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1 **Title**

2 A comparison of medium-term heat acclimation by post-exercise hot water immersion or
3 exercise in the heat: Adaptations, overreaching, and thyroid hormones

4

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17

18 **Running head**

19 Post-exercise hot water immersion adaptations and mechanisms

20 **Abstract**

21 This research compared thermal and perceptual adaptations, endurance capacity, and
22 overreaching markers in men after 3, 6, and 12-days of post-exercise hot water immersion
23 (HWI) or exercise heat acclimation (EHA) with a temperate exercise control (CON), and
24 examined thyroid hormones as a mechanism for the reduction in resting and exercising core
25 temperature (T_{re}) after HWI. HWI involved a treadmill run at 65% $\dot{V}O_{2peak}$ in 19°C followed
26 by a 40°C bath. EHA and CON involved a work-matched treadmill run at 65% $\dot{V}O_{2peak}$ in 33°C
27 or 19°C, respectively. Compared with CON, resting mean body temperature (T_b), resting and
28 end-exercise T_{re} , T_{re} at sweating onset, thermal sensation and perceived exertion were lower
29 and whole-body sweat rate (WBSR) was higher after 12-days of HWI (all $P \leq 0.049$, resting
30 T_b : CON $-0.11 \pm 0.15^\circ\text{C}$, HWI $-0.41 \pm 0.15^\circ\text{C}$). Moreover, resting T_b and T_{re} at sweating onset
31 were lower after HWI than EHA ($P \leq 0.015$, resting T_b : EHA $-0.14 \pm 0.14^\circ\text{C}$). No differences
32 were identified between EHA and CON ($P \geq 0.157$) except WBSR which was greater after
33 EHA ($P = 0.013$). No differences were observed between interventions for endurance capacity
34 or overreaching markers (mood, sleep, Stroop, $P \geq 0.190$). Thermal adaptations observed after
35 HWI were not related to changes in thyroid hormone concentrations ($P \geq 0.086$). In conclusion,
36 12-days of post-exercise hot water immersion conferred more complete heat acclimation than
37 exercise heat acclimation without increasing overreaching risk, and changes in thyroid
38 hormones are not related to thermal adaptations after post-exercise hot water immersion.

39

40 **Keywords**

41 Hot bath, core temperature, thermoregulation, triiodothyronine, thyroxine.

42 **Introduction**

43 It is well established that exercise in hot and hot-humid environments is detrimental to
44 endurance capacity (1, 2) and may expose individuals to the risk of exertional heat illness (3).

45 To reduce these deleterious effects of heat stress, athletes, military personnel, and occupational
46 workers should prepare by completing a period of heat acclimation (4, 5).

47

48 Previous research in both recreationally active (6) and endurance-trained individuals (7)
49 demonstrates that taking a hot bath for up to 40 min immediately after submaximal exercise in

50 temperate conditions on six consecutive days reduces resting core body temperature. This

51 reduction in resting core body temperature leads to a subsequent reduction in core body

52 temperature during exercise-heat-stress, a hallmark heat acclimation adaptation. Post-exercise

53 hot water immersion (HWI) also presents a more practical heat acclimation strategy than

54 conventional exercise heat acclimation (EHA), as it eliminates the requirement for access to an

55 environmental chamber and can be more easily incorporated into normal training and an

56 athlete's taper (8). Moreover, McIntyre *et al.* (9) recently demonstrated that despite a similar

57 endogenous thermal stimulus for adaptation, 6 days of HWI elicited larger thermal adaptations

58 than EHA. While the 6-day HWI intervention presents an effective, practical, and time-efficient

59 short-term (< 7 days) heat acclimation strategy, previous literature suggests that medium- (7–

60 14 days) (10, 11) and long-term (> 14-day) (10) interventions provide a more complete state

61 of heat acclimation. It is yet to be determined whether extending the 6-day HWI intervention

62 provides additional thermal benefits. In addition, the true benefit of medium-term conventional

63 exercise-based heat acclimation strategies beyond exercising in temperate conditions is

64 unknown due to the lack of work-matched interventions within the literature and hence further

65 research is warranted. In contrast to the beneficial adaptations of medium-term heat

66 acclimation, the physical demands of prolonged interventions can disrupt training and may

67 trigger overreaching, which has detrimental effects on exercise performance and mood (4, 12).
68 The effects of medium-term heat acclimation on overreaching are currently unknown and,
69 given the applied implications, warrant investigation.

70

71 The reduction in thermal strain after HWI heat acclimation can be largely attributed to a
72 reduction in resting core temperature (6, 7, 9, 13, 14). The underlying mechanism for this
73 reduction in resting core temperature is currently unknown but may involve a reduction in
74 metabolic heat production via reduced circulating thyroid hormone concentrations (15), a
75 decrease in the thermoregulatory balance point (16, 17), or hypothalamic neural network
76 remodeling (18, 19). The release of thyroid-stimulating hormone by the anterior pituitary gland
77 stimulates the release of two protein-iodine-bound hormones: triiodothyronine (T3) and
78 thyroxine (T4). When unbound, free thyroid hormones are metabolically active and stimulate
79 glucose uptake, gluconeogenesis, lipolysis, and thermogenesis (20). Reductions in thyroid
80 hormones have been demonstrated after 3 weeks of heat exposure in rats (21, 22) and previous
81 research also shows rats with lower circulating thyroid hormones have a lower core temperature
82 at rest and during heat stress (23, 24). However, no study to date in humans has investigated
83 the effect of heat acclimation on thyroid hormone concentrations or thyroid hormone influences
84 on heat acclimation thermal adaptations. Specifically, it is unknown whether reductions in
85 thyroid hormones are responsible for the pronounced reduction in resting core temperature
86 observed after HWI heat acclimation (6, 7, 9).

