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3

4 **Title**

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

6

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

38 Results: ANCOVA, using baseline values as the covariate, revealed no interaction effects but
39 significant group effects demonstrated that compared to EHA, HWI elicited larger reductions in
40 resting rectal temperature (T_{re} ; $p=0.021$), T_{re} at sweating onset ($p=0.011$), and end-exercise T_{re} during
41 exercise-heat-stress (-0.47°C ; $p=0.042$). Despite a similar endogenous thermal stimulus to HWI, EHA
42 elicited a modest reduction in end-exercise T_{re} (-0.26°C), which was not different from TNE
43 (-0.25°C , $p=1.000$). There were no main effects or interaction effects for end-exercise T_{sk} , heart rate,
44 physiological strain index, RPE, thermal sensation, plasma volume, or TTE (all $p\geq 0.154$).

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.

48

49 INTRODUCTION

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

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

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

75 by including a work-matched thermoneutral exercise intervention (TNE), we investigated the
76 individual contributions of daily submaximal exercise and heat stress to adaptation following EHA.
77 We hypothesised that post-exercise HWI would accelerate the speed of adaptation compared to EHA,
78 and that the benefits of EHA beyond that of TNE would be modest.

79

80 METHODS

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

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

	HWI	EHA	TNE
Age (years)	22 \pm 3	20 \pm 2	21 \pm 2
Height (cm)	177 \pm 5	181 \pm 5	178 \pm 6
Body mass (kg)	73 \pm 7	74 \pm 7	70 \pm 7
$\dot{V}\text{O}_{2\text{peak}}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	53 \pm 6	54 \pm 3	53 \pm 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
101 experimental trial. $\dot{V}O_{2peak}$ was assessed using a continuous maximal incremental exercise test
102 performed on a motorised treadmill (HP Cosmos Mercury 4.0, Nussdorf-Traunstein, Germany) in a
103 temperate laboratory (19°C) to volitional exhaustion. $\dot{V}O_{2peak}$ was determined as the highest oxygen
104 uptake attained over a 30-s period. A running speed that elicited 65% $\dot{V}O_{2peak}$ in temperate conditions
105 was determined by the interpolation of the running speed– $\dot{V}O_2$ relationship. Participants were then
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).

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

116 On the day of the experimental trials (Fig. 1), participants arrived at the laboratory at 0730-h and were
117 provided with a standardised breakfast (2091 kilojoules, 71 g carbohydrate, 18 g fat, 17 g protein) and
118 a bolus of water (7 mL·kg⁻¹ of body mass). Following 20-min seated rest in temperate conditions
119 (19°C, 45% RH), participants completed the Profile of Mood States questionnaire¹⁹ to determine total
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
122 volume were assessed using the optimised carbon monoxide rebreathing technique.²⁰ Briefly, aliquots
123 of whole blood were used for the immediate determination of haemoglobin concentration (g·dL⁻¹), in
124 duplicate (Hemocue, Sheffield, UK) and haematocrit (%), in triplicate (capillary tube method). Total

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

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

152 (expressed in milligrams per square centimetre per minute). T_{re} at sweating onset was determined by
153 plotting the relationship between local forearm sweat rate and T_{re} (recorded at 20-s intervals) before
154 using segmented linear regression to identify the breakpoint in the two line segments.²³ Rating of
155 perceived exertion (RPE),²⁴ thermal sensation,²⁵ oxygen uptake ($\dot{V}O_2$) and respiratory exchange ratio
156 (RER), assessed by the Douglas bag method, were recorded every 10-min. On completion of the
157 exercise, participants rested for 20-min in temperate conditions (19°C, 45% RH), during which they
158 completed a modified Stroop test,²⁶ and provided a nude body mass to estimate whole-body sweat
159 rate.

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

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_{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% $\dot{V}O_{2peak}$; 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.

