

A comparison of heat acclimation by post-exercise hot water immersion and exercise in the heat

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26 ABSTRACT

27 Objectives: To compare heat acclimation adaptations after three and six days of either post-exercise

28 hot water immersion (HWI) or exercise-heat-acclimation (EHA) in recreationally active individuals.

29 Design: Randomised, mixed model, repeated measures.

30 Methods: Post-exercise HWI involved a daily 40-min treadmill-run at 65% VO_{2peak} in temperate conditions (19°C, 45% RH) followed by HWI ($\leq 40 \text{ min}$, 40°C water; *n*=9). Daily EHA involved a 31 \leq 60-min treadmill-run in the heat (65% $\dot{V}O_{2peak}$; 33°C, 40% RH; *n*=9), chosen to elicit a similar 32 endogenous thermal stimulus to HWI. A thermoneutral exercise intervention (TNE, 19°C, 45% RH; 33 n=9), work-matched to EHA, was also included to determine thermoregulatory adaptations to daily 34 exercise in temperate conditions. An exercise heat-stress-test was performed before and after three and 35 six intervention days and involved a 40-min treadmill-run and time-to-exhaustion (TTE) at 65% 36 37 VO_{2peak} in the heat (33°C, 40% RH).

Results: ANCOVA, using baseline values as the covariate, revealed no interaction effects but significant group effects demonstrated that compared to EHA, HWI elicited larger reductions in resting rectal temperature (T_{re} ; p=0.021), T_{re} at sweating onset (p=0.011), and end-exercise T_{re} during exercise-heat-stress (-0.47°C; p=0.042). Despite a similar endogenous thermal stimulus to HWI, EHA elicited a modest reduction in end-exercise T_{re} (-0.26°C), which was not different from TNE (-0.25°C, p=1.000). There were no main effects or interaction effects for end-exercise T_{sk} , heart rate, physiological strain index, RPE, thermal sensation, plasma volume, or TTE (all p≥0.154).

45 Conclusion: Compared with conventional short-term exercise heat acclimation, short-term post-

46 exercise hot water immersion elicited larger thermal adaptations.

47 **Keywords:** acclimatization, endurance training, running, thermotolerance, performance, hot bath.

INTRODUCTION 49

50 workers who reside in temperate conditions are advised to complete a period of heat acclimation.^{1, 2} 51 Heat acclimation adaptations, that improve endurance capacity and reduce susceptibility to exertional 52 heat illness,³ include an earlier onset of cutaneous vasodilatation and sweating, an increase in sweating 53 rate, and a reduction in resting and exercising body temperature.^{4, 5} Recommendations to maximise 54 adaptations are to complete ≥ 15 daily exercise heat acclimation exposures (long-term heat 55 acclimation), which initiate profuse sweating and maintain an elevated body temperature for ≥ 60 56 min.^{3, 6} However, protocols can be costly, impractical, ineffective as trained individuals are considered 57 partially heat acclimatised,^{7,8} and the physical demands of daily exercise-heat-stress can disrupt 58 training and lead to fatigue.^{6,9} As a consequence, athlete engagement with long-term exercise heat 59 acclimation is poor.¹ To reduce the time commitment, sport scientists have designed short-term heat 60 acclimation interventions (\leq 7-days), supported by the premise that ~80% of adaptations occur in 7-61 62 days.¹⁰ However, research investigations report inconsistent reductions in core body temperature at rest and during exercise-heat-stress following short-term exercise heat acclimation.¹¹ 63

In preparation for competing or working in the heat, athletes, military personnel and occupational

Post-exercise passive heating, such as sauna bathing¹² and hot water immersion (HWI),¹³⁻¹⁵ provide 64 65 alternative, more accessible and time efficient heat acclimation strategies compared to conventional exercise-based approaches. These exposures to hot air/water can be incorporated into normal training, 66 e.g., HWI as part of a post-exercise washing routine, and may also support muscle recovery.¹⁶ Six days 67 of post-exercise HWI presents a short-term heat acclimation strategy, which provides reductions in 68 thermal strain that compare favourably with long-term interventions.¹¹ HWI exposes individuals to a 69 70 large dual thermal stimulus (i.e. elevated core and skin temperatures), which is purported to induce a more complete state of heat acclimation.¹⁷ Furthermore, exposure to high skin temperatures has been 71 shown to accelerate heat acclimation adaptation in females.¹⁸ 72

The primary aim of the current study was to compare thermal adaptations from three and six days of 73 post-exercise HWI with exercise heat acclimation (EHA) in recreationally active males. In addition, 74

- by including a work-matched thermoneutral exercise intervention (TNE), we investigated the
- 77 We hypothesised that post-exercise HWI would accelerate the speed of adaptation compared to EHA,
- and that the benefits of EHA beyond that of TNE would be modest.

