Heat Acclimation by Post-Exercise Hot Water Immersion

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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**
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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% $\dot{V}O_{2max}$ 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    $\dot{V}O_{2max}$) 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 ± 0.24°C, PM; -
54   0.27 ± 0.23°C; $P = 0.002$), T_{re} at sweating onset (AM; -0.34 ±
55   0.24°C, PM; -0.31 ± 0.25°C; $P = 0.001$), and end-exercise T_{re}
56   (AM; -0.47 ± 0.33°C, PM; -0.43 ± 0.29°C; $P = 0.001$), heart
57   rate (AM; -14 ± 7 beats·min^{-1}, PM; -13 ± 6 beats·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 adaptions 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.

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}); ≥ 38.5°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} ~0.2°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-

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heat acclimation, performed in the afternoon (1500 to 1700 h), initiates reductions in thermal strain (T_{re}; -0.3°C) and cardiovascular strain (heart rate (HR); -13 beats·min^{-1}) during exercise-heat stress tests performed in the morning (0900 to 1200 h).[14]

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

Methods

Participants

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

Study design

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

Preliminary measurements

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

Experimental trials

Participants completed a food diary 24-h prior to the PRE experimental trial and were instructed to replicate this diet 24-h prior to the POST experimental trial. The food diary verified 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 MJ·kg^{-1}) and a bolus of water (7
202 mL·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 is 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% $\dot{V}O_{2max}$ (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 sensation[21] 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 ($\dot{V}O_2$), 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 ($\leq$ 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.[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 $T_{re} \leq 38.5$°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 ≥ 38.5°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 data[15], 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 ±
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. Cohen[26] 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

Results

Post-exercise HWI heat acclimation

All participants completed a 40 min treadmill run at 65% $\dot{V}O_{2max}$, followed by HWI ($\leq$ 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 $39.34 \pm 0.29°C$ and daily area under the curve averaged $27 \pm 13°C \cdot min^{-1}$. 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 previous[15] 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 acclimation[16] 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 matched[28] 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 previously[10], may be explained by the mild thermal stimulus for adaptation during daily passive heat exposures (+0.7°C change in T_{re}[10]). The large, daily disruption to homeostasis during post-exercise HWI