180 *Statistical Analysis:* A sample size estimation (G*Power 3.1.9)²⁷ was performed using data from post-
181 exercise HWI (-0.36°C),¹⁵ exercise heat acclimation (-0.22°C)²⁸ and thermoneutral exercise (0.00°C),
182 with a pooled SD of 0.2°C. A one-way analysis of variance (ANOVA; alpha = 0.05, power = 0.8,
183 correlation = 0.7) estimated that eight participants per group was required to detect a difference in the
184 change in end-exercise T_{re} between groups. However, following statistical advice during the review
185 process, a two-way mixed-methods analysis of covariance (ANCOVA) was considered the more
186 appropriate and statistically powerful approach for comparing the effectiveness of interventions. To
187 ensure adequate power and allowing for dropout, nine participants per group were recruited. All data
188 were checked for normality and sphericity, presented as mean and standard deviation (SD), and
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
192 T_{re}) across time (post three days vs. post six days) and between groups (HWI vs. EHA vs. TNE). The
193 endogenous thermal stimulus and physical activity during each of the daily interventions was
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
197 effects, respectively.²⁹ Pearson's correlations determined the strength of the relationship between
198 hallmark adaptations and changes in TTE. To assess endogenous thermal stimulus, the AUC was
199 performed on the daily intervention T_{re} (time and magnitude T_{re} was >38.5°C) in each group using the
200 trapezoid method.³⁰ A statistically meaningful change in end-exercise T_{re} was defined as -0.34°C
201 based on the large beneficial effect observed in a recent meta-analysis.¹¹ Data were analysed using
202 SPSS version 27 (IBM Corporation, NY, USA), or GraphPad Prism Version 9 (GraphPad Software
203 Inc. La Jolla, USA).

204

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 $T_{re} > 38.5^{\circ}\text{C}$ was similar in HWI and
209 EHA (HWI, $17 \pm 3^{\circ}\text{C}\cdot\text{min}^{-1}$; EHA, $17 \pm 7^{\circ}\text{C}\cdot\text{min}^{-1}$; $p=1.000$) but lower in TNE ($2 \pm 3^{\circ}\text{C}\cdot\text{min}^{-1}$;
210 $p<0.001$; Supplement A). The daily endogenous thermal stimulus was maintained throughout the six-
211 day intervention (main effect of time, $f=0.035$, $p=0.853$; interaction effect, $f=1.019$, $p=0.376$), owing
212 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
214 significant main effect of group ($f=4.315$, $p=0.025$), *post hoc* pairwise comparisons revealed that total
215 external work was lower in HWI (37 ± 6 km) compared to EHA (48 ± 9 km, $p=0.026$), but no
216 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
218 study protocol, evidenced by no main effects of time or group, and no interaction effect (all $p\geq 0.423$;
219 Supplement A).

220 *Hallmark heat acclimation adaptations:* No differences were detected between groups for sleep
221 duration (6 ± 1 h), sleep efficiency ($86 \pm 9\%$) or urine specific gravity (1.019 ± 0.007) before
222 experimental trials, evidenced by no main effects of time or group, and no interaction effects (all
223 $p\geq 0.336$). A two-way mixed model ANCOVA, with baseline as the covariate, detected a main effect
224 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
225 and D), T_{re} at sweating onset ($f=7.633$, $p=0.003$), and whole-body sweat rate ($f=7.633$, $p=0.001$,
226 Supplement B); there were no main effects of time or interaction effects (all $p\geq 0.144$). *Post hoc*
227 pairwise comparisons revealed that HWI elicited a larger reduction in resting T_{re} (baseline-adjusted:
228 $-0.38 \pm 0.23^{\circ}\text{C}$, CI: -0.26 to -0.49°C , $d=1.6$) compared to EHA ($-0.14 \pm 0.23^{\circ}\text{C}$, CI: -0.03 to
229 -0.26°C , $p=0.021$, $d=0.6$) and TNE ($-0.12 \pm 0.23^{\circ}\text{C}$, CI: -0.01 to -0.24°C , $p=0.011$, $d=0.5$; Fig. 2A).
230 Similarly, the reduction in end-exercise T_{re} was larger following HWI ($-0.47 \pm 0.23^{\circ}\text{C}$, CI: -0.36 to
231 -0.58°C , $d=2.1$) compared to EHA ($-0.26 \pm 0.24^{\circ}\text{C}$, CI: -0.15 to -0.38°C , $p=0.042$, $d=1.1$) and TNE

232 $(-0.25 \pm 0.23^{\circ}\text{C}$, 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. $\geq 0.34^{\circ}\text{C}$ 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_{re} at sweating onset was reduced in accordance with resting T_{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 $\dot{V}\text{O}_2$ or mean RER
245 (all $p \geq 0.154$; Supplement B).