80 METHODS

81 Participants: Twenty-seven recreationally active and non-heat-acclimatised males provided written informed consent to participate. The study received local and Ministry of Defence Research Ethics 82 Committee approval and was conducted following the Declaration of Helsinki (2013; although was 83 not pre-registered) and received Defence Science and Technology Laboratory permission to publish. 84 Participants were matched for fitness characteristics in groups of three and randomly assigned to either 85 86 HWI, EHA, or TNE (randomiser.org; see Table 1 for participant characteristics). HWI involved a 40min treadmill-run in temperate conditions (19°C) followed by hot water immersion (≤ 40-min, 40°C 87 water). To elicit a similar endogenous thermal stimulus to HWI (i.e. area under the curve, AUC, time 88 and magnitude $T_{\rm re}$ was >38.5°C, °C·min⁻¹; Supplement A), EHA involved a \leq 60-min treadmill-run in 89 the heat (33°C, 40% RH). Pilot data demonstrated a similar AUC from post-exercise HWI¹² vs. a 60-90 min treadmill run in the heat (65% VO_{2peak}; 33°C, 40% RH). We deemed it unnecessary to include a 91 thermoneutral water immersion intervention as we have previously demonstrated that it provides no 92 93 heat acclimation benefits.¹⁵ We did however include a thermoneutral exercise intervention (TNE) to account for the effect of daily submaximal exercise on thermoregulatory adaptations. To enable work-94 95 matching with EHA. TNE participants completed the same external work \geq 1-day after EHA 96 participants.

- **Table 1.** Participant characteristics of post-exercise hot water immersion (HWI), exercise heat
- 98 acclimation (EHA) and thermoneutral exercise (TNE).

	HWI	ЕНА	TNE
Age (years)	22 ± 3	20 ± 2	21 ± 2
Height (cm)	177 ± 5	181 ± 5	178 ± 6
Body mass (kg)	73 ± 7	74 ± 7	70 ± 7
^{VO} _{2peak} (ml·kg ^{−1} ·min ^{−1})	53 ± 6	54 ± 3	53 ± 4

Data are displayed as mean \pm SD. N = 9, each group.

99 ***Fig. 1 near here***

100 Fitness assessment: Participants completed a fitness assessment within a week before their first experimental trial. VO_{2peak} was assessed using a continuous maximal incremental exercise test 101 102 performed on a motorised treadmill (HP Cosmos Mercury 4.0, Nussdorf-Traunstein, Germany) in a temperate laboratory (19°C) to volitional exhaustion. VO_{2peak} was determined as the highest oxygen 103 104 uptake attained over a 30-s period. A running speed that elicited 65% VO_{2peak} in temperate conditions was determined by the interpolation of the running speed-VO₂ relationship. Participants were then 105 106 familiarised with the treadmill running speed and experimental trial procedures. As temperate training 107 influences heat acclimation adaptations,⁷ participants' physical activity time (> 3 METS) for the 108 duration of the study was assessed (Fitbit Flex, San Francisco, USA).

Experimental trials: Participants completed three experimental trials: before and after three, and six
days of their assigned intervention (Fig. 1). Twenty-four hours before experimental trials, participants
refrained from exercise, alcohol, diuretics, and caffeine. Before the first experimental trial, participants
completed a diet diary and replicated this food and fluid intake before subsequent experimental trials.
To ensure a similar circadian pattern, participants were instructed to sleep between 2200-h and 0700-h
before experimental trials; sleep duration and quality were confirmed (Actigraph wGT3X-BT,
Actigraph, Pensacola, USA).

On the day of the experimental trials (Fig. 1), participants arrived at the laboratory at 0730-h and were 116 provided with a standardised breakfast (2091 kilojoules, 71 g carbohydrate, 18 g fat, 17 g protein) and 117 a bolus of water (7 mL·kg⁻¹ of body mass). Following 20-min seated rest in temperate conditions 118 (19°C, 45% RH), participants completed the Profile of Mood States questionnaire¹⁹ to determine total 119 120 mood disturbance and energy index (vigour-fatigue), to detect perceived training-induced fatigue. A 121 venous blood sample was taken without stasis and total haemoglobin mass, blood volume, and plasma volume were assessed using the optimised carbon monoxide rebreathing technique.²⁰ Briefly, aliquots 122 of whole blood were used for the immediate determination of haemoglobin concentration ($g \cdot dL^{-1}$), in 123 duplicate (Hemocue, Sheffield, UK) and haematocrit (%), in triplicate (capillary tube method). Total 124

