

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

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30 **Preferred Running Head:**

31 Hot water immersion heat acclimation

Abstract

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

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

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

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

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

Introduction

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

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

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

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

Sweating responses: Local forearm sweat rate was measured by dew point hygrometry during all experimental trials as previously described.¹⁸ Sweating threshold was calculated by plotting individual relationships between local forearm sweat rate and T_{re} , as previously described.²⁴ Changes in dry nude body mass were used to estimate WBSR during all intervention days and experimental trials.

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

Statistical analysis

Using previous data¹⁵, a sample size estimation (G*Power 3.1.2) with an alpha level of 0.05 and power of 0.95, determined that eight participants were required to detect a significant difference in resting T_{re} (-0.27°C) and end-exercise T_{re} (-0.36°C) following post-exercise HWI heat acclimation. To ensure adequate power and allowing for dropout, 10 participants were recruited. Data is presented as mean \pm standard deviation (SD) and statistical significance was accepted at $P < 0.05$. All data were checked for normality and sphericity. Paired sample t-tests were used to assess the differences between the heat acclimation status (changes from PRE to POST) in the morning and afternoon (AM and PM). Two-way repeated measures analysis of variance (ANOVA) with Greenhouse Geisser correction to the degrees of freedom (where necessary) were used to assess differences between the heat acclimation status (changes from PRE and POST) and the time of day (AM and PM). Friedman test was used to assess differences between the PRE and POST, AM and PM trials for measures of RPE and thermal sensation. When statistical significance was found, Wilcoxon Signed Rank tests were used to identify where the difference occurred. Partial η^2 (ηp^2) were reported to analyse the magnitude of the effects. Cohen²⁶ has provided benchmarks to define small ($\eta p^2 = 0.01$), medium ($\eta p^2 = 0.06$), 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

Results

Post-exercise HWI heat acclimation

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

Experimental trials

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

Discussion

The novel findings of the current study confirm and advance those previous¹⁵ by showing that hallmark heat acclimation adaptations by post-exercise HWI are not restricted to the clock-time of daily heat exposures. These data provide clear evidence that post-exercise HWI can be performed in the morning to reduce thermal strain in both the morning and mid-afternoon (end-exercise T_{re} AM -0.47°C ; PM -0.43°C ; Figure 2B). The observed reduction in thermal strain during exercise-heat stress performed in the morning and afternoon was achieved, at least in part, through a reduction in T_{re} at rest in temperate conditions (AM -0.34°C ; PM -0.27°C ; Figure 2A). Other hallmark heat acclimation adaptations were evident during exercise-heat stress in both the morning and afternoon; these included a reduction in T_{re} at sweating onset and a reduction in end-exercise HR, RPE, thermal sensation and T_{sk} . However, in line with short-term exercise-heat acclimation¹⁶ and our previous work,¹⁵ six days of post-exercise HWI did not alter WBSR during submaximal exercise in the heat.

Current heat acclimation recommendations, based upon the work of Shido and colleagues,^{10,12,27} performed across comparable clock-times as the current study, state that to acquire the greatest benefit daily heat exposures should be scheduled at the anticipated clock-time of future exercise-heat stress.^{1,5-9} However, the present data demonstrate that 6-days post-exercise HWI heat acclimation does not need to be constrained to the same clock-time of future exercise-heat stress, when performed between 0900-h and 1540-h (Figure 1). The magnitude of adaptation appears to be slightly smaller in the afternoon compared with the morning for hallmark adaptations (Figure 2, Table 2). However, a recent meta-analysis considered a 0.3°C reduction to be a meaningful change in exercising T_{re} ;¹⁶ as such, the -0.47°C (AM) and -0.43°C (PM) reduction in end-exercise T_{re} observed in the current study can both be considered meaningful adaptations. Indeed, the currently available evidence from short-term exercise-heat acclimation studies challenges the notion that heat acclimation adaptations are clock-time dependent. For example, comparable reductions in thermal and cardiovascular strain were demonstrated during exercise-heat stress when the clock-time of the daily intervention and the exercise-heat stress was either matched²⁸ or performed at different times of the day;¹⁴ albeit these studies were not specifically designed to assess whether heat acclimation adaptations are clock-time dependent. It is conceivable that the subtle, clock-time dependent reduction in resting T_{re} shown previously¹⁰, may be explained by the mild thermal stimulus for adaptation during daily passive heat exposures ($+0.7^{\circ}\text{C}$ change in T_{re} ¹⁰). The large, daily disruption to homeostasis during post-exercise HWI

