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Heat Acclimation by Postexercise Hot-Water Immersion: Reduction of Thermal Strain During Morning and Afternoon Exercise-Heat Stress After Morning Hot-Water Immersion

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

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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% $\dot{V}O_{2max}$ treadmill run) before (PRE) and after (POST) heat acclimation. The 6-day heat acclimation intervention involved a daily, 40 min treadmill-run (65% $\dot{V}O_{2max}$) 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 ± 0.24°C, PM; -0.27 ± 0.23°C; P = 0.002), T_{re} at sweating onset (AM; -0.34 ± 0.24°C, PM; -0.31 ± 0.25°C; P = 0.001), and end-exercise T_{re} (AM; -0.47 ± 0.33°C, PM; -0.43 ± 0.29°C; P = 0.001), heart rate (AM; -14 ± 7 beats·min^{-1}, PM; -13 ± 6 beats·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 adaptions 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}); ≥ 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-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. Twentyfour hours prior to, and on the day of the experimental trials participants were also instructed to refrain from any additional exercise. As sleeping patterns can influence thermoregulation,[1] participants were instructed to sleep between 2200-h and 0700-h to ensure a similar circadian pattern prior to each experimental trial. This was confirmed by monitoring sleep, using an Actigraph worn on the non-dominant arm with epoch length set to 1 min (Actigraph GT3X Version 4.4.0, Actigraph, Pensacola, USA). Data was subsequently analysed for sleep efficiency (number of sleep min, divided by total number of min in bed, multiplied by 100 to convert to percentage) and sleep duration using Actilife+Sleep Version 6 (Actigraph, Pensacola, USA).

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

At 0945-h dressed in running shorts, socks and trainers, participants entered the environmental chamber (33°C, 40% relative humidity; Delta Environmental Systems, Chester, UK) to complete the AM trial which involved running for 40 min at 65% $\dot{V}O_{2max}$ (1% gradient) as previously described.[15] During this time, no fluids were consumed. T_{re}, mean skin temperature (T_{sk}), and HR were monitored continuously and rating of perceived exertion (RPE)[20] and thermal sensation[21] were recorded every 10 min. Local forearm sweating rate was measured every 20 s for the first 15 min of exercise to assess the onset of sweating as previously described.[15] Oxygen uptake ($\dot{V}O_2$), and respiratory exchange ratio (RER) were assessed from 60 s expired gas samples collected by Douglas bag method immediately prior to 10th, 20th, 30th and 40th min of 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 $MJ \cdot kg^{-1}$) and a bolus of water (7 $mL \cdot kg^{-1}$ 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.

Post-exercise HWI heat acclimation

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

Measurement and instrumentation

Body temperatures: T_{re} was measured using a flexible, sterile, disposable thermistor (Henleys Medical Supplies Ltd., Herts, UK) and recorded using a data logger (YSI model 4000A, YSI, Dayton, USA). Prior to insertion, a bead was fixed to the rectal thermistor 10 cm from the inserted end; this ensured the thermistor remained inserted to the same depth throughout the trial. To assess cumulative hyperthermia, an area under the curve analysis (time T_{re} was ≥ 38.5°C) was performed on the 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 data[15], 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 ± 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. Cohen[26] 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 using SPSS version 20 (IBM Corporation, NY, USA), or GraphPad Prism Version 5.02 (GraphPad Software Inc. La Jolla, USA).

Results

Post-exercise HWI heat acclimation

All participants completed a 40 min treadmill run at 65% $\dot{V}O_{2max}$, 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 ± 0.29°C and daily area under the curve averaged 27 ± 13°C·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 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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Conflicts of interest