haemoglobin mass was estimated from the percentage change in carboxyhaemoglobin concentration 125 (ABL80 CO-OX Flex hemoximeter Radiometer; Copenhagen, Denmark) measured in duplicate from 126 earlobe capillary blood samples collected before and after rebreathing a mixed bolus of (0.8 mL·kg⁻¹ 127 body mass) carbon monoxide (99.9%) and oxygen (3 L, 99.5%). Total haemoglobin mass, 128 haemoglobin concentration and haematocrit (%) was used to calculate blood volume (mL; 129 [haemoglobin mass / haemoglobin concentration] \times 100) and red cell mass (mL; blood volume \times 130 [haematocrit / 100]) for the calculation of plasma volume (mL; = blood volume – red cell mass).²⁰ A 131 132 urine sample was analysed using a handheld refractometer (Atago Uricon-Ne refractometer, NSG Precision cells, New York, USA); exercise began when urine specific gravity was < 1.03.²¹ A rectal 133 134 thermistor (Henleys Medical Supplies Ltd., Herts, UK), fitted 10 cm beyond the anal sphincter, and a 135 data logger (YSI model 4000A; YSI, Dayton, Ohio, USA) provided a measure of rectal core temperature (T_{re}). A pre-exercise nude body mass was recorded using a digital platform scale (Model 136 703; Seca, Hamburg, Germany). Skin thermistors (Grant EUS-U, Cambridge, UK) were attached on 137 138 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 data logger (Grant 139 140 SQ2020, Cambridge, UK); mean skin temperature (T_{sk}) was calculated using the following four-site weighted equation: $T_{sk} = 0.3(T_{chest} + T_{arm}) + 0.2(T_{thigh} + T_{calf})^{22}$ Following instrumentation, participants 141 rested for a further 30-min in temperate conditions (19°C, 45% RH) to establish baseline measures. 142

143 At 0945-h, dressed in shorts, socks, and trainers, participants entered the environmental chamber (Delta Environmental Systems, Chester, UK; 33°C, 40% RH; 0.2 m·s⁻¹ wind velocity) to complete a 144 40-min treadmill run at 65% $\dot{V}O_{2peak}$. No fluids were consumed and T_{re} , skin temperatures, and heart 145 146 rate (Polar FT1, Polar Electro, Kempele, Finland) were monitored continuously. Local forearm sweat 147 rate was measured by dew point hygrometry. Anhydrous compressed nitrogen was passed through a 5cm² capsule affixed to the lower arm ventral surface (halfway between the antecubital fossa and 148 carpus) and connected to a hygrometry system (DS2000; Alpha Moisture Systems, UK). Local 149 150 forearm sweating rate was calculated using the difference in water content between effluent and 151 influent air and the flow rate (1 $L \cdot min^{-1}$) and normalised for the skin surface area under the capsule

(expressed in milligrams per square centimetre per minute). Tre at sweating onset was determined by 152 plotting the relationship between local forearm sweat rate and T_{re} (recorded at 20-s intervals) before 153 using segmented linear regression to identify the breakpoint in the two line segments.²³ Rating of 154 perceived exertion (RPE),²⁴ thermal sensation,²⁵ oxygen uptake (VO₂) and respiratory exchange ratio 155 (RER), assessed by the Douglas bag method, were recorded every 10-min. On completion of the 156 157 exercise, participants rested for 20-min in temperate conditions (19°C, 45% RH), during which they completed a modified Stroop test,²⁶, and provided a nude body mass to estimate whole-body sweat 158 159 rate.

Participants then re-entered the environmental chamber and completed a TTE on a motorised treadmill 160 at 65% VO_{2peak}. Participants were instructed to "run for as long as possible". TTE was terminated 161 when participants stopped running owing to volitional exhaustion, thermal discomfort, or when T_{re} 162 exceeded 39.5°C. No fluids were consumed, no feedback was provided, and Tre and heart rate were 163 monitored continuously. Following the cessation of exercise, capillary blood lactate concentrations 164 165 were assessed (Lactate Pro 2TM, Arkray, Australia) as a marker of short-term overreaching. Participants were provided with a bolus of water and were free to leave the laboratory when $T_{\rm re} \leq$ 166 38.5°C. 167

168 *Daily intervention:* Each participant completed two, three consecutive day blocks of their assigned 169 intervention (Fig. 1), during which they consumed their normal diet and fluid intake. Each day, 170 participants arrived at the laboratory (0600-h and 1300-h), fitted a rectal thermistor to monitor $T_{\rm re}$, and 171 completed a 15-min seated rest in temperate conditions. Participants commenced their assigned 172 intervention dressed in shorts, socks, and trainers. A bolus of water (5 mL·kg⁻¹ of nude body mass) 173 was consumed during the first 20-min of exercise.

174 HWI involved a 40-min treadmill run in temperate conditions (65% VO_{2peak}; 19°C, 45% RH; 0.2 m·s⁻¹

175 wind velocity) followed by a semi-recumbent \leq 40-min hot water immersion (40°C) to the neck, as

176 described.¹⁵ EHA and TNE involved a \leq 60-min treadmill run at the predetermined speed that reflected

177 65% $\dot{V}O_{2peak}$ (in temperate conditions) in hot (33°C, 40% RH; 0.2 m·s⁻¹ wind velocity) or temperate

178 conditions (19°C, 45% RH; 0.2 m·s⁻¹ wind velocity). Intervention sessions were terminated if maximal 179 immersion or exercise duration was reached, at the participant's volition, or if T_{re} exceeded 39.5°C.