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

Practical applications

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

Conclusion

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

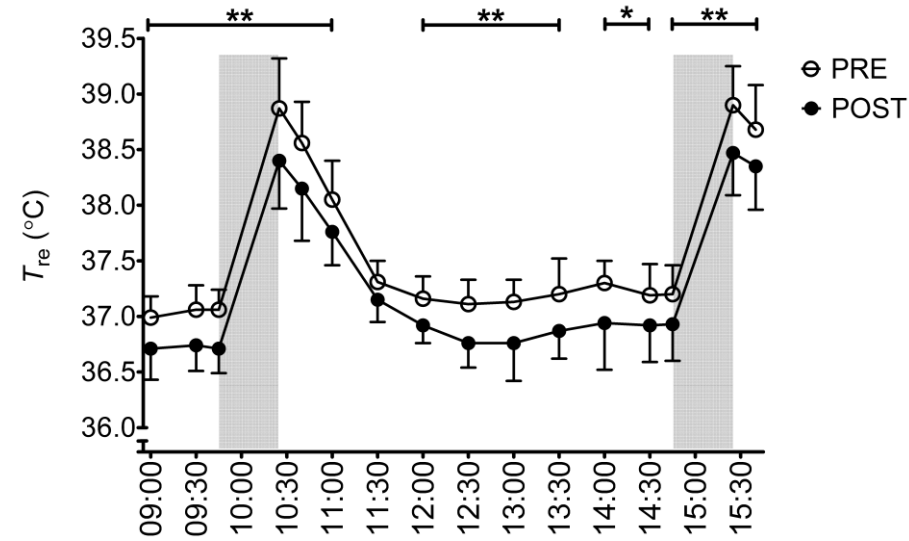
495 **References**

- 496 1. Périard JD, Racinais S, Sawka MN. Adaptations and
497 mechanisms of human heat acclimation: Applications for
498 competitive athletes and sports. *Scand J Med Sci Sports*.
499 2015;25:20-38. doi:10.1111/sms.12408.
- 500 2. Lorenzo S, Halliwill JR, Sawka MN, Minson CT. Heat
501 acclimation improves exercise performance. *J Appl*
502 *Physiol*. 2010;109(4):1140-1147.
503 doi:10.1152/jappphysiol.00495.2010.
- 504 3. Gonzalez RR, Gagge AP. Warm discomfort and
505 associated thermoregulatory changes during dry and
506 humid heat acclimatization. *Isr J Med Sci*.
507 1976;12(8):804-807.
- 508 4. Frank A, Belokopytov M, Moran D, Shapiro Y, Epstein
509 Y. Changes in heart rate variability following
510 acclimation to heat. *J Basic Clin Physiol Pharmacol*.
511 2001;12(1):19-32. doi:10.1007/s00421-014-2935-5.
- 512 5. Taylor NS. Human heat adaptation. *Compr Physiol*.
513 2014;4(1):325-365. doi:10.1002/cphy.c130022.
- 514 6. Cable NT, Drust B, Gregson WA. The impact of altered
515 climatic conditions and altitude on circadian physiology.
516 *Physiol Behav*. 2007;90(2-3):267-273.
517 doi:10.1016/j.physbeh.2006.09.002.
- 518 7. Chalmers S, Esterman A, Eston R, Bowering KJ, Norton
519 K. Short-term heat acclimation training improves
520 physical performance: a systematic review, and
521 exploration of physiological adaptations and application
522 for team sports. *Sports Med*. 2014;44(7):971-988.
523 doi:10.1007/s40279-014-0178-6.
- 524 8. Beaudin AE, Clegg ME, Walsh ML, White MD.
525 Adaptation of exercise ventilation during an actively-
526 induced hyperthermia following passive heat
527 acclimation. *Am J Physiol Regul Integr Comp Physiol*.
528 2009;297(3):R605-R614.
529 doi:10.1152/ajpregu.90672.2008.
- 530 9. Patterson MJ, Stocks JM, Taylor N a S. Whole-body
531 fluid distribution in humans during dehydration and
532 recovery, before and after humid-heat acclimation
533 induced using controlled hyperthermia. *Acta Physiol*.
534 2014;210:899-912. doi:10.1111/apha.12214.
- 535 10. Shido O, Sugimoto N, Tanabe M, Sakurada S. Core
536 temperature and sweating onset in humans acclimated to
537 heat given at a fixed daily time. *Am J Physiol*.
538 1999;276:R1095-101.
- 539 11. Sakurada S, Shido O, Sugimoto N, Fujikake K,
540 Nagasaka T. Changes in hypothalamic temperature of
541 rats after daily exposure to heat at a fixed time. *Pflugers*
542 *Arch Eur J Physiol*. 1994;429:291-293.
543 doi:10.1007/BF00374326.
- 544 12. Shido O, Yoneda Y, Nagasaka T. Shifts in the