87 This research is presented in two parts. Part 1 compared heat acclimation thermal and
88 endurance capacity adaptations, overreaching markers, and changes in plasma thyroid
89 hormones concentrations after 3, 6, and 12 days of HWI and EHA with a work-matched
90 temperate exercise control (CON) in 21 active males. Given larger thermal adaptations were
91 observed after short-term HWI than short-term EHA (9), we hypothesized that extending the

92 HWI intervention to 12 days would augment thermal adaptations and that these would confer
93 more complete heat acclimation than EHA. In addition, we expected that compared to CON
94 the high physical demands of daily exercise and heat-stress during HWI and EHA would lead
95 to increased markers of overreaching (i.e., low mood and physical/cognitive performance
96 decrements). Part 2 examined, in a larger cohort of 48 active males, the effect of 6 days of HWI
97 in comparison to CON on plasma thyroid hormone concentrations, and additionally examined
98 the relationship of thyroid hormone changes with hallmark heat acclimation adaptations. We
99 hypothesized that 6 days of HWI would elicit reductions in plasma thyroid hormone
100 concentrations and that these reductions would be associated with heat acclimation thermal
101 adaptations, in particular a reduction in resting and exercising core temperature.

102 **Methods**

103 **Experimental approaches**

104 In Part 1, a mixed-methods (between and within) repeated measures design was used to assess
105 the effect of 12 days of HWI, EHA, and CON on thermal and perceptual adaptations,
106 overreaching markers, and plasma thyroid hormone concentrations in 21 recreationally active
107 males. This is a subset of participants of a larger cohort that completed six intervention days
108 (9). Participants in Part 1 completed experimental trials before (PRE) and after 3 (POST3), 6
109 (POST6), and 12 days (POST12) of their assigned intervention (Fig. 1). To enable work-
110 matching with EHA, CON participants completed the same external work ≥ 1 day after EHA
111 participants. In Part 2, data from four previously published heat acclimation studies from our
112 laboratory (6, 7, 9, 14) were amalgamated in a between-groups design to assess the effect of 6
113 days of HWI and CON on thermal adaptations, thyroid hormone concentrations, and the
114 relationship between plasma thyroid hormones and thermal adaptations. Thyroid hormones
115 were not previously investigated in these studies. Amalgamating the four studies enabled the
116 relationship between thyroid hormones and thermal adaptations to be examined more robustly
117 in a larger sample. Testing was halted during summer months (June–August) to reduce the
118 potential effect of seasonal heat acclimatization. All studies received ethical approval
119 (829/MoDREC/17, PO5-17/18, S/PhD10-15/16, PhD19-13/14), were conducted in accordance
120 with the Declaration of Helsinki (2013) but were not registered in a database.

121

122 **Participant recruitment and randomization**

123 Part 1 participant flow and attrition before protocol completion, and biochemical and statistical
124 analyses are summarized in Fig. 2. Participants were matched for $\dot{V}O_{2\text{peak}}$ in groups of three
125 and randomly assigned to either HWI, EHA, or CON (randomizer.org). Participants were
126 excluded from the final analysis if they failed to complete the 12-day study protocol. The

127 participant characteristics of the 21 male participants included in the final analysis are
128 summarized in Table 1. A sample size of 21 (7 participants per group) was estimated (G*Power
129 3.1.9) (25) as adequate to detect a significant difference in the change in end-exercise rectal
130 core temperature (T_{re}) between heat acclimation and temperate exercise control interventions
131 using a mixed-model analysis of covariance (ANCOVA), standard alpha (0.05) and power
132 (0.80), and a Cohen's F effect size of 0.88. This effect size was calculated from the average
133 reduction in end-exercise T_{re} change after HWI (-0.36°C) (6) and exercise heat acclimation
134 (-0.44°C (26) and -0.49°C (27)) compared to exercise in temperate conditions (0.00°C) (6)
135 and a pooled SD of 0.21°C (control group) (6). Part 2 participants were 48 active males (age,
136 22 ± 3 years; height, 178 ± 6 cm; body mass, 72 ± 7 kg; $\dot{V}\text{O}_{2\text{peak}}$, 58 ± 8 $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Data
137 of fourteen participants (HWI, $n = 7$; CON, $n = 7$) were included in both Parts 1 and 2. All
138 participants in Parts 1 and 2 provided written informed consent and were healthy, non-smokers,
139 free from any known cardiovascular or metabolic diseases, were not taking any medication,
140 and had not been regularly ($>$ once a week) exposed to the heat (including sauna and hot bath
141 use) in the 6 weeks before commencing testing.

142

143 **Preliminary measurements and familiarization**

144 Participants completed a fitness assessment within a week before their first experimental trial
145 (PRE; Fig. 1). $\dot{V}\text{O}_{2\text{peak}}$ was assessed using a continuous maximal incremental exercise test
146 performed on a motorized treadmill (HP Cosmos Mercury 4.0, Nussdorf-Traunstein, Germany)
147 in a temperate laboratory (19°C , 45% RH) to volitional exhaustion. $\dot{V}\text{O}_{2\text{peak}}$ was determined as
148 the highest oxygen uptake attained over a 30-s period. The average values of breath-by-breath
149 $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ during the final minute of each submaximal stage were used to calculate
150 running economy, expressed as kilocalories per kilogram per min (28). A running speed that
151 elicited 65% $\dot{V}\text{O}_{2\text{peak}}$ in temperate conditions was subsequently determined by the interpolation

152 of the running speed– $\dot{V}O_2$ relationship and confirmed via Douglas bag method. All participants
153 ran at a speed below their anaerobic threshold as determined by the onset of blood lactate
154 accumulation (29). Participants were then familiarized with the treadmill running speed, Stroop
155 test, venepuncture, and Profile of Mood States (POMS) questionnaire.

156

157 **Experimental trials**

158 Twenty-four hours before the first experimental trial, participants were instructed to refrain
159 from exercise, alcohol, diuretics, and caffeine and to complete a diet diary. Twenty-four hours
160 before all subsequent experimental trials, participants were instructed to replicate this food and
161 fluid intake. To ensure a similar circadian pattern, participants were instructed to sleep between
162 2200 h and 0700 h before experimental trials with their sleep duration and efficiency assessed
163 by an Actigraph (Actigraph GT3X Version 4.4.0, Actigraph, Pensacola, USA). Sleep duration
164 and efficiency were also assessed as overreaching markers (30).

