$ ). However, there were no  
364 main effects for the time of day for measures of: end-exercise  
365  $T_{re}$ ; RPE; thermal sensation;  $T_{sk}$ ;  $\dot{V}O_2$ ; RER and WBSR ( $P >$   
366  $0.05$ ). Main effects for heat acclimation status (PRE vs. POST)  
367 were observed during experimental trials between 0900-h to  
368 1540-h, evidenced by reductions in core body temperature  
369 (Figure 1). In addition, reductions from PRE to POST were  
370 observed for measures of: resting  $T_{re}$  ( $P = 0.002$ ,  $np^2 = 0.68$ ;  
371 Figure 2A); end-exercise  $T_{re}$  ( $P = 0.001$ ,  $np^2 = 0.75$ ; Figure 2B);  
372  $T_{re}$  at sweating onset ( $P = 0.001$ ;  $np^2 = 0.71$ ); end-exercise HR  
373 ( $P < 0.001$ ;  $np^2 = 0.85$ ); RPE ( $P = 0.01$ ); thermal sensation ( $P =$   
374  $0.005$ );  $T_{sk}$  ( $P = 0.01$ ;  $np^2 = 0.51$ ) and mean  $\dot{V}O_2$  ( $P =$   
375  $0.02$ ;  $np^2 = 0.46$ ). No differences were observed from PRE to  
376 POST for measure of RER and WBSR (Table 2:  $P > 0.05$ ) and  
377 relative changes in plasma volume were not significant from  
378 PRE to POST (+2.6%;  $P > 0.05$ ). Control data from Zurawlew  
379 et al.,<sup>16</sup> provides confidence that the adaptations shown are  
380 attributed to bathing in hot water after exercise, since daily  
381 exercise in temperate conditions followed by thermoneutral  
382 water immersion ( $34^\circ\text{C}$ ) did not affect thermoregulatory  
383 outcomes (Figure 2; data shown for comparison only).

384

385 **Discussion**

386 The novel findings of the current study confirm and advance  
387 those previous<sup>15</sup> by showing that hallmark heat acclimation  
388 adaptations by post-exercise HWI are not restricted to the  
389 clock-time of daily heat exposures. These data provide clear  
390 evidence that post-exercise HWI can be performed in the  
391 morning to reduce thermal strain in both the morning and mid-  
392 afternoon (end-exercise  $T_{re}$  AM  $-0.47^{\circ}\text{C}$ ; PM  $-0.43^{\circ}\text{C}$ ; Figure  
393 2B). The observed reduction in thermal strain during exercise-  
394 heat stress performed in the morning and afternoon was  
395 achieved, at least in part, through a reduction in  $T_{re}$  at rest in  
396 temperate conditions (AM  $-0.34^{\circ}\text{C}$ ; PM  $-0.27^{\circ}\text{C}$ ; Figure 2A).  
397 Other hallmark heat acclimation adaptations were evident  
398 during exercise-heat stress in both the morning and afternoon;  
399 these included a reduction in  $T_{re}$  at sweating onset and a  
400 reduction in end-exercise HR, RPE, thermal sensation and  $T_{sk}$ .  
401 However, in line with short-term exercise-heat acclimation<sup>16</sup>  
402 and our previous work,<sup>15</sup> six days of post-exercise HWI did not  
403 alter WBSR during submaximal exercise in the heat.

404  
405 Current heat acclimation recommendations, based upon the  
406 work of Shido and colleagues,<sup>10,12,27</sup> performed across  
407 comparable clock-times as the current study, state that to  
408 acquire the greatest benefit daily heat exposures should be  
409 scheduled at the anticipated clock-time of future exercise-heat  
410 stress.<sup>1,5-9</sup> However, the present data demonstrate that 6-days  
411 post-exercise HWI heat acclimation does not need to be  
412 constrained to the same clock-time of future exercise-heat  
413 stress, when performed between 0900-h and 1540-h (Figure 1).  
414 The magnitude of adaptation appears to be slightly smaller in  
415 the afternoon compared with the morning for hallmark  
416 adaptations (Figure 2, Table 2). However, a recent meta-  
417 analysis considered a  $0.3^{\circ}\text{C}$  reduction to be a meaningful  
418 change in exercising  $T_{re}$ ;<sup>16</sup> as such, the  $-0.47^{\circ}\text{C}$  (AM) and -  
419  $0.43^{\circ}\text{C}$  (PM) reduction in end-exercise  $T_{re}$  observed in the  
420 current study can both be considered meaningful adaptations.  
421 Indeed, the currently available evidence from short-term  
422 exercise-heat acclimation studies challenges the notion that  
423 heat acclimation adaptations are clock-time dependent. For  
424 example, comparable reductions in thermal and cardiovascular  
425 strain were demonstrated during exercise-heat stress when the  
426 clock-time of the daily intervention and the exercise-heat stress  
427 was either matched<sup>28</sup> or performed at different times of the  
428 day;<sup>14</sup> albeit these studies were not specifically designed to  
429 assess whether heat acclimation adaptations are clock-time  
430 dependent. It is conceivable that the subtle, clock-time  
431 dependent reduction in resting  $T_{re}$  shown previously<sup>10</sup>, may be  
432 explained by the mild thermal stimulus for adaptation during  
433 daily passive heat exposures ( $+0.7^{\circ}\text{C}$  change in  $T_{re}$ <sup>10</sup>). The  
434 large, daily disruption to homeostasis during post-exercise HWI