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

247 Seven participants were removed from the TTE analysis owing to: reaching the T_{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
251 $p \geq 0.416$) were observed in the remaining 20 participants who completed the TTE protocol (7 HWI; 8
252 EHA; 5 TNE; Supplement C). Correlational analysis revealed that the change in TTE was associated
253 with the magnitude of adaptation in end-exercise T_{re} ($r = -0.47$, $p=0.019$), end-exercise physiological
254 strain index ($r = -0.54$, $p=0.008$), and whole-body sweat rate ($r = 0.49$, $p=0.013$). There were no main
255 effects of time or group, and no interaction effects detected for markers of short-term overreaching (all
256 $p \geq 0.172$), including: mood disturbance, energy index, Stroop reaction time, Stroop accuracy, end-TTE
257 heart rate, end-TTE blood lactate, and sleep efficiency. Although interestingly, the three EHA
258 participants who experienced no improvement or a decline in endurance capacity (Supplement C)

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

261

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
265 exercise and heat stress to the adaptations following EHA were investigated. The novel finding is that
266 short-term post-exercise HWI elicits larger thermal adaptations compared with short-term EHA. For
267 example, resting T_{re} was lower following HWI (-0.38°C) compared to EHA (-0.14°C), which
268 translated to a lower end-exercise T_{re} (-0.47°C) during exercise-heat-stress. Despite a similar daily
269 endogenous thermal stimulus during HWI and EHA (Supplement A), the benefits of exercising in the
270 heat beyond exercising in temperate conditions appear modest (end-exercise T_{re} reduction: EHA,
271 -0.26°C ; TNE, -0.25°C).

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

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

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

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

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

315 such, future work with a larger sample size is required to confirm (or reject) whether post-exercise
316 HWI provides favourable improvements in endurance capacity in the heat compared to EHA. We also
317 recognise that while the approach used in the current study allowed us to examine the importance of
318 the dual thermal stimulus for heat acclimation adaptation, mean body temperature (calculated from
319 core and skin temperature) was likely higher during HWI compared to EHA. Hence, further research
320 is required to determine whether post-exercise HWI compares favourably to EHA when mean body
321 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**

- 327 • Conventional short-term fixed intensity exercise heat acclimation initiates inconsistent and
328 relatively modest thermal adaptations.
- 329 • Short-term post-exercise hot bath intervention initiates larger thermal adaptations compared with
330 conventional short-term exercise heat acclimation.
- 331 • Taking a hot bath submerged to the neck, for up to 40 min, following habitual training in
332 temperate conditions, presents a practical and economical heat acclimation intervention —
333 eliminating the requirement for an increased training load, access to an environmental chamber or
334 relocation to a hot climate.
- 335 • To facilitate adaptations from the post-exercise hot bath intervention, exposure to a large dual
336 thermal stimulus (i.e. maintained elevation in both core temperature $> 38.5^{\circ}\text{C}$ and skin
337 temperature $\sim 40^{\circ}\text{C}$) is required.