Statistical Analysis: A sample size estimation (G*Power 3.1.9)²⁷ was performed using data from post-180 exercise HWI (-0.36° C),¹⁵ exercise heat acclimation (-0.22° C)²⁸ and thermoneutral exercise (0.00° C), 181 with a pooled SD of 0.2° C. A one-way analysis of variance (ANOVA; alpha = 0.05, power = 0.8, 182 correlation = 0.7) estimated that eight participants per group was required to detect a difference in the 183 184 change in end-exercise T_{re} between groups. However, following statistical advice during the review process, a two-way mixed-methods analysis of covariance (ANCOVA) was considered the more 185 appropriate and statistically powerful approach for comparing the effectiveness of interventions. To 186 ensure adequate power and allowing for dropout, nine participants per group were recruited. All data 187 were checked for normality and sphericity, presented as mean and standard deviation (SD), and 188 189 statistical significance was accepted at p<0.050. Uncertainty in the true (population) values of effects 190 is presented as 95% confidence intervals (CI). A two-way mixed model ANCOVA with baseline 191 (PRE) as the covariate was used to compare hallmark heat acclimation adaptations (e.g., end-exercise $T_{\rm re}$) across time (post three days vs. post six days) and between groups (HWI vs. EHA vs. TNE). The 192 endogenous thermal stimulus and physical activity during each of the daily interventions was 193 194 compared using a two-way mixed model analysis of variance (ANOVA). Bonferroni-adjusted pairwise 195 comparisons were used where appropriate to determine where differences occurred. The magnitude of 196 effect was reported using Cohen's d, where 0.2, 0.5, and 0.8 represent small, medium, and large effects, respectively.²⁹ Pearson's correlations determined the strength of the relationship between 197 hallmark adaptations and changes in TTE. To assess endogenous thermal stimulus, the AUC was 198 199 performed on the daily intervention $T_{\rm re}$ (time and magnitude $T_{\rm re}$ was >38.5°C) in each group using the trapezoid method.³⁰ A statistically meaningful change in end-exercise $T_{\rm re}$ was defined as $-0.34^{\circ}{\rm C}$ 200 based on the large beneficial effect observed in a recent meta-analysis.¹¹ Data were analysed using 201 SPSS version 27 (IBM Corporation, NY, USA), or GraphPad Prism Version 9 (GraphPad Software 202 Inc. La Jolla, USA). 203

205 **RESULTS**

206 Daily intervention: All participants completed six days of their assigned intervention. Differences in

207 the daily endogenous thermal stimulus were observed between groups (main effect of group, f=29.756,

208 p<0.001; Supplement A), for example, mean daily AUC for $T_{\rm re} > 38.5^{\circ}$ C was similar in HWI and

209 EHA (HWI, $17 \pm 3^{\circ}$ C·min⁻¹; EHA, $17 \pm 7^{\circ}$ C·min⁻¹; p=1.000) but lower in TNE ($2 \pm 3^{\circ}$ C·min⁻¹;

- 210 p<0.001; Supplement A). The daily endogenous thermal stimulus was maintained throughout the six-
- day intervention (main effect of time, f=0.035, p=0.853; interaction effect, f=1.019, p=0.376), owing
- to an increase (main effect of time, f=7.897, p=0.010) in mean daily HWI (days 1-3, 31 ± 6 min; days
- 213 6-8, 35 ± 5 min) and EHA duration (days 1-3, 49 ± 9 min; days 6-8, 54 ± 8 min). Following a
- significant main effect of group (f=4.315, p=0.025), post hoc pairwise comparisons revealed that total
- external work was lower in HWI (37 ± 6 km) compared to EHA (48 ± 9 km, p=0.026), but no

differences were detected between TNE (45 ± 9 km) and EHA (p=1.000), or between TNE and HWI

217 (p=0.169). No differences were observed for daily physical activity time (> 3 METS) throughout the

study protocol, evidenced by no main effects of time or group, and no interaction effect (all $p \ge 0.423$;