- hypothalamic temperature of rats acclimated to direct internal heat load with different schedules. *J Therm Biol.* 1991;16(5):267-271. doi:10.1016/0306-4565(91)90015-t.
13. Maruyama M, Hara T, Katakura M, et al. Contribution of the suprachiasmatic nucleus to the formation of a time memory for heat exposure in rats. *J Physiol Sci.* 2007;57(2):107-114. doi:10.2170/physiolsci.RP014506.
 14. Garrett AT, Goosens NG, Rehrer NJ, Rehrer NG, Patterson MJ, Cotter JD. Induction and decay of short-term heat acclimation. *Eur J Appl Physiol.* 2009;107(6):659-670. doi:10.1007/s00421-009-1182-7.
 15. Zurawlew MJ, Walsh NP, Fortes MB, Potter C. Post-exercise hot water immersion induces heat acclimation and improves endurance exercise performance in the heat. *Scand J Med Sci Sport.* 2016;26(7):745-754. doi:10.1111/sms.12638.
 16. Tyler CJ, Reeve T, Hodges GJ, Cheung SS. The effects of heat adaptation on physiology, perception and exercise performance in the heat: A meta-analysis. *Sport Med.* 2016;46(11):1699-1724. doi:10.1007/s40279-016-0538-5.
 17. Aschoff J. Circadian control of body temperature. *J Therm Biol.* 1983;8:143-147.
 18. Fortes MB, Di Felice U, Dolci A, et al. Muscle-damaging exercise increases heat strain during subsequent exercise heat stress. *Med Sci Sports Exerc.* 2013;45(10):1915-1924. doi:10.1249/MSS.0b013e318294b0f8.
 19. Armstrong L. Hydration assessment techniques. *Nutr Rev.* 2005;63(3):S40-S54.
 20. Borg G. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med.* 1970;2(2):92-98.
 21. Hollies N, Goldman R. Psychological scaling in comfort assessment. In: Hollies NRS, Goldman RFG (eds) *Clothing Comfort: Interaction of Thermal, Ventilation, Construction, and Assessment Factors.* 1977:107-120.
 22. Cheuvront SN, Chinevere TD, Ely BR, et al. Serum S-100 β response to exercise-heat strain before and after acclimation. *Med Sci Sports Exerc.* 2008;40(8):1477-1482. doi:10.1249/MSS.0b013e31816d65a5.
 23. Ramanathan NL. A new weighting system for mean surface temperature of the human body. *J Appl Physiol.* 1964;19:531-533.
 24. Cheuvront S, Bearden S, Kenefick R, et al. A simple and valid method to determine thermoregulatory sweating threshold and sensitivity. *J Appl Physiol.* 2009;107(1):69-75. doi:10.1152/jappphysiol.00250.2009.
 25. Dill DB, Costill DL. Calculation of percentage changes

595 in volumes of blood, plasma, and red cells in
596 dehydration. *J Appl Physiol.* 1974;37(2):247-248.
597 26. Cohen J. *A Statistical Power Analysis for the*
598 *Behavioural Sciences.* New York: Routledge Academic;
599 1988.
600 27. Sugimoto N, Shido O, Sakurada S. Thermoregulatory
601 responses of rats acclimated to heat given daily at a fixed
602 time. *J Appl Physiol.* 1995;78(5):1720-1724.
603 28. Gibson OR, Mee JA, Tuttle JA, Taylor L, Watt PW,
604 Maxwell NS. Isothermic and fixed intensity heat
605 acclimation methods induce similar heat adaptation
606 following short and long-term timescales. *J Therm Biol.*
607 2015;49-50:55-65. doi:10.1016/j.jtherbio.2015.02.005.
608 29. Hill DW, Leiferman JA, Lynch NA, Dangelmaier BS,
609 Burt SE. Temporal specificity in adaptations to high-
610 intensity exercise training. *Med Sci Sports Exerc.*
611 1998;30(3):450-455. doi:10.1097/00005768-199803000-
612 00017.
613