435 heat acclimation (e.g. +2.1°C change in  $T_{re}$ <sup>15</sup>) and controlled  
436 hyperthermia, exercise-heat acclimation (e.g. +1.7°C change in  
437  $T_{re}$ <sup>28</sup>), provides a greater stimulus for adaptation. This larger  
438 stimulus, may account for the reduction in  $T_{re}$  at rest and  
439 reduction in thermal strain during exercise-heat stress in both  
440 the morning and afternoon performed on the same day.  
441 Notwithstanding, before any changes can be made to current  
442 heat acclimation recommendations, further research is required  
443 specifically to assess the purported clock-time dependency of  
444 exercise-heat acclimation adaptations.

445

#### 446 **Practical applications**

447 Heat acclimation recommendations state that to acquire the  
448 greatest benefit daily heat exposures should be scheduled at the  
449 anticipated clock-time of future exercise-heat stress.<sup>1,5-9</sup> The  
450 data from the current study shows that post-exercise HWI on  
451 six consecutive days in the morning reduces thermal strain  
452 during exercise-heat stress in both the morning and afternoon.  
453 As such, when the time of day of future exercise-heat stress is  
454 unknown (e.g. in military or other occupational settings) post-  
455 exercise HWI could be considered as a practical heat  
456 acclimation strategy. The post-exercise HWI heat acclimation  
457 intervention presents an accessible strategy to alleviate thermal  
458 strain during exercise-heat stress that could be incorporated into  
459 post-exercise washing routines, reducing the interference with  
460 daily training.<sup>15</sup> Future research should determine the extent of  
461 adaptation across the full daily circadian rhythm of core  
462 temperature. Specifically, trials would be performed from the  
463 mid-point of the nadir phase (~0600-h) to the acrophase  
464 (~1800-h)<sup>17</sup>; ideally on different days. Appropriately controlled  
465 studies, in highly trained males and females, should also  
466 determine the effect of afternoon heat acclimation on morning  
467 exercise-heat stress and determine whether any improvements  
468 translate to an enhanced endurance performance and reduced  
469 susceptibility to heat illness. It is important these studies assess  
470 exercise performance because temporal specificity in  
471 adaptations and performance outcomes to exercise training  
472 have been demonstrated.<sup>29</sup> To improve the practical relevance  
473 of these findings, future research should investigate whether  
474 adaptations are beneficial across different time zones that  
475 replicate international travel for competition.

476

#### 477 **Conclusion**

478 Hot water immersion after exercise in temperate conditions in  
479 the morning on six consecutive days induced heat acclimation  
480 adaptations evident at rest and during morning and mid-afternoon  
481 exercise-heat stress performed on the same day. Thus, this heat  
482 acclimation method is a strategy that could be adopted to  
483 reduce heat strain when it is unknown if future exercise-heat  
484 stress will occur in the morning or afternoon.