219 Supplement A).

- 220 Hallmark heat acclimation adaptations: No differences were detected between groups for sleep
- duration (6 ± 1 h), sleep efficiency (86 ± 9%) or urine specific gravity (1.019 ± 0.007) before
- experimental trials, evidenced by no main effects of time or group, and no interaction effects (all
- 223 $p \ge 0.336$). A two-way mixed model ANCOVA, with baseline as the covariate, detected a main effect
- of group for resting T_{re} (*f*=6.438, p=0.006, Fig. 2A and C), end-exercise T_{re} (*f*=5.299, p=0.013, Fig. 2B
- and D), T_{re} at sweating onset (f=7.633, p=0.003), and whole-body sweat rate (f=7.633, p=0.001,
- Supplement B); there were no main effects of time or interaction effects (all $p \ge 0.144$). Post hoc
- pairwise comparisons revealed that HWI elicited a larger reduction in resting $T_{\rm re}$ (baseline-adjusted:
- 228 $-0.38 \pm 0.23^{\circ}$ C, CI: -0.26 to -0.49° C, d=1.6) compared to EHA ($-0.14 \pm 0.23^{\circ}$ C, CI: -0.03 to
- 229 -0.26° C, p=0.021, d=0.6) and TNE ($-0.12 \pm 0.23^{\circ}$ C, CI: -0.01 to -0.24° C, p=0.011, d=0.5; Fig. 2A).
- Similarly, the reduction in end-exercise $T_{\rm re}$ was larger following HWI (-0.47 ± 0.23 °C, CI: -0.36 to
- 231 -0.58° C, d=2.1) compared to EHA ($-0.26 \pm 0.24^{\circ}$ C, CI: -0.15 to -0.38° C, p=0.042, d=1.1) and TNE

232	$(-0.25 \pm 0.23$ °C, CI: -0.14 to -0.37 °C; p=0.025, d=1.1; Fig. 2B). Furthermore, HWI elicited a
233	statistically meaningful decrease in end-exercise T_{re} (i.e. $\geq 0.34^{\circ}$ C reduction) ¹¹ after only three days.
234	No differences were observed between EHA and TNE for resting T_{re} (p=1.000) or end-exercise T_{re}
235	(p=1.000). $T_{\rm re}$ at sweating onset was reduced in accordance with resting $T_{\rm re}$, with reductions being
236	larger following HWI compared with EHA (p=0.011) and TNE (p=0.005; Supplement B); no
237	differences were observed between EHA and TNE (p=1.000). Whole-body sweat rate was greater
238	following HWI (p=0.001) and EHA (p=0.009) compared to TNE, but no difference was detected
239	between HWI and EHA (p=0.950; Supplement B). The change in T_{re} during the 40-min treadmill-run
240	in the heat was lower at POST6 compared to at POST3, evidenced by a main effect of time (f=4.444,
241	p=0.046, Supplement B); however, no differences were detected between groups (main effect of
242	group, f=1.046, p=0.368; interaction effect, f=1.046, p=0.368). No main effects of time or group, and
243	no interaction effects were detected for end-exercise T_{sk} , heart rate, physiological strain index, RPE,
244	thermal sensation, plasma volume, blood volume, total haemoglobin mass, mean $\dot{V}O_2$ or mean RER
245	(all p≥0.154; Supplement B).

246 ***Fig. 2 near here***

247 Seven participants were removed from the TTE analysis owing to: reaching the $T_{\rm re}$ ethical cut-off 248 (HWI, n=2); going to the toilet (EHA, n=1); lower limb discomfort (TNE, n=1); exercise-induced 249 bronchoconstriction (TNE, n=1); nausea (TNE, n=1); and an obvious lack of effort without markers of 250 overreaching at rest (TNE, n=1). No main effects of time or group, and no interaction effects (all $p \ge 0.416$) were observed in the remaining 20 participants who completed the TTE protocol (7 HWI; 8 251 252 EHA; 5 TNE; Supplement C). Correlational analysis revealed that the change in TTE was associated with the magnitude of adaptation in end-exercise T_{re} (r = -0.47, p=0.019), end-exercise physiological 253 strain index (r = -0.54, p=0.008), and whole-body sweat rate (r = 0.49, p=0.013). There were no main 254 255 effects of time or group, and no interaction effects detected for markers of short-term overreaching (all p≥0.172), including: mood disturbance, energy index, Stroop reaction time, Stroop accuracy, end-TTE 256 heart rate, end-TTE blood lactate, and sleep efficiency. Although interestingly, the three EHA 257 participants who experienced no improvement or a decline in endurance capacity (Supplement C) 258

- showed some signs of overreaching, evidenced by an increase in total mood disturbance (+29), and
- decreases in energy index (-15), sleep efficiency (-13%), and Stroop accuracy (-3%).