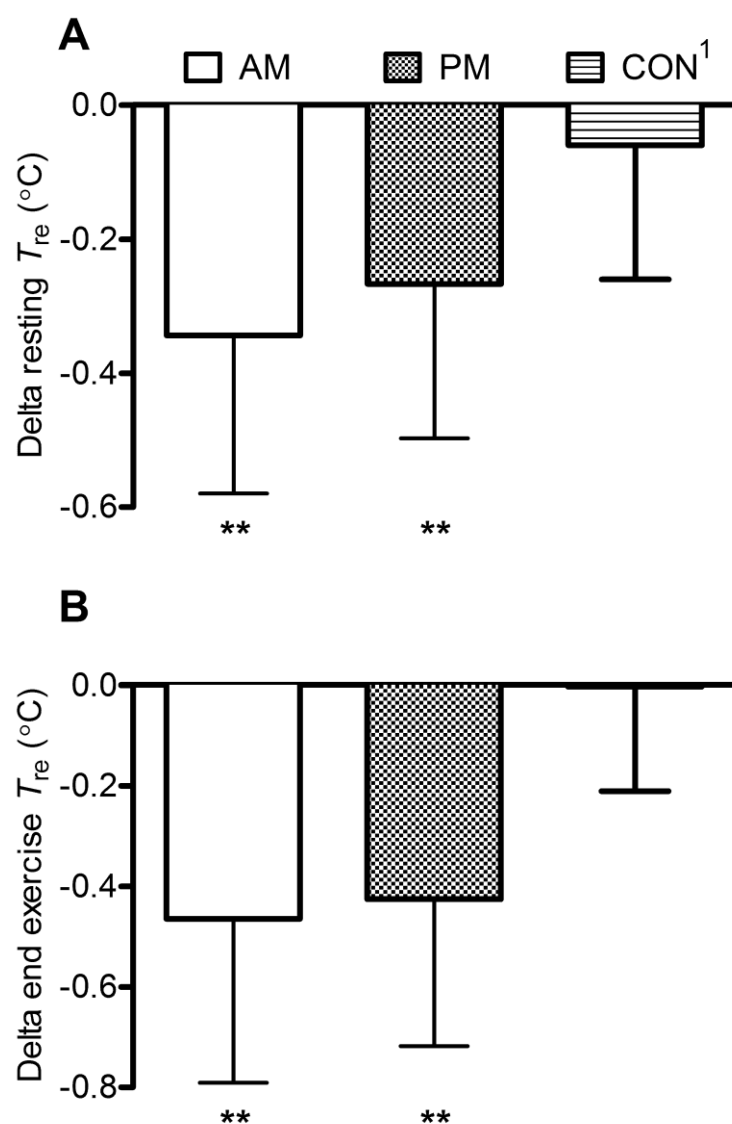
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617 **Figure 1** Effect of 6-day post-exercise hot water immersion heat acclimation on rectal temperature (T_{re}) responses between 0900-h and 1540-h.
 618 Filled grey boxes on x-axis represents period of exercise. * $P < 0.05$ and ** $P < 0.01$ indicates 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 only.¹⁵ Data displayed as
 628 mean \pm SD. * $P < 0.05$ and ** $P < 0.01$ indicates POST less
 629 than PRE.
 630

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 in 40°C on daily thermoregulatory variables, heart rate, and immersion time.

	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**

Notes: HR, heart rate; HWI, hot water immersion, T_{re} , rectal temperature; WBSR, whole body sweating rate.

** $P < 0.01$ indicates a significant difference between Day 1 and Day 6. Data displayed as Mean ± SD.

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

	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 $\dot{V}\text{O}_2$ ($\text{L} \cdot \text{min}^{-1}$)	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	-	-

Notes: T_{re} , rectal temperature; HR, heart rate; RPE, rating of perceived exertion; T_{sk} , mean skin temperature; RER, respiratory exchange ratio; 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 PRE. Data displayed as Mean \pm SD.