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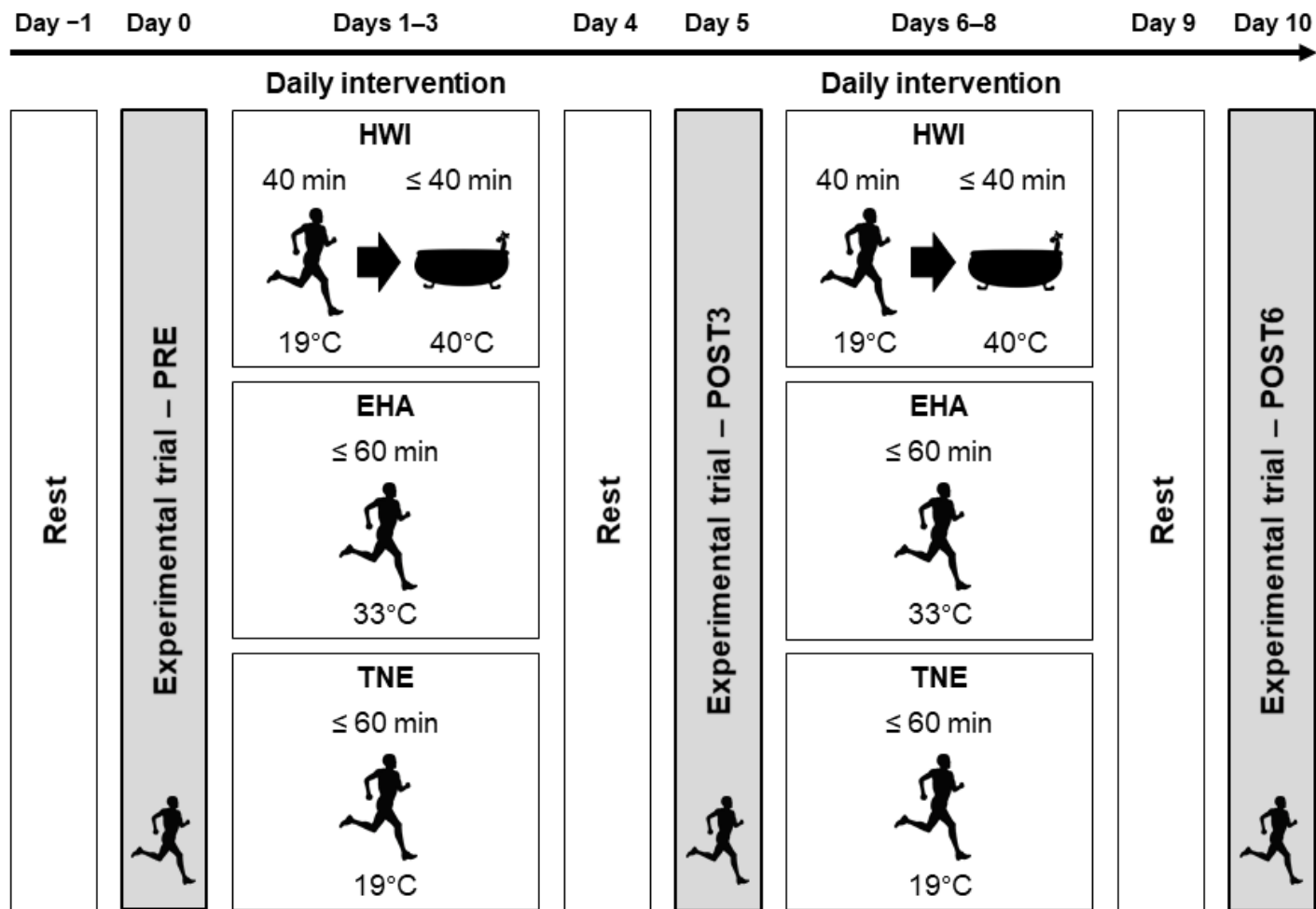
433 **FIGURE LEGENDS**

434

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

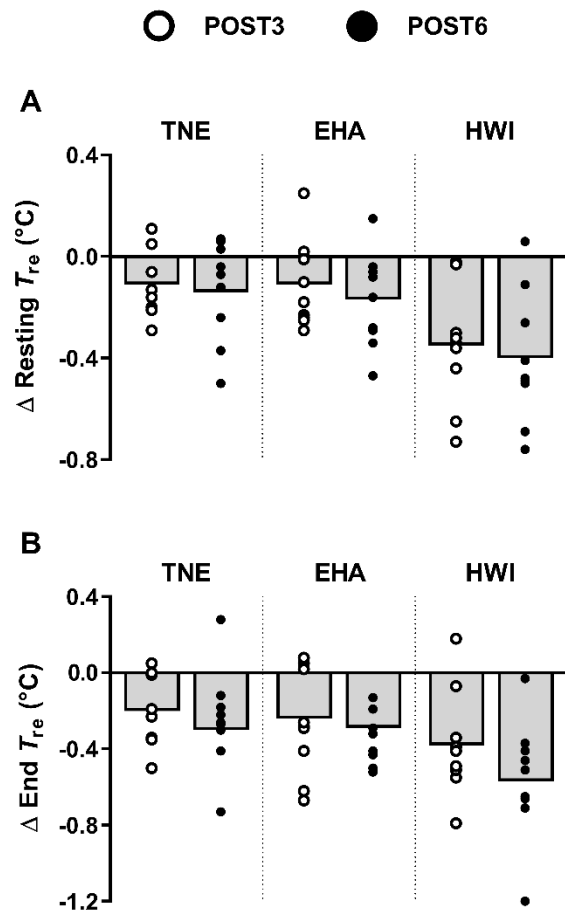
437

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
440 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.