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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 adaptions 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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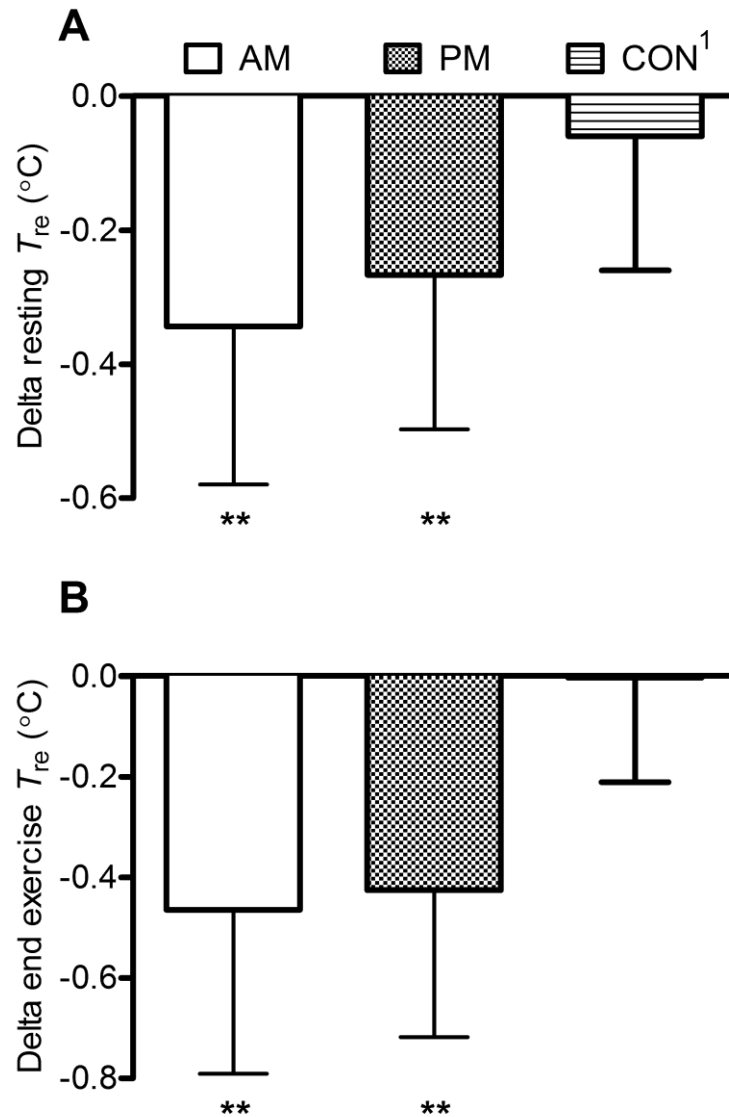612   00017.
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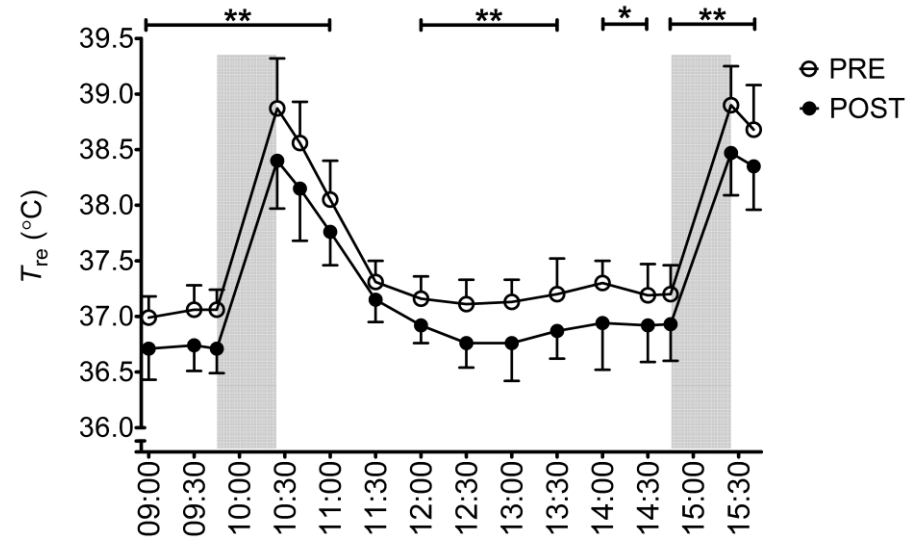
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617 **Figure 1** Effect of 6-day post-exercise hot water immersion heat acclimation on rectal temperature (T_{re}) responses between 0900-h and 1540-h.
618 Filled grey boxes on x-axis represents period of exercise. * $P < 0.05$ and ** $P < 0.01$ indicates POST less than PRE. Data displayed as Mean ±
619 SD.

620

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).[1]Morning 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 ± SD. * $P < 0.05$ and ** $P < 0.01$ indicates POST less
629 than PRE.
630

Table 1. The influence of submaximal running at 65% $\dot{V}O_{2max}$ 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 $\pm$ 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 (°C)	37.03 ± 0.21 [#]	36.68 ± 0.28 [#] **	37.23 ± 0.28	36.92 ± 0.32 **
End-exercise HR (beats·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} (°C)	35.01 ± 0.93	34.11 ± 0.85 *	34.86 ± 1.08	34.17 ± 1.04 *
Mean $\dot{V}O_2$ (L·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 (L·h⁻¹)	1.04 ± 0.41	0.97 ± 0.28	0.92 ± 0.20	0.96 ± 0.25
Haemoglobin (g·dL)	14.8 ± 0.6	14.6 ± 0.6	-	-
Haematocrit (%)	45 ± 1	44 ± 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. [#] $P < 0.05$ and [##] $P < 0.01$ indicates AM less than PM. * $P < 0.05$ and ** $P < 0.01$ indicates POST less than
643 PRE. Data displayed as Mean ± SD.