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491

492 **Conflicts of interest**

493 The authors of the study declare that they have no conflicts of  
494 interest.

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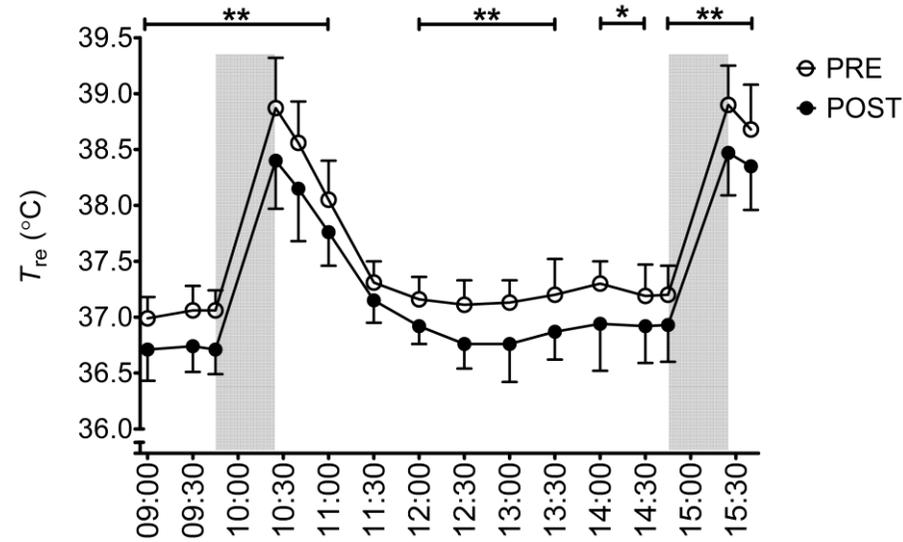
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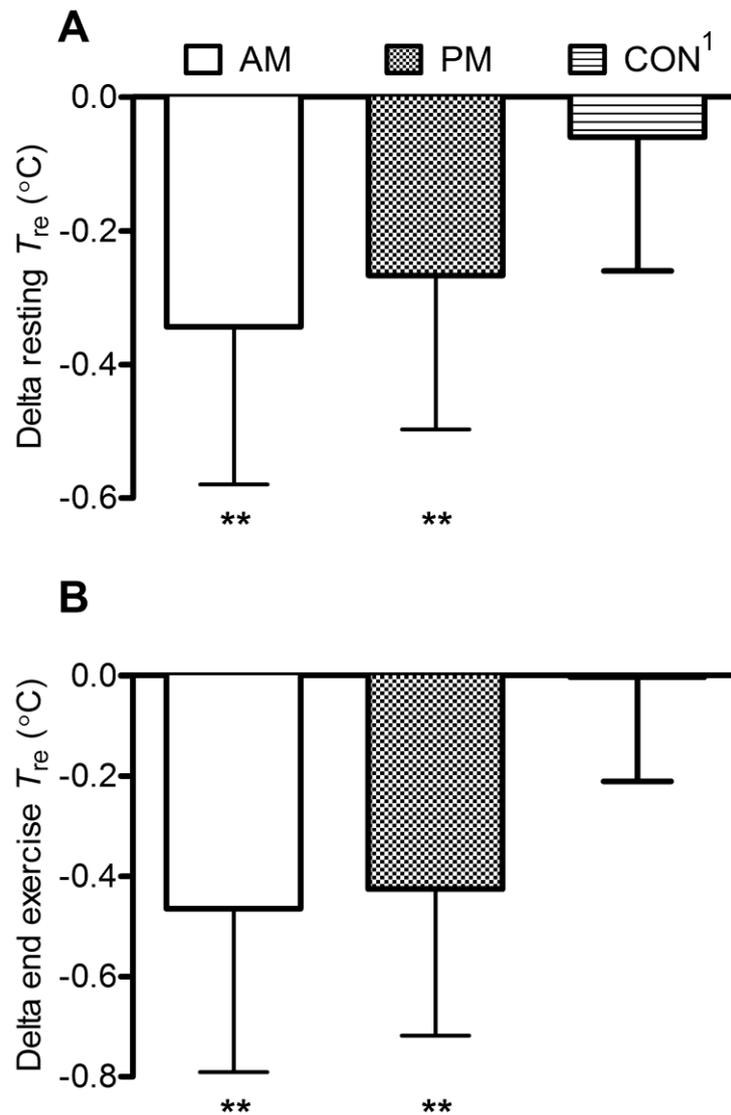
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617 **Figure 1** Effect of 6-day post-exercise hot water immersion heat acclimation on rectal temperature ( $T_{re}$ ) responses between 0900-h and 1540-h.  
618 Filled grey boxes on x-axis represents period of exercise. \*  $P < 0.05$  and \*\*  $P < 0.01$  indicates POST less than PRE. Data displayed as Mean  $\pm$   
619 SD.



621  
 622 **Figure 2** Change in resting (A) and end-exercise (B) rectal  
 623 temperature ( $T_{re}$ ) following 6-day post-exercise hot water  
 624 immersion (40°C) heat acclimation in the morning (AM) and  
 625 afternoon (PM).<sup>1</sup>Morning control data (CON) following 6-day  
 626 post-exercise thermoneutral water (34°C) immersion  
 627 intervention shown for comparison.<sup>15</sup> Data displayed as  
 628 mean  $\pm$  SD. \*  $P < 0.05$  and \*\*  $P < 0.01$  indicates POST less  
 629 than PRE.  
 630

631 **Table 1.** The influence of submaximal running at 65%  $\dot{V}O_{2\max}$  for 40 min in temperate conditions (20°C) and post-exercise hot water immersion  
 632 in 40°C on daily thermoregulatory variables, heart rate, and immersion time.  
 633

