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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

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10

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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°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 ± 7 beats $\cdot\text{min}^{-1}$, PM; -13 ± 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.¹ 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.²⁻⁴ 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 ≥ 60 min.⁵
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.^{1,5-9} 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.¹⁰ 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^{11,12} and then in
98 humans.¹⁰ Six adult men and women heat acclimated via 9-10
99 daily, 4-h passive heat exposures commencing in the afternoon
100 (46°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°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.^{6,13} 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.^{1,5-9} 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°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).¹⁴

123

124 Post-exercise hot water immersion (HWI) completed on 6
125 consecutive days represents a practical, economical, and
126 effective heat acclimation strategy¹⁵ which elicits adaptations
127 that compare favourably to exercise heat acclimation
128 strategies.¹⁶ 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 ± 4 years;
139 body mass: 72.8 ± 7.8 kg; $\dot{V}O_{2\max}$ 58.2 ± 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,¹⁰
157 where there is a meaningful difference in resting core
158 temperature (~ 0.3 – 0.4°C between AM and PM).¹⁷ Heat
159 acclimation involved six consecutive daily post-exercise HWI
160 in the morning between 0630-h and 1100-h, as described
161 previously.¹⁵ To control for any training and/or hydrostatic
162 effects Zurawlew et al.¹⁵ demonstrated that six consecutive
163 daily post-exercise (18°C) thermoneutral water immersion
164 (34°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 ± 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°C) as described previously.¹⁸ 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,¹
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.¹⁵ 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°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).¹⁹ A rectal
212 thermistor was fitted and T_{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°C).

219

220 At 0945-h dressed in running shorts, socks and trainers,
221 participants entered the environmental chamber (33°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.¹⁵ During
225 this time, no fluids were consumed. T_{re} , mean skin temperature
226 (T_{sk}), and HR were monitored continuously and rating of
227 perceived exertion (RPE)²⁰ and thermal sensation²¹ 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.¹⁵ 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 10th, 20th, 30th and 40th 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⁻¹) and a bolus
242 of water (7 mL·kg⁻¹ 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.³
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 (≤ 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.¹⁵ In the first
262 20 min of exercise, a bolus of water (5 mL·kg⁻¹ 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.¹⁵ 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.²² 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.²³

291

292 *Sweating responses:* Local forearm sweat rate was measured by
293 dew point hygrometry during all experimental trials as
294 previously described.¹⁸ Sweating threshold was calculated by
295 plotting individual relationships between local forearm sweat
296 rate and T_{re} , as previously described.²⁴ 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.²⁵

309

310 **Statistical analysis**

311 Using previous data¹⁵, 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 η^2 (ηp^2) were
332 reported to analyse the magnitude of the effects. Cohen²⁶ has
333 provided benchmarks to define small ($\eta p^2 = 0.01$), medium (ηp^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 (≤ 40 min) on six consecutive days.
344 HWI time increased from 30 ± 6 min on day 1 to 40 ± 0 min on
345 day 6 (Table 1). Daily end T_{re} averaged $39.34 \pm 0.29^{\circ}C$ and
346 daily area under the curve averaged $27 \pm 13^{\circ}C \cdot min^{-1}$. 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.,¹⁶ 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 ($34^{\circ}C$) 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 previous¹⁵ 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°C ; PM -0.43°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°C ; PM -0.27°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 acclimation¹⁶
402 and our previous work,¹⁵ 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,^{10,12,27} 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.^{1,5-9} 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°C reduction to be a meaningful
418 change in exercising T_{re} ;¹⁶ as such, the -0.47°C (AM) and -
419 0.43°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 matched²⁸ or performed at different times of the
428 day;¹⁴ 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 previously¹⁰, 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} ¹⁰). The
434 large, daily disruption to homeostasis during post-exercise HWI

435 heat acclimation (e.g. +2.1°C change in T_{re} ¹⁵) and controlled
436 hyperthermia, exercise-heat acclimation (e.g. +1.7°C change in
437 T_{re} ²⁸), 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.^{1,5-9} 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.¹⁵ 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)¹⁷; 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.²⁹ 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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490 and co-operation.