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

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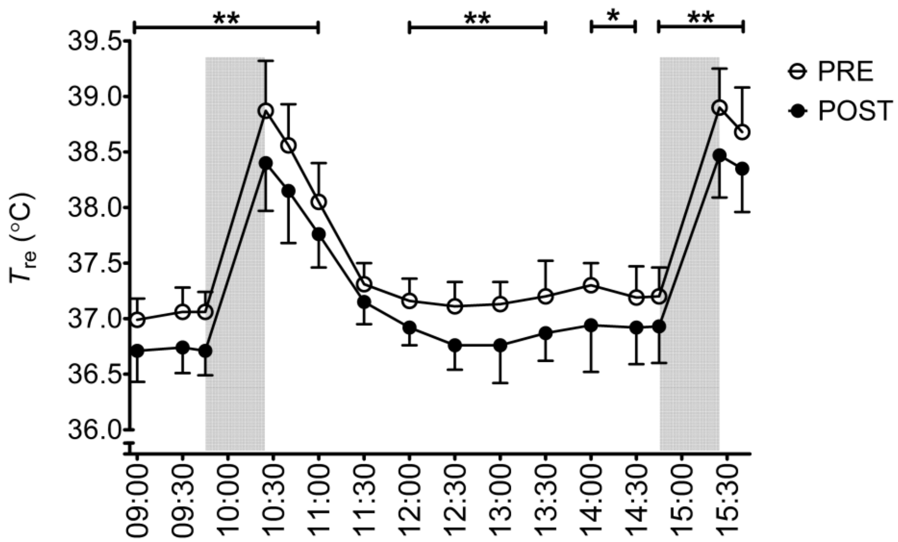

Figure 1 Effect of 6-day post-exercise hot water immersion heat acclimation on rectal temperature (T_{re}) responses between 0900-h and 1540-h. Filled grey boxes on x-axis represents period of exercise. * $P < 0.05$ and ** $P < 0.01$ indicates POST less than PRE. Data displayed as Mean ± SD.

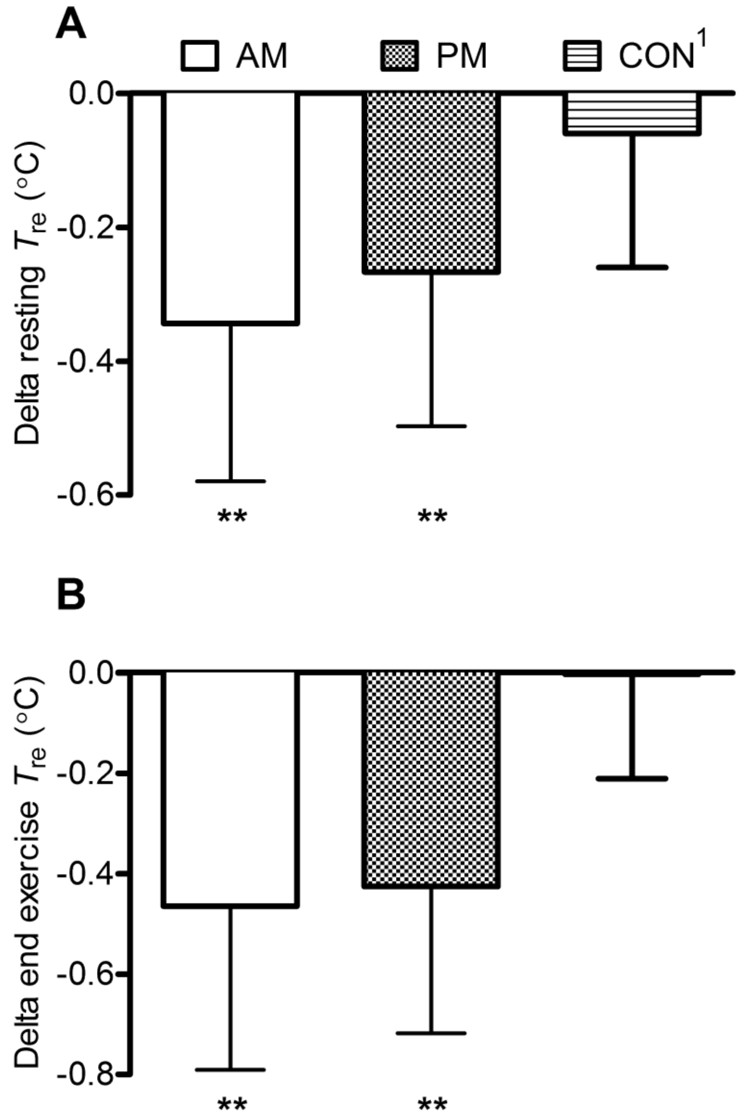

Figure 2 Change in resting (A) and end-exercise (B) rectal temperature (T_{re}) following 6-day post-exercise hot water immersion (40°C) heat acclimation in the morning (AM) and afternoon (PM).[1]Morning control data (CON) following 6-day post-exercise thermoneutral water (34°C) immersion intervention shown for comparison only.[15] Data displayed as mean ± SD. * $P < 0.05$ and ** $P < 0.01$ indicates POST less than PRE.

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 ± 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 (°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	-	-

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 ± SD.