	HWI intervention day					
	1	2	3	4	5	6
<b>Submaximal exercise</b>						
Change in $T_{re}$ (°C)	1.17 ± 0.28	1.19 ± 0.28	1.14 ± 0.26	1.13 ± 0.32	1.05 ± 0.24	1.11 ± 0.30
End HR (beats·min <sup>-1</sup> )	154 ± 7	150 ± 9	149 ± 8	146 ± 8	145 ± 8	143 ± 9**
<b>HWI</b>						
Change in $T_{re}$ (°C)	0.84 ± 0.30	0.86 ± 0.16	1.05 ± 0.21	1.00 ± 0.20	0.92 ± 0.15	0.99 ± 0.16
Immersion time (min)	30 ± 6	37 ± 4	38 ± 4	38 ± 4	39 ± 2	40 ± 0**
Participants completing 40 min (n)	1 of 10	6 of 10	8 of 10	6 of 10	8 of 10	10 of 10
<b>Submaximal exercise and HWI</b>						
Area under the curve (°C·min <sup>-1</sup> )	27 ± 17	27 ± 16	30 ± 12	27 ± 15	23 ± 14	27 ± 14
WBSR (L·h <sup>-1</sup> )	0.94 ± 0.29	0.92 ± 0.20	0.97 ± 0.25	1.03 ± 0.27	1.04 ± 0.25	1.09 ± 0.23**

634  
 635 **Notes:** HR, heart rate; HWI, hot water immersion,  $T_{re}$ , rectal temperature; WBSR, whole body sweating rate.  
 636 \*\*  $P < 0.01$  indicates a significant difference between Day 1 and Day 6. Data displayed as Mean ± SD.

637 **Table 2.** Physiological and perceptual responses during exercise-heat stress in both the morning (AM) and afternoon (PM) following 6-day post-  
 638 exercise hot water immersion heat acclimation.  
 639

	AM		PM	
	PRE	POST	PRE	POST
$T_{re}$ at sweating onset ( $^{\circ}\text{C}$ )	$37.03 \pm 0.21$ <sup>#</sup>	$36.68 \pm 0.28$ <sup>#**</sup>	$37.23 \pm 0.28$	$36.92 \pm 0.32$ <sup>**</sup>
End-exercise HR ( $\text{beats}\cdot\text{min}^{-1}$ )	$178 \pm 11$	$164 \pm 11$ <sup>##**</sup>	$180 \pm 12$	$167 \pm 9$ <sup>**</sup>
End-exercise RPE	$15 \pm 2$	$13 \pm 1$ <sup>*</sup>	$15 \pm 3$	$13 \pm 1$ <sup>*</sup>
End-exercise thermal sensation	$10 \pm 2$	$9 \pm 1$ <sup>**</sup>	$11 \pm 1$	$9 \pm 1$ <sup>**</sup>
End-exercise $T_{sk}$ ( $^{\circ}\text{C}$ )	$35.01 \pm 0.93$	$34.11 \pm 0.85$ <sup>*</sup>	$34.86 \pm 1.08$	$34.17 \pm 1.04$ <sup>*</sup>
Mean $\dot{V}\text{O}_2$ ( $\text{L}\cdot\text{min}^{-1}$ )	$2.99 \pm 0.42$	$2.84 \pm 0.47$ <sup>*</sup>	$2.98 \pm 0.37$	$2.87 \pm 0.49$ <sup>*</sup>
Mean RER	$0.87 \pm 0.03$	$0.86 \pm 0.02$	$0.86 \pm 0.04$	$0.86 \pm 0.03$
WBSR ( $\text{L}\cdot\text{h}^{-1}$ )	$1.04 \pm 0.41$	$0.97 \pm 0.28$	$0.92 \pm 0.20$	$0.96 \pm 0.25$
Haemoglobin ( $\text{g}\cdot\text{dL}$ )	$14.8 \pm 0.6$	$14.6 \pm 0.6$	-	-
Haematocrit (%)	$45 \pm 1$	$44 \pm 2$	-	-

640  
 641 **Notes:**  $T_{re}$ , rectal temperature; HR, heart rate; RPE, rating of perceived exertion;  $T_{sk}$ , mean skin temperature; RER, respiratory exchange ratio;  
 642 WBSR, whole body sweating rate. <sup>#</sup>  $P < 0.05$  and <sup>##</sup>  $P < 0.01$  indicates AM less than PM. <sup>\*</sup>  $P < 0.05$  and <sup>\*\*</sup>  $P < 0.01$  indicates POST less than  
 643 PRE. Data displayed as Mean  $\pm$  SD.