491

492 **Conflicts of interest**

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

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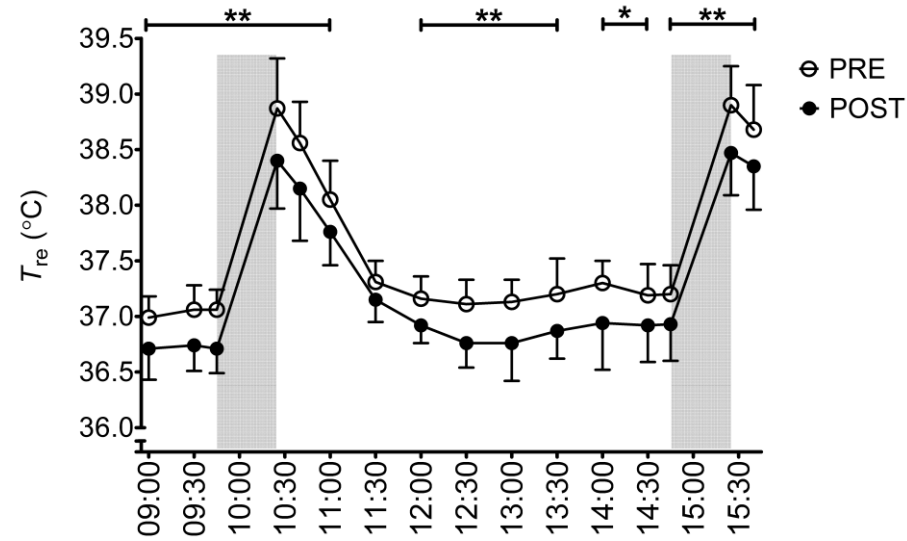
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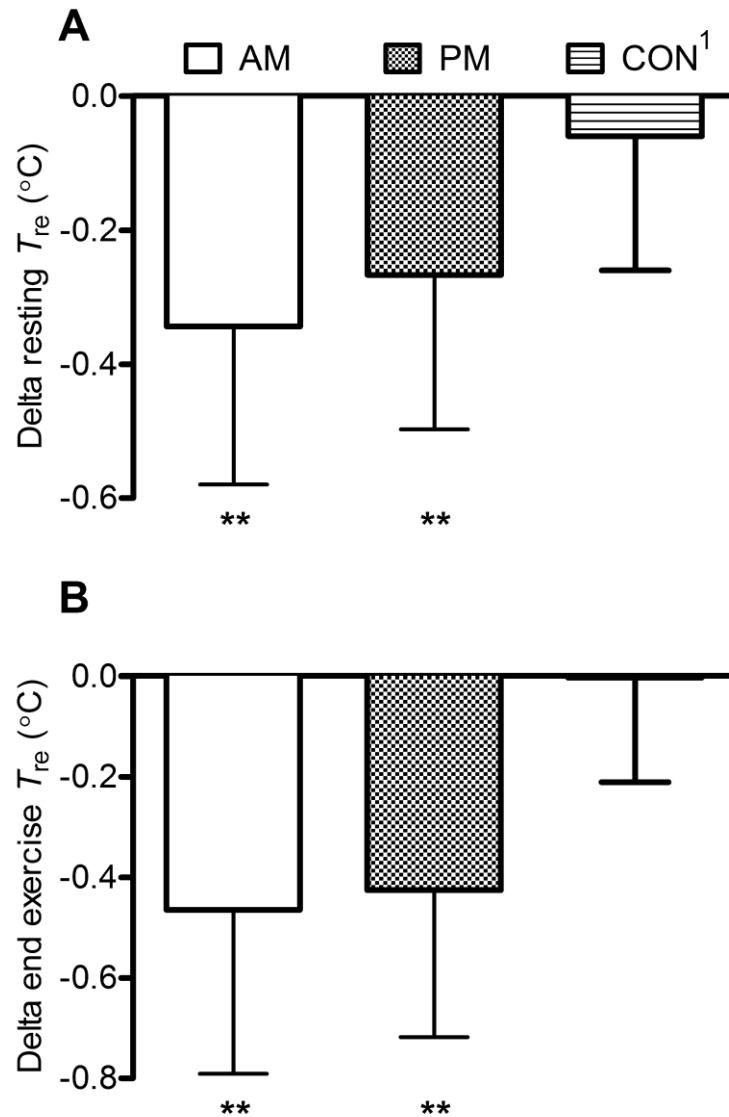
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617 **Figure 1** Effect of 6-day post-exercise hot water immersion heat acclimation on rectal temperature (T_{re}) responses between 0900-h and 1540-h.

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).¹Morning control data (CON) following 6-day
 626 post-exercise thermoneutral water (34°C) immersion
 627 intervention shown for comparison.¹⁵ 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
Submaximal exercise						
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 ⁻¹)	154 ± 7	150 ± 9	149 ± 8	146 ± 8	145 ± 8	143 ± 9**
HWI						
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
Submaximal exercise and HWI						
Area under the curve (°C·min ⁻¹)	27 ± 17	27 ± 16	30 ± 12	27 ± 15	23 ± 14	27 ± 14
WBSR (L·h ⁻¹)	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 ± 0.21 [#]	36.68 ± 0.28 ^{#**}	37.23 ± 0.28	36.92 ± 0.32 ^{**}
End-exercise HR ($\text{beats}\cdot\text{min}^{-1}$)	178 ± 11	164 ± 11 ^{##**}	180 ± 12	167 ± 9 ^{**}
End-exercise RPE	15 ± 2	13 ± 1 [*]	15 ± 3	13 ± 1 [*]
End-exercise thermal sensation	10 ± 2	9 ± 1 ^{**}	11 ± 1	9 ± 1 ^{**}
End-exercise T_{sk} ($^{\circ}\text{C}$)	35.01 ± 0.93	34.11 ± 0.85 [*]	34.86 ± 1.08	34.17 ± 1.04 [*]
Mean $\dot{V}O_2$ ($\text{L}\cdot\text{min}^{-1}$)	2.99 ± 0.42	2.84 ± 0.47 [*]	2.98 ± 0.37	2.87 ± 0.49 [*]
Mean RER	0.87 ± 0.03	0.86 ± 0.02	0.86 ± 0.04	0.86 ± 0.03
WBSR ($\text{L}\cdot\text{h}^{-1}$)	1.04 ± 0.41	0.97 ± 0.28	0.92 ± 0.20	0.96 ± 0.25
Haemoglobin ($\text{g}\cdot\text{dL}$)	14.8 ± 0.6	14.6 ± 0.6	-	-
Haematocrit (%)	45 ± 1	44 ± 2	-	-

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