165

166 On the day of the experimental trials, participants arrived at the laboratory at 0730 h and were
167 provided with a standardized breakfast (2091 kilojoules, 71 g carbohydrate, 18 g fat, 17 g
168 protein) and a bolus of water (7 mL·kg⁻¹ of nude body mass). At 0800 h, dressed in a t-shirt,
169 shorts, socks and trainers, participants rested for 20 min in temperate conditions (19°C, 45%
170 RH). Following the seated rest, participants completed the abbreviated POMS questionnaire
171 (31) to determine total mood disturbance and energy index (vigor–fatigue) as markers of
172 overreaching (30). A venous blood sample was then taken without stasis for the determination
173 of plasma volume and plasma concentrations of free T3, free T4, total T3, and total T4. A urine
174 sample was then analyzed to confirm urine specific gravity was <1.03 (32) and a flexible,
175 sterile, single-use rectal thermistor (Henleys Medical Supplies Ltd., Herts, UK) was self-
176 inserted 10 cm beyond the anal sphincter to measure T_{re} . A pre-exercise nude body mass was

177 recorded using a digital platform scale (Model 703; Seca, Hamburg, Germany) and skin
178 thermistors were attached on the right side of the body for the determination of mean skin
179 temperature (T_{sk}), as previously described (33). Mean body temperature (T_b) was estimated
180 using the formula: $T_b = 0.64 \cdot T_{re} + 0.36 \cdot T_{sk}$ (34). Following instrumentation, participants rested
181 for a further 30 min in temperate conditions (19°C, 45% RH) to establish baseline measures.
182 Body surface area (A_D) by the Du Bois equation (35), and $\dot{V}O_2$ and respiratory exchange ratio
183 (RER) from a 60-s expired gas collection by Douglas bag method between 29–30 min of seated
184 rest were used to estimate resting metabolic heat production (H) as follows (36):

185

$$186 \quad H (\text{W} \cdot \text{m}^{-2}) = [0.23(\text{RER}) + 0.77] \cdot [5.873(\dot{V}O_2)] \cdot (60 / A_D).$$

187

188 At 0945 h, dressed in shorts, socks, and trainers, participants entered the environmental
189 chamber (33°C, 40% RH, 0.2 m·s⁻¹ wind velocity) to complete a 40-min treadmill run at 65%
190 $\dot{V}O_{2\text{peak}}$. T_{re} , skin temperatures, and heart rate were monitored continuously. Local forearm
191 sweat rate was measured by dew point hygrometry (DS2000; Alpha Moisture Systems, UK).
192 Anhydrous compressed nitrogen at a flow rate of 1 L·min⁻¹ was passed through a 5-cm²
193 capsule, affixed to the ventral surface of the lower arm (halfway between the antecubital fossa
194 and carpus). Local forearm sweat rate was calculated as the difference in water content between
195 effluent and influent air, divided by the skin surface area under the capsule (expressed in
196 milligrams per square centimeter per minute). T_{re} at sweating onset was determined by plotting
197 the relationship between local forearm sweat rate and T_{re} (recorded at 20-s intervals) before
198 using segmented linear regression to identify the breakpoint in the two line segments (37).
199 Rating of perceived exertion (RPE) (38), thermal sensation (TS) (39), $\dot{V}O_2$, and RER (40) were
200 recorded every 10 min. On completion of the exercise, participants rested for 20 min in
201 temperate conditions (19°C, 45% RH), during which they completed a modified Stroop test

202 (41) to assess cognitive function as a marker of overreaching (30), and provided a nude body
203 mass to estimate whole-body sweat rate.

204 Participants then re-entered the environmental chamber and completed a time to exhaustion
205 (TTE) on a motorized treadmill at 65% $\dot{V}O_{2peak}$. Participants were instructed to “run for as long
206 as possible”. TTE was terminated when participants stopped running owing to volitional
207 exhaustion, thermal discomfort, or when T_{re} exceeded 39.5 °C. No fluids were consumed, no
208 feedback was provided, and T_{re} and heart rate were monitored continuously. Following the
209 cessation of exercise, capillary blood lactate concentration was assessed (Lactate Pro 2™,
210 Arkray, Australia) as a marker of overreaching (42, 43). Participants were provided with a
211 bolus of water and were free to leave the laboratory when $T_{re} \leq 38.5^{\circ}C$.

212 **Daily intervention**

213 All participants in Part 1 and Part 2 completed 12 and 6 days of their assigned intervention,
214 respectively. During the intervention, participants were instructed to consume their normal diet
215 and fluid intake, including caffeine and alcohol (≤ 3 units per day). Participants arrived at the
216 laboratory each day between 0600 h and 1300 h. Before exercise, a nude body mass was taken,
217 and a rectal thermistor and heart rate monitor were fitted. Following instrumentation,
218 participants completed a 15-min seated rest in temperate conditions (19°C, 45% RH) to
219 establish baseline measures, before commencing their assigned intervention protocol. A bolus
220 of water (5 mL·kg⁻¹ of nude body mass) was consumed during the first 20 min of exercise.

221 Participants assigned to HWI completed a 40-min treadmill run dressed in shorts, socks, and
222 trainers at a speed equivalent to their 65% $\dot{V}O_{2peak}$ (9.1 ± 1.6 km·h⁻¹) in temperate conditions
223 (19°C, 45% RH, 0.2 m·s⁻¹ wind velocity). Following exercise (2–3 min transition), dressed in
224 shorts, participants began a semi-recumbent ≤ 40 -min HWI (40°C) to the neck, as previously
225 described (6). Participants assigned to EHA completed a ≤ 60 -min treadmill run at a speed

226 equivalent to their 65% $\dot{V}O_{2\text{peak}}$ ($9.1 \pm 1.1 \text{ km}\cdot\text{h}^{-1}$) in an environmental chamber (33°C, 40%
227 RH, $0.2 \text{ m}\cdot\text{s}^{-1}$ wind velocity). Participants assigned to CON completed a daily submaximal
228 treadmill run equivalent to 65% $\dot{V}O_{2\text{peak}}$ and work-matched to EHA ($8.8 \pm 0.9 \text{ km}\cdot\text{h}^{-1}$) in
229 temperate conditions (19°C, 45% RH, $0.2 \text{ m}\cdot\text{s}^{-1}$ wind velocity). Owing to the nature of these
230 interventions, it was not possible to blind the participants. In Part 1, to maintain the endogenous
231 thermal stimulus for adaptation after the first six intervention sessions (Days 1–3 and Days 6–
232 8, Fig. 1), maximum immersion (HWI) and exercise duration (EHA and CON) increased by
233 25%, as of the seventh intervention session (Days 11–16, intervention sessions 7–12), to ≤ 50
234 min and ≤ 75 min, respectively. All intervention sessions were terminated if the maximal
235 immersion/exercise duration was reached, at the participant's volition, or if T_{re} exceeded
236 39.5°C. Upon removal from the hot water/environmental chamber, participants rested in a
237 seated position for 5 min in a temperate laboratory, were provided with a bolus of water, and
238 were free to leave the laboratory when $T_{\text{re}} \leq 38.5^\circ\text{C}$.