262 DISCUSSION

263 The current study sought to compare adaptations after three and six days of post-exercise HWI and 264 EHA in recreationally active males. In addition, the individual contributions of daily submaximal exercise and heat stress to the adaptations following EHA were investigated. The novel finding is that 265 short-term post-exercise HWI elicits larger thermal adaptations compared with short-term EHA. For 266 example, resting $T_{\rm re}$ was lower following HWI (-0.38°C) compared to EHA (-0.14°C), which 267 268 translated to a lower end-exercise $T_{\rm re}$ (-0.47°C) during exercise-heat-stress. Despite a similar daily endogenous thermal stimulus during HWI and EHA (Supplement A), the benefits of exercising in the 269 heat beyond exercising in temperate conditions appear modest (end-exercise T_{re} reduction: EHA, 270 -0.26°C; TNE, -0.25°C). 271

Post-exercise HWI initiated a large reduction in resting T_{re} (-0.38°C), which accounted for most of the 272 reduction in end-exercise $T_{\rm re}$ during exercise-heat-stress (~81%). The induction of a large reduction in 273 274 resting $T_{\rm re}$ following HWI is likely due to exposure to a large dual thermal stimulus (average endimmersion $T_{\rm re}$ was 39.3°C and $T_{\rm sk}$ was 40°C), which is purported to induce a more complete state of 275 heat acclimation.¹⁷ We contend that this dual thermal stimulus is necessary for meaningful heat 276 acclimation adaptations to arise; a recent post-exercise HWI study eliciting an end-intervention $T_{\rm re}$ of 277 only 38.4°C observed no further benefit compared to exercise in the heat alone.³¹ Furthermore, the 278 279 HWI protocol in the current study likely elicited a greater peripheral stimulus as skin temperature was 280 continuously elevated for the whole immersion duration. In the present study, EHA had no effect on resting $T_{\rm re}$ beyond that of exercise in temperate conditions, despite eliciting a similar endogenous 281 282 thermal stimulus to HWI. The larger reductions in resting T_{re} , end-exercise T_{re} and T_{re} at sweating 283 onset after HWI compared to EHA are likely due to a higher skin temperature during the daily intervention (40°C vs. \sim 35°C). This finding is supported by previous work that demonstrated an 284 accelerated rate of phenotypic adaptation when a high skin temperature was employed in conjunction 285 with conventional exercise heat acclimation.¹⁸ Research has linked the repeated elevation in skin 286 temperature and activation of warm-sensitive neurons to the induction of hypothalamic neural network 287 changes that reduce resting core temperature.³² Accordingly, the induction of meaningful heat 288

acclimation benefits is dependent upon the magnitude of both the endogenous thermal stimulus and
skin temperature.¹⁷ In addition, HWI may elicit haematological adaptations (e.g., plasma volume
expansion) distinct from conventional exercise heat acclimation;³³ however, no differences were
observed in plasma volume following the interventions.

The inclusion of a work-matched thermoneutral exercise intervention allows for insights into the 293 individual influence of daily submaximal exercise and heat stress on adaptations in recreationally 294 active participants. For example, after adjusting for baseline, ~96% of the reduction in end-exercise $T_{\rm re}$ 295 following six days of EHA, was observed from daily thermoneutral exercise alone (-0.26°C vs. 296 -0.25°C). Aligning with previous research,¹¹ EHA had a larger effect on increasing whole-body sweat 297 rate compared to daily exercise in temperate conditions, but this did not translate to an improvement in 298 endurance capacity in the heat. It is however worth noting that three EHA participants experienced 299 300 either a decline or no change in TTE, coinciding with evidence of short-term overreaching (e.g. total mood disturbance and sleep efficiency).³⁴ The combined stressors of daily exercise-heat-stress could 301 302 have exhibited these abnormal training responses within our recreationally active population.³⁴ As such, interventions should be undertaken with caution, while ensuring adequate time for recovery to 303 minimise fatigue and ensure adaptations are fully realised.⁶ 304

305 The reduction in end-exercise $T_{\rm re}$ following six days of post-exercise HWI in the present study (-0.47°C) exceeds that previously reported (-0.36°C) .¹⁵ This is likely explained by the lower aerobic 306 fitness of the participants in the present study (53 ml·kg⁻¹·min⁻¹ vs. 61 ml·kg⁻¹·min⁻¹);¹⁴ endurance-307 trained individuals are considered partially heat acclimatised and to have a reduced adaptation 308 potential.^{7,8} Furthermore, the current study involved an additional exercise-heat exposure 309 310 (experimental trial) and rest-day after three days (Fig. 1). It is possible that previous research, adopting a limited recovery period between the final heat acclimation bout and the exercise-heat-stress test, may 311 underplay heat acclimation benefits, as the full effects may manifest after adequate recovery.³⁵ 312

While the data clearly demonstrate heat acclimation adaptations, findings on the effectiveness of theinterventions for improving TTE should be considered with caution owing to the small sample size. As

such, future work with a larger sample size is required to confirm (or reject) whether post-exercise HWI provides favourable improvements in endurance capacity in the heat compared to EHA. We also recognise that while the approach used in the current study allowed us to examine the importance of the dual thermal stimulus for heat acclimation adaptation, mean body temperature (calculated from core and skin temperature) was likely higher during HWI compared to EHA. Hence, further research is required to determine whether post-exercise HWI compares favourably to EHA when mean body temperature is matched.