443

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



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Fig. 2. Influence of three (POST3) and six days (POST6) of thermoneutral exercise (TNE), exercise 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 (33°C, 40% 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	EHA	HWI
Duration $T_{re} \geq 38.5^{\circ}\text{C}$ (min) ^{##}	9 ± 15	36 ± 12 ^{\$\$}	35 ± 5 ^{\$\$}	7 ± 11	37 ± 10 ^{\$\$}	39 ± 7 ^{\$\$}
AUC ($^{\circ}\text{C}\cdot\text{min}^{-1}$) ^{##}	2 ± 5	18 ± 9 ^{\$\$}	16 ± 4 ^{\$\$}	2 ± 4	16 ± 7 ^{\$\$}	18 ± 3 ^{\$\$}
End intervention T_{re} ($^{\circ}\text{C}$) ^{##}	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

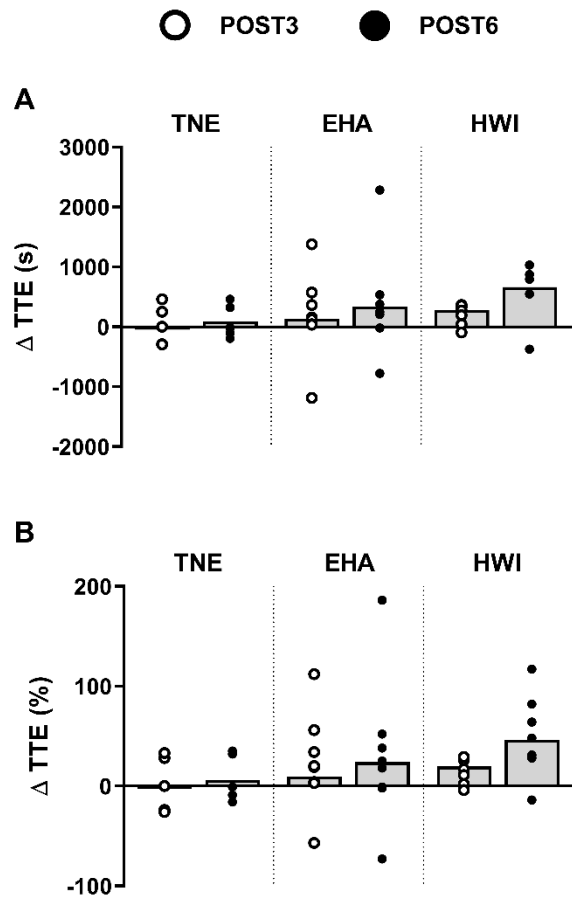
T_{re}; rectal core temperature, AUC; area under the curve for $T_{re} > 38.5^{\circ}\text{C}$. Data are displayed as mean ± SD of days 1–3 and days 6–8. ^{##} $p < 0.01$ denotes main effect of group; ^{\$\$} $p < 0.01$ denotes group different from TNE overall.

453

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 adaptations at rest and during a 40-min treadmill run at 65% $\dot{V}O_{2peak}$ in the heat (33°C, 40% RH).

	TNE			EHA			HWI		
	PRE	POST3	POST6	PRE	POST3	POST6	PRE	POST3	POST6
Resting T_{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 ± 0.19 ^{†, §}	36.68 ± 0.22 ^{†, §}
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 ± 0.26 ^{†, §}	38.25 ± 0.20 ^{†, §}
T_{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 ± 0.18 ^{†, §} §§	36.58 ± 0.20 ^{†, §} §§
ΔT_{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·h ⁻¹) ^{###}	0.84 ± 0.12	0.85 ± 0.08	0.83 ± 0.06	0.91 ± 0.16	0.94 ± 0.08 ^{§§}	0.93 ± 0.06 ^{§§}	0.92 ± 0.20	0.95 ± 0.08 ^{§§}	0.97 ± 0.06 ^{§§}
End-exercise T_{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

T_{re}, rectal core temperature; *T_{sk}*, mean skin temperature; *RER*, respiratory exchange ratio; *RPE*, rating of perceived exertion. Data are mean ± SD at PRE and baseline-adjusted mean ± 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.



457

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 ($65\% \dot{V}O_{2\text{peak}}$) time to exhaustion (TTE) in the heat (33°C , $40\% \text{RH}$). Bars represent the baseline-
 460 adjusted mean change (A) and percentage change (B) from baseline; circles represent individual participant responses.