239 **Blood sample collection and analysis**

240 Venous blood samples were collected from an antecubital vein without stasis into two 6-mL
241 EDTA vacutainers (BD, Oxford, UK). Aliquots of whole blood were used for the immediate
242 determination of hemoglobin in duplicate (Hemocue, Sheffield, UK) and hematocrit in
243 triplicate using a microcentrifuge and micro-hematocrit reader (Hawksley & Sons Limited,
244 Lancing, UK). The change in plasma volume was estimated by correcting the initial plasma
245 volume at PRE for the percentage change in plasma volume ($\%\Delta\text{PV}$) at POST3, POST6 and
246 POST12, as previously described (44). The remaining whole blood was then centrifuged, and
247 the plasma frozen at -80°C for later analysis.

248

249 Plasma concentrations of free and total triiodothyronine (T3) and thyroxine (T4) were
250 measured in duplicate by ELISA (free T3: Cat. No. RE55231, detection limit: $0.1 \text{ pmol}\cdot\text{L}^{-1}$;

251 free T4: Cat. No. RE55241, detection limit: 0.6 pmol·L⁻¹; total T3: Cat. No. RE55251,
252 detection limit: 0.2 nmol·L⁻¹; total T4: Cat. No. RE55261, detection limit: 0.1 nmol·L⁻¹; IBL
253 International, Hamburg, Germany). The intra-assay coefficient of variation for duplicates were
254 free T3, 5.1%; free T4, 2.6%; total T3, 5.6%; total T4, 5.9%. Thyroid hormone concentrations
255 were adjusted for plasma volume changes using the following formula (45):

256

257
$$\text{Corrected value} = \text{Uncorrected value} \cdot ((100 + \% \Delta \text{PV}) / 100).$$

258

259 **Statistical analysis**

260 Data were analyzed using SPSS version 27 (IBM Corporation, NY, USA) or GraphPad Prism
261 Version 9.1 (GraphPad Software Inc. La Jolla, USA). All data were checked for normality and
262 sphericity; plasma free T4 data was reciprocal transformed to address statistical assumptions
263 of sphericity. Data are presented as untransformed mean and SD unless otherwise stated, and
264 statistical significance was accepted at $P < 0.05$. In Part 1, the mean daily endogenous thermal
265 stimulus and external work during HWI, EHA, and CON were compared using a two-way
266 mixed model ANOVA. A two-way mixed model ANCOVA, with baseline (PRE) as the
267 covariate, was used to detect differences in heat acclimation adaptations, endurance capacity,
268 overreaching markers, and plasma thyroid hormone concentrations after 3, 6, and 12 days of
269 HWI, EHA, or CON. Bonferroni-adjusted pairwise comparisons were used where appropriate
270 to determine where differences occurred. The size of the between-intervention differences was
271 calculated using Cohen's d effect size with values greater than 0.2, 0.5, and 0.8 representing
272 small, medium, and large effects, respectively (46). In Part 2, the mean daily endogenous
273 thermal stimulus and external work during HWI and CON were compared using t -tests and a
274 one-way ANCOVA was used to detect differences in heat acclimation adaptations and plasma
275 thyroid hormone concentrations after 6 days of HWI or CON. Bonferroni-adjusted pairwise

276 comparisons were used where appropriate to determine where differences occurred. Pearson's
277 correlations determined the strength of the relationship between the endogenous thermal
278 stimulus, changes in resting T_{re} and plasma thyroid hormone concentrations after 12 days of
279 heat acclimation by HWI and EHA. Pearson correlation coefficients of 0.00–0.19 were
280 regarded as very weak, 0.20–0.39 as weak, 0.40–0.59 as moderate, and 0.60–0.79 as strong
281 relationships (47).

282 **Results**

283 **Part 1 daily intervention thermal stimulus and external work**

284 Throughout the 12-day intervention the daily endogenous thermal stimulus for adaptation was
285 similar between HWI and EHA (Table 2; all $P \geq 0.407$), but lower in CON ($P < 0.001$); there
286 were no main effects of time or interaction effects ($P \geq 0.252$). The daily endogenous thermal
287 stimulus was maintained throughout the 12 days by an increase in mean daily immersion on
288 HWI (Days 1–3, 33 ± 4 min; Days 6–8, 35 ± 5 min; Days 11–16, 39 ± 5 min, $P < 0.001$) and
289 an increase in exercise duration on EHA (Days 1–3, 51 ± 9 min; Days 6–8, 55 ± 8 min; Days
290 11–16, 61 ± 11 min, $P < 0.001$). The similar daily thermal stimulus during HWI and EHA was
291 achieved with a lower mean daily external work in HWI than EHA (Table 2; $P = 0.006$), and
292 mean daily external work also tended to be lower in HWI than CON ($P = 0.053$).

293

294 **Part 1 experimental trials**

295 Prior experimental trial standardization ensured sleep duration (6 ± 1 h, $P \geq 0.184$) and
296 hydration status, as assessed by urine specific gravity (1.020 ± 0.007 , $P \geq 0.268$), were similar
297 between the interventions, as evidenced by no main effects of group or time, and no interaction
298 effects.

299

300 *Thermal responses at rest in temperate conditions*

301 Thermal responses at rest in temperate conditions were different between interventions after
302 12 days. Resting T_b was lower after HWI than EHA (Fig. 3A, $P = 0.009$, $d = 1.86$) and CON
303 ($P = 0.005$, $d = 2.04$). Resting T_b was not different between EHA and CON over the 12 days
304 ($P = 1.000$, $d = 0.20$). The average reduction in resting T_b over the 12-days was $-0.41 \pm 0.15^\circ\text{C}$
305 for HWI, $-0.14 \pm 0.14^\circ\text{C}$ for EHA, and $-0.11 \pm 0.15^\circ\text{C}$ for CON. Resting T_{re} was lower after
306 HWI (Fig. 3B, $-0.41 \pm 0.15^\circ\text{C}$) than CON ($-0.12 \pm 0.15^\circ\text{C}$, $P = 0.007$, $d = 1.93$), but not EHA

307 $(-0.20 \pm 0.15^{\circ}\text{C}, P = 0.061, d = 1.37)$. Resting T_{re} was not different between EHA and CON
308 over the 12 days ($P = 0.936, d = 0.56$). Conversely, there were no differences between
309 interventions for resting T_{sk} (Fig. 3C), resting $T_{\text{re}}-T_{\text{sk}}$ gradient, resting H (Fig. 3D), or plasma
310 volume (all $P \geq 0.096$; Table 3).

311

312 *Thermal and perceptual responses to exercise in the heat*

313 Thermal and perceptual responses to submaximal exercise in the heat were different between
314 the interventions after 12 days. End-exercise T_{re} following exercise-heat-stress was lower after
315 HWI (Fig. 4B, $-0.50 \pm 0.19^{\circ}\text{C}$) than CON ($-0.33 \pm 0.13^{\circ}\text{C}; P = 0.049, d = 1.13$), but not EHA
316 ($-0.37 \pm 0.13^{\circ}\text{C}; P = 0.196, d = 0.88$); no difference was observed between EHA and CON (P
317 $= 1.000, d = 0.30$). T_{re} at sweating onset was lower after HWI (Fig. 4C, $-0.43 \pm 0.12^{\circ}\text{C}$) than
318 EHA ($-0.22 \pm 0.12^{\circ}\text{C}; P = 0.015, d = 1.75$) and CON ($-0.16 \pm 0.12^{\circ}\text{C}; P = 0.002, d = 2.27$).
319 Conversely, EHA did not reduce T_{re} at sweating onset compared to CON ($P = 1.000, d = 0.52$).
320 Whole-body sweat rate was greater after HWI (Fig. 4D, $+0.08 \text{ L}\cdot\text{h}^{-1}; P = 0.003, d = 2.13$) and
321 EHA ($+0.06 \text{ L}\cdot\text{h}^{-1}; P = 0.013, d = 1.78$) than CON ($-0.06 \text{ L}\cdot\text{h}^{-1}$), but no difference was detected
322 between HWI and EHA ($P = 1.000, d = 0.35$). In accordance with thermal adaptations,
323 perceptual responses to exercise-heat-stress were lower after HWI (RPE Fig. 4E, -2 ± 1 ; TS
324 Fig. 4F, -1 ± 1) than CON (RPE, $0 \pm 1, P = 0.036, d = 1.57$; TS, $0 \pm 1, P = 0.047, d = 1.55$)
325 but not EHA (RPE, $-1 \pm 1, P = 0.951, d = 0.54$; TS, $-1 \pm 1, P = 1.000, d = 0.55$); no differences
326 were observed between EHA and CON ($P \geq 0.157, d = 1.07$). There were no differences
327 between interventions for the change in T_{re} during the 40-min treadmill run in the heat, end-
328 exercise T_{b} (Fig. 4A), end-exercise T_{sk} , end-exercise $T_{\text{re}}-T_{\text{sk}}$ gradient, end-exercise heart rate,
329 exercising $\dot{V}\text{O}_2$, or exercising RER (Table 3; all $P \geq 0.059$). The rate of thermal and perceptual
330 adaptations was not different between HWI, EHA or CON from POST3 to POST12, as

331 indicated by no interaction effects (all $P \geq 0.087$). There were also no main effects of time (all
332 $P \geq 0.148$).

333

334 *Overreaching markers and endurance capacity*

335 There was no evidence to suggest that 12 days of HWI or EHA induced overreaching to a
336 greater extent than CON, with no interaction effects, main effects of group or time detected
337 for total mood disturbance, energy index, Stroop reaction time, Stroop accuracy, sleep
338 duration, or sleep efficiency (Table 4; all $P \geq 0.190$). Five participants were removed from
339 the TTE endurance capacity test analysis owing to: reaching the T_{re} ethical cut-off (HWI, $n =$
340 2); going to the toilet (EHA, $n = 1$); exercise-induced bronchoconstriction (CON, $n = 1$); and
341 an obvious lack of effort without markers of overreaching at rest (CON, $n = 1$). Analysis of
342 the remaining 16 participants (HWI, $n = 5$; EHA, $n = 6$; CON, $n = 5$) who completed the TTE
343 revealed no statistical differences between interventions or across time (Table 4; $P \geq 0.219$).
344 In addition, no differences were detected between interventions for end-TTE T_{re} , end-TTE
345 heart rate, or end-TTE blood lactate concentration (Table 4; all $P \geq 0.198$).

346

347 *Thyroid hormones*

348 Twelve days of HWI elicited a reduction in thyroid hormones, evidenced by an interaction
349 effect for plasma concentrations of free T3 ($P = 0.006$). Follow-up analyses showed that free
350 T3 was lower after 12 days of HWI (-23%) than EHA ($+4\%$, $P = 0.008$) and CON ($+1\%$, $P =$
351 0.015 ; Fig. 5A). No differences were detected for free T3 between EHA and CON ($P = 1.000$).
352 Conversely, there were no interaction effects or main effects of group or time detected for
353 plasma concentrations of free T4 ($P \geq 0.148$, Fig. 5B), total T3 ($P \geq 0.057$, Fig. 5C), or total
354 T4 ($P \geq 0.156$, Fig. 5D).

355

356 **Part 2 daily intervention thermal stimulus and external work**

357 All 48 participants completed 6 days of their assigned intervention. The HWI intervention
358 caused a greater daily endogenous thermal stimulus than CON as indicated by greater daily
359 duration $T_{re} > 38.5^{\circ}\text{C}$ (HWI, 41 ± 13 min; CON, 7 ± 8 ; $P < 0.001$), AUC for $T_{re} > 38.5^{\circ}\text{C}$ (HWI,
360 23 ± 10 $^{\circ}\text{C}\cdot\text{min}^{-1}$; CON, 1 ± 2 $^{\circ}\text{C}\cdot\text{min}^{-1}$; $P < 0.001$), and end-intervention T_{re} (HWI, $39.3 \pm$
361 0.2°C ; CON, $38.3 \pm 0.4^{\circ}\text{C}$; $P < 0.001$). Daily external work was similar between HWI and
362 CON (HWI, 7.0 ± 1.1 km; CON 7.3 ± 1.3 km; $P = 0.065$).

363

364 **Part 2 experimental trials**

365 *Thermal responses at rest in temperate conditions*

366 Resting T_b was lower after 6 days of HWI than CON (HWI, $-0.31 \pm 0.32^{\circ}\text{C}$; CON, $-0.04 \pm$
367 0.32°C ; $P = 0.009$). In accordance with resting T_b , resting T_{re} was also lower after 6 days of
368 HWI than CON (HWI, $-0.33 \pm 0.20^{\circ}\text{C}$; CON, $-0.09 \pm 0.21^{\circ}\text{C}$; $P = 0.001$, Fig. 6A). Conversely,
369 no differences were detected for resting T_{sk} ($P = 0.083$), resting $T_{re}-T_{sk}$ gradient ($P = 0.509$),
370 resting H ($P = 0.711$, Fig. 6B), or plasma volume ($P = 0.387$).

371

372 *Thermal and perceptual responses to exercise in the heat*

373 Compared to CON, 6 days of HWI also resulted in a lower end-exercise T_b (HWI, $-0.54 \pm$
374 0.24°C ; CON, $-0.18 \pm 0.24^{\circ}\text{C}$; $P < 0.001$), end-exercise T_{re} (HWI, $-0.42 \pm 0.24^{\circ}\text{C}$; CON,
375 $-0.13 \pm 0.24^{\circ}\text{C}$; $P < 0.001$), T_{re} at sweating onset (HWI, $-0.31 \pm 0.20^{\circ}\text{C}$; CON, $-0.08 \pm 0.19^{\circ}\text{C}$;
376 $P = 0.01$), end-exercise T_{sk} (HWI, $-0.74 \pm 0.54^{\circ}\text{C}$; CON, $-0.30 \pm 0.54^{\circ}\text{C}$; $P < 0.001$), end-
377 exercise RPE (HWI, -1 ± 1 ; CON, 0 ± 1 ; $P = 0.010$), and end-exercise TS (HWI, -1 ± 1 ; CON,
378 0 ± 1 ; $P = 0.003$). No differences were detected for whole-body sweat rate ($P = 0.228$).

379 *Thyroid hormones*

380 Despite 6-days of HWI causing pronounced heat acclimation adaptations, including reductions
381 in T_b and T_{re} at rest and during exercise in the heat, no differences between HWI and CON were
382 detected in resting plasma thyroid hormone concentrations; free T3 (HWI, $0 \pm 12\%$; CON, -1
383 $\pm 12\%$; $P = 0.802$; Fig. 6C), free T4 (HWI, $-8 \pm 10\%$; CON, $-3 \pm 10\%$; $P = 0.108$; Fig. 6D),
384 total T3 (HWI, $-3 \pm 10\%$; CON, $-2 \pm 17\%$; $P = 0.873$; Fig. 6E), or total T4 (HWI, $-4 \pm 8\%$;
385 CON, $-1 \pm 8\%$; $P = 0.180$; Fig. 6F). Moreover, after 6-days of HWI, only weak non-significant
386 relationships were observed between the reduction in resting T_b , resting T_{re} (Fig. 7), resting T_{sk} ,
387 resting $T_{re}-T_{sk}$ gradient, end-exercise T_b , end-exercise T_{re} , T_{re} at sweating onset, end-exercise
388 T_{sk} or end-exercise TS and changes in free T3 ($r \leq 0.21$, $P \geq 0.269$), free T4 ($r \leq 0.20$, $P \geq$
389 0.274), total T3 ($r \leq 0.31$, $P \geq 0.086$), and total T4 ($r \leq 0.24$, $P \geq 0.193$).

390 **Discussion**

391 This research is the first to compare hallmark heat acclimation adaptations, endurance capacity,
392 and overreaching markers after 12 days of HWI and EHA with work-matched CON. This study
393 is also the first in humans to examine the potential role of plasma thyroid hormone changes as
394 a mechanism for the thermal adaptations after heat acclimation, specifically HWI heat
395 acclimation. The three primary findings of this research conducted in recreationally active men
396 are: 1. In line with our hypothesis, HWI elicited larger and a greater number of thermal
397 adaptations, and reductions in perceived strain during exercise-heat-stress compared to CON
398 and EHA over the 12-day interventions. Conventional EHA provided only modest further heat
399 acclimation benefits to work-matched CON. 2. Contrary to our hypothesis, and previous
400 literature examining short-term heat acclimation (12), there was no evidence to suggest that
401 HWI or EHA induced overreaching risk more than with exercise in temperate conditions. 3.
402 Also contrary to our hypothesis, changes in plasma thyroid hormone concentrations were not
403 significantly associated with changes in thermal adaptations over the 12 days of HWI,
404 indicating that a reduction in thyroid hormones is unlikely the cause of the pronounced
405 reduction in resting and end-exercise core temperature observed consistently after HWI heat
406 acclimation. Instead, we provide evidence that the reduction in core temperature elicited by
407 post-exercise HWI intervention represents the establishment of a new lower thermal balance
408 point (17).

409

410 Previous research has demonstrated that short-term (< 7 days) HWI provides beneficial heat
411 acclimation adaptations in comparison with CON and conventional EHA in recreationally
412 active males (6, 9, 13, 14). The current study furthers this work by showing that 12 days of
413 HWI heat acclimation led to more pronounced resting and exercising thermal adaptations than
414 EHA and CON (Fig. 3 and 4). Resting T_b and T_{re} at sweating onset were lower over the 12-day

415 HWI intervention than the 12-day EHA intervention. Compared to CON, HWI led to a greater
416 number of thermal adaptations than EHA, i.e., HWI reduced resting T_b , resting T_{re} , end-exercise
417 T_{re} , T_{re} at sweating onset, end-exercise RPE, end-exercise TS, and increased whole-body sweat
418 rate whereas EHA increased whole-body sweat rate only. The data also suggests that
419 improvements in endurance capacity in the heat may be more readily observed after HWI than
420 EHA, which has practical implications for applied practitioners and coaches. However, due to
421 dropout, future studies are required to confirm (or reject) this preliminary finding. In
422 combination, these findings indicate that HWI leads to larger and more complete heat
423 acclimation than conventional EHA, even when the endogenous thermal stimulus for
424 adaptation is similar. Heat acclimation adaptations developed throughout the 12 days, with the
425 largest proportion of the adaptations occurring within the first 3 days, for example, ~58% of
426 the 12-day reduction in end-exercise T_{re} was observed on day 3 (Fig. 4B). Nevertheless, we
427 observed no further statistically significant thermal benefits or improvements in endurance
428 capacity by extending the 6-day heat acclimation interventions to 12 days. These findings align
429 with the majority of previous studies that show no further thermal adaptations in males after
430 medium- compared to short-term interventions (10, 26, 48, 49), even when a progressive heat
431 acclimation method was employed (27). Far less studied is the influence of additional heat
432 acclimation days on exercise performance. In contrast with our findings, previous research
433 showed additional improvements in exercise performance in the heat when extending exercise
434 heat acclimatization from 6 to 14 days (50). The disparity with our findings may be explained
435 by the small sample size for the TTE outcome in the current study and/or by differences in
436 intervention methods and/or participants' training status (recreationally active vs competitive)
437 (51).
438

439 The change in T_{re} during the 40-min submaximal treadmill run in the heat was similar on all
440 interventions; hence, the lower end-exercise T_{re} (i.e., lower thermal strain) after HWI can be
441 attributed to larger reductions in resting T_{re} than observed after CON. The induction of large
442 reductions in resting T_{re} after HWI are likely due to exposure to a large dual thermal stimulus
443 (i.e., maintained elevation in both core and skin temperature), as it is purported to induce a
444 more complete state of heat acclimation (52). We anticipated the larger thermal adaptations
445 from HWI would be associated with larger reductions in thyroid hormone concentrations in
446 accordance with previous literature, which demonstrate a lower core temperature in
447 hypothyroid compared to control rats (23, 24). However, despite large reductions in resting and
448 end-exercise T_{re} after 3, 6 and 12 days of HWI, a concomitant reduction in plasma thyroid
449 hormone concentrations (free T3) was only observed after 12 days (Fig. 5A). The temporal
450 disconnect and the absence of significant relationships between changes in thyroid hormones
451 and thermal adaptations indicate that circulating thyroid hormone changes are unlikely the
452 cause of short- and medium-term heat acclimation adaptations. Indeed, the change in free T3
453 observed after 12 days appears a consequence of HWI heat acclimation. We can further refute
454 the notion that HWI heat acclimation reduces core temperature via alterations in thyroid
455 hormones and metabolism as we did not observe differences between interventions or a
456 reduction in resting H after HWI (Fig. 3D and 6B). The lower resting core temperature after
457 HWI is also unlikely explained by increased heat loss mechanisms as skin temperature, an
458 index of skin blood flow, was not higher after HWI. In fact, a trend ($P < 0.1$) was observed for
459 lower skin temperature after HWI in both Part 1 and 2 (Fig. 3C). The large reduction in resting
460 core temperature observed after HWI heat acclimation may alternatively be explained by the
461 establishment of a new lower thermal balance point (17). In this study, the new lower thermal
462 balance point is indicated by a lower resting whole-body temperature with no change in resting
463 core to skin temperature gradient (Fig. 3A).

464

465 A combined stimulus of exercise and heat stress is generally considered the “gold standard”
466 method for inducing heat acclimation adaptations (53). As expected, we found that
467 conventional EHA caused thermal adaptations in comparison to baseline (end-exercise: T_{re}
468 $-0.37 \pm 0.13^{\circ}\text{C}$, Table 3). However, there is a dearth of medium-term heat acclimation studies
469 with an appropriate control intervention; hence, the true effect of conventional exercise heat
470 acclimation is poorly understood. In the current study, the inclusion of a work-matched
471 temperate exercise intervention allowed the independent effectiveness of the exercise and heat
472 stress stimuli to be determined. We found that, aside from an increase in whole-body sweat
473 rate, which was greater after EHA, no additional heat acclimation adaptations existed between
474 EHA and CON. Our findings align with studies that demonstrate aerobic training in temperate
475 conditions initiates adaptations commonly associated with heat acclimation in recreationally
476 active individuals (54-57). These studies suggest it is principally the endogenous heat
477 production incurred during exercise rather than the external environmental temperature that is
478 important for initiating heat acclimation adaptations. When considered together with these
479 investigations, the benefits of conventional exercise-based heat acclimation beyond work-
480 matched exercise in temperate conditions are modest.

481

482 Previous research has shown that intensified training during exercise heat acclimation can
483 trigger markers of overreaching including increased perceived fatigue and decreased
484 performance (12). In contrast we observed no evidence of overreaching after EHA or HWI; a
485 discrepancy that might be explained by the lower exercise intensity and the inclusion of three
486 rest days in our study compared with previous research. More participants did however
487 withdraw with lower limb discomfort (i.e., knee/ankle pain, etc.) in EHA (25%) and CON
488 (25%) than in HWI (7%; Fig. 2); a finding that might be explained by the ~35% greater external

489 work during EHA and CON interventions than HWI. This finding provides insight into the
490 practical feasibility of these interventions but is difficult to compare with previous research as
491 heat acclimation studies do not often report participant flow and attrition. Based on our
492 findings, athletes and coaches may be more inclined to choose HWI in the knowledge it carries
493 less injury risk than EHA. Although this is a reasonable hypothesis, future studies with
494 adequate sample sizes are required to specifically evaluate the injury and illness risks of heat
495 acclimation.

496
497 Athletes and coaches should consider HWI rather than EHA before traveling to hot climates as
498 it leads to a more complete state of heat acclimation, can be incorporated into the post-exercise
499 washing routine, and eliminates the requirement for an increased training load or access to an
500 environmental chamber. These benefits reduce the disruption to normal training compared with
501 conventional exercise-based strategies, which is especially important during tapering in the
502 lead-up to sporting events. Whilst adverse events after HWI, including syncope, have not been
503 observed by us (6, 7, 13, 14), or reported by others (58, 59), practitioners should follow protocol
504 guidelines carefully. In particular, hot water immersions should be terminated at the
505 participant's volition or if T_{re} exceeds 39.5°C rather than attempting to complete 40 min. In our
506 study this led to a gradual daily increase in hot water immersion duration up to a maximum of
507 40 min for the first six (Days 1–3, 33 ± 4 min; Days 6–8, 35 ± 5 min), and then 50 min for the
508 seventh to twelfth immersions (Days 11–16, 39 ± 5 min). The current and previous studies
509 demonstrate the effectiveness of HWI to prepare young, healthy, active males (6, 7, 9, 13, 14)
510 and elderly males and females for heat stress (59). Further research is required to confirm that
511 HWI is effective to cause beneficial thermal, perceptual and performance adaptations in
512 pediatric, female, and older athletic populations. We hypothesize that HWI will be an effective
513 strategy in these populations as Mee et al. (60) demonstrated that combining both active and

514 passive heat acclimation strategies can accelerate thermal adaptations in females. The large
515 dual thermal stimulus from 6 days of HWI should be sufficient to initiate heat acclimation
516 adaptations in these populations as they typically have smaller body masses than adult males
517 and consequently gain heat more quickly (61). Due to the smaller body masses these future
518 investigations might require shorter maximum HWI durations to cause the beneficial thermal
519 adaptations.

520

521 **Perspectives and Significance**

522 Our findings show that medium-term post-exercise HWI confers more complete heat
523 acclimation than conventional exercise heat acclimation, without increasing the risk of
524 overreaching. Compared to conventional exercise heat acclimation, post-exercise HWI caused
525 a greater reduction in resting whole-body temperature (core and periphery), which highlights
526 the importance of a large dual (endogenous and exogenous) thermal stimulus for optimizing
527 adaptation to the heat. The consistently reported large reduction in resting core temperature
528 after HWI is most likely explained by the establishment of a new lower thermal balance point
529 and not initiated by thyroid hormone alterations, changes in heat production, or heat loss
530 mechanisms. In addition to lowering resting whole-body temperature, post-exercise HWI also
531 caused more pronounced beneficial exercising thermal and perceptual adaptations than
532 conventional exercise heat acclimation. Future research should assess whether the reduction in
533 thermal strain after post-exercise HWI translates to ‘real-world’ performance improvements
534 and reduces the incidence of exertional heat illness.

535 **References**

- 536 1. **Galloway SD, and Maughan RJ.** Effects of ambient temperature on the capacity to
537 perform prolonged cycle exercise in man. *Med Sci Sports Exerc* 29: 1240-1249, 1997.
- 538 2. **Maughan RJ, Otani H, and Watson P.** Influence of relative humidity on prolonged
539 exercise capacity in a warm environment. *Eur J Appl Physiol* 112: 2313-2321, 2012.
- 540 3. **Casa DJ, DeMartini JK, Bergeron MF, Csillan D, Eichner ER, Lopez RM, Ferrara**
541 **MS, Miller KC, O'Connor F, Sawka MN, and Yeargin SW.** National Athletic Trainers'
542 Association Position Statement: Exertional Heat Illnesses. *J Athl Train* 50: 986-1000, 2015.
- 543 4. **Saunders PU, Garvican-Lewis LA, Chapman RF, and Periard JD.** Special
544 Environments: Altitude and Heat. *Int J Sport Nutr Exerc Metab* 29: 210-219, 2019.
- 545 5. **Ashworth ET, Cotter JD, and Kilding AE.** Methods for improving thermal tolerance
546 in military personnel prior to deployment. *Mil Med Res* 7: 58, 2020.
- 547 6. **Zurawlew MJ, Walsh NP, Fortes MB, and Potter C.** Post-exercise hot water
548 immersion induces heat acclimation and improves endurance exercise performance in the heat.
549 *Scand J Med Sci Sports* 26: 745-754, 2016.
- 550 7. **Zurawlew MJ, Mee JA, and Walsh NP.** Post-exercise Hot Water Immersion Elicits
551 Heat Acclimation Adaptations in Endurance Trained and Recreationally Active Individuals.
552 *Front Physiol* 9: 1824, 2018.

- 553 8. **Casadio JR, Kilding AE, Siegel R, Cotter JD, and Laursen PB.** Periodizing heat
554 acclimation in elite Laser sailors preparing for a world championship event in hot conditions.
555 *Temperature (Austin)* 3: 437-443, 2016.
- 556 9. **McIntyre RD, Zurawlew MJ, Oliver SJ, Cox AT, Mee JA, and Walsh NP.** A
557 comparison of heat acclimation by post-exercise hot water immersion and exercise in the heat.
558 *J Sci Med Sport* 24: 729-734, 2021.
- 559 10. **Moss JN, Bayne FM, Castelli F, Naughton MR, Reeve TC, Trangmar SJ,**
560 **Mackenzie RWA, and Tyler CJ.** Short-term isothermic heat acclimation elicits beneficial
561 adaptations but medium-term elicits a more complete adaptation. *Eur J Appl Physiol* 120: 243-
562 254, 2020.
- 563 11. **Cheung SS, and McLellan TM.** Heat acclimation, aerobic fitness, and hydration
564 effects on tolerance during uncompensable heat stress. *J Appl Physiol (1985)* 84: 1731-1739,
565 1998.
- 566 12. **Schmit C, Duffield R, Hausswirth C, Brisswalter J, and Le Meur Y.** Optimizing
567 Heat Acclimation for Endurance Athletes: High- vs Low-Intensity Training. *Int J Sports*
568 *Physiol Perform* 13: 816-823, 2018.
- 569 13. **Zurawlew MJ, Mee JA, and Walsh NP.** Post-exercise Hot Water Immersion Elicits
570 Heat Acclimation Adaptations That Are Retained for at Least Two Weeks. *Front Physiol* 10:
571 1080, 2019.

- 