322 Conclusion

323 Short-term post-exercise HWI intervention elicited larger thermal adaptations compared with

324 conventional short-term exercise heat acclimation. In addition, the thermal benefits of conventional

325 short-term exercise heat acclimation beyond exercising in temperate conditions appear modest.

326 Practical Implications

Conventional short-term fixed intensity exercise heat acclimation initiates inconsistent and
 relatively modest thermal adaptations.

Short-term post-exercise hot bath intervention initiates larger thermal adaptations compared with
 conventional short-term exercise heat acclimation.

• Taking a hot bath submerged to the neck, for up to 40 min, following habitual training in

temperate conditions, presents a practical and economical heat acclimation intervention —

- eliminating the requirement for an increased training load, access to an environmental chamber orrelocation to a hot climate.
- To facilitate adaptations from the post-exercise hot bath intervention, exposure to a large dual
- thermal stimulus (i.e. maintained elevation in both core temperature > 38.5°C and skin
- temperature $\sim 40^{\circ}$ C) is required.

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433 FIGURE LEGENDS

434

Fig. 1. Schematic of study design. HWI; post-exercise hot water immersion, EHA; exercise heat
acclimation and TNE; work-matched thermoneutral exercise.

- 438 Fig. 2. Influence of three (POST3) and six days (POST6) of thermoneutral exercise (TNE), exercise
- 439 heat acclimation (EHA), or post-exercise hot water immersion (HWI) on resting rectal core
- temperature (T_{re}) (A) and end-exercise T_{re} following a 40-min treadmill run at 65% $\dot{V}O_{2peak}$ in the heat
- 441 (33°C, 40% RH) (B). Bars represent the baseline-adjusted mean change from baseline; circles
- 442 represent individual participant responses.





444 Fig. 1. Schematic of study design. HWI; post-exercise hot water immersion, EHA; exercise heat acclimation and TNE; work-matched thermoneutral exercise.



446 Fig. 2. Influence of three (POST3) and six days (POST6) of thermoneutral exercise (TNE), exercise heat acclimation (EHA), or post-exercise hot water

- 447 immersion (HWI) on resting rectal core temperature (T_{re}) (A) and end-exercise T_{re} following a 40-min treadmill run at 65% $\dot{V}O_{2peak}$ in the heat (33°C, 40%)
- 448 RH) (B). Bars represent the baseline-adjusted mean change from baseline; circles represent individual participant responses.

449 Supplementary material

450

451 Supplement A The daily thermal stimulus for adaptation and physical activity during days 1–3 and days 6–8 of thermoneutral exercise (TNE), exercise heat

452 acclimation (EHA) and post-exercise hot water immersion (HWI).

	1–3				4–6			
	TNE	EHA	HWI	TNE	ЕНА	HWI		
Duration $T_{\rm re} \ge 38.5^{\circ}$ C (min) ^{##}	9 ± 15	36 ± 12 §§	$35\pm5\ ^{\$\$}$	7 ± 11	$37\pm10^{~\$\$}$	$39\pm7~^{\$\$}$		
AUC (°C·min ⁻¹) ##	2 ± 5	$18\pm9~^{\$\$}$	16 ± 4	2 ± 4	$16\pm7~^{\$\$}$	18 ± 3 §§		
End intervention $T_{\rm re}$ (°C) ^{##}	38.2 ± 0.5	39.2 ± 0.3 §§	39.2 ± 0.2 §§	38.2 ± 0.5	39.2 ± 0.2 §§	39.3 ± 0.1		
Physical activity > 3 METS (min)	121 ± 80	138 ± 81	138 ± 55	120 ± 46	125 ± 43	135 ± 66		

 T_{re} ; rectal core temperature, AUC; area under the curve for $T_{re} > 38.5$ °C. Data are displayed as mean \pm SD of days 1–3 and days 6–8. ^{##}p<0.01 denotes main effect of group; $\frac{\delta\delta}{\delta}p$ <0.01 denotes group different from TNE overall.

454 Supplement B Influence of three (POST3) and six days (POST6) of thermoneutral exercise (TNE), exercise heat acclimation (EHA) or post-exercise hot water

455	immersion (HWI) on heat acclimation ada	ptations at rest and during	a 40-min treadmill run at (65%	_{ak} in the heat (33°C, 40% RF	H).
			8		2pec	AIL () -	

		TNE			EHA			HWI	
	PRE	POST3	POST6	PRE	POST3	POST6	PRE	POST3	POST6
Resting $T_{\rm re}$ (°C) ^{##}	37.05 ± 0.25	36.97 ± 0.19	36.94 ± 0.22	37.08 ± 0.29	36.96 ± 0.19	36.90 ± 0.22	37.10 ± 0.24	$36.72\pm0.19^{\dagger,\$}$	$36.68\pm0.22^{\dagger,\$}$
End-exercise T_{re} (°C) [#]	38.68 ± 0.49	38.62 ± 0.26	38.52 ± 0.21	38.98 ± 0.31	38.58 ± 0.26	38.53 ± 0.21	38.80 ± 0.27	$38.44\pm0.26^{\dagger,\$}$	$38.25\pm0.20^{\dagger,\$}$
$T_{\rm re}$ at sweating onset (°C) ^{##}	36.99 ± 0.25	36.92 ± 0.18	36.89 ± 0.20	37.03 ± 0.32	36.93 ± 0.18	36.83 ± 0.20	37.08 ± 0.27	$36.70 \pm 0.18^{\dagger,}_{_{\S\S}}$	$36.58 \pm 0.20^{\dagger,}$
$\Delta T_{\rm re}$ during exercise (°C) *	1.63 ± 0.49	1.66 ± 0.26	1.58 ± 0.28	1.91 ± 0.19	1.62 ± 0.26	1.64 ± 0.29	1.69 ± 0.31	1.72 ± 0.25	1.57 ± 0.28
Whole-body sweat rate $(L \cdot h^{-1})^{\#\#}$	0.84 ± 0.12	0.85 ± 0.08	0.83 ± 0.06	0.91 ± 0.16	$0.94\pm0.08^{\$\$}$	$0.93\pm0.06^{\$\$}$	0.92 ± 0.20	$0.95\pm0.08^{\text{SS}}$	$0.97\pm0.06^{\text{SS}}$
End-exercise $T_{\rm sk}$ (°C)	35.63 ± 0.62	35.15 ± 0.49	35.06 ± 0.45	35.78 ± 0.79	35.22 ± 0.50	34.97 ± 0.46	35.19 ± 0.62	34.91 ± 0.51	34.86 ± 0.47
End-exercise heart rate (beats min ⁻¹)	182 ± 16	177 ± 6	173 ± 6	189 ± 11	175 ± 6	170 ± 6	183 ± 11	173 ± 6	167 ± 6
End-exercise physiological strain	7.6 ± 1.2	7.4 ± 0.6	7.1 ± 0.5	8.5 ± 0.9	7.2 ± 0.6	7.0 ± 0.5	8.0 ± 0.4	7.1 ± 0.5	6.6 ± 0.5
Plasma volume (ml)	3031 ± 365	3139 ± 269	3197 ± 228	3203 ± 248	3287 ± 270	3314 ± 228	3108 ± 659	3169 ± 268	3249 ± 227
Blood volume (ml)	5647 ± 604	5711 ± 343	5771 ± 328	5625 ± 755	5864 ± 347	5930 ± 331	5688 ± 853	5819 ± 344	5925 ± 329
Total haemoglobin mass (g)	861 ± 90	847 ± 49	851 ± 46	847 ± 103	878 ± 50	869 ± 46	851 ± 125	899 ± 49	843 ± 46
Mean $\dot{V}O_2$ (L·min ⁻¹)	2.72 ± 0.26	2.65 ± 0.13	2.65 ± 0.15	3.01 ± 0.35	2.98 ± 0.13	2.96 ± 0.15	2.90 ± 0.50	2.86 ± 0.13	2.86 ± 0.14
Mean RER	0.92 ± 0.03	0.91 ± 0.04	0.91 ± 0.03	0.92 ± 0.08	0.93 ± 0.05	0.92 ± 0.03	0.93 ± 0.04	0.92 ± 0.05	0.93 ± 0.03
End-exercise RPE (6-20 scale)	16 ± 2	15 ± 1	15 ± 0	15 ± 3	14 ± 1	14 ± 0	15 ± 2	14 ± 1	14 ± 0
End-exercise thermal sensation (1–13 scale)	11 ± 1	11 ± 0	11 ± 0	11 ± 1	10 ± 0	10 ± 0	10 ± 1	10 ± 0	10 ± 0
Time to exhaustion (s)	1300 ± 349	1327 ± 497	1395 ± 664	1156 ± 423	1342 ± 515	1512 ± 687	1821 ± 936	2031 ± 532	2466 ± 710

Tre, rectal core temperature; Tsk, mean skin temperature; RER, respiratory exchange ratio; RPE, rating of perceived exertion. Data are mean \pm SD at PRE and baselineadjusted mean \pm SD at POST3 and POST6. *p<0.05 denotes main effect of time; #p<0.05, ##p<0.01; denotes main effect of group; [†]p<0.05 denotes HWI different from EHA overall; [§]p<0.05, ^{§§}p<0.01 denotes group different from TNE overall.



458 **Supplement C** Influence of three (POST3) and six days (POST6) of thermoneutral exercise (TNE, n = 5), exercise heat acclimation (EHA, n = 8) or post-459 exercise hot water immersion (HWI, n = 7) on treadmill (65% \dot{VO}_{2peak}) time to exhaustion (TTE) in the heat (33°C, 40% RH). Bars represent the baseline-460 adjusted mean change (A) and percentage change (B) from baseline; circles represent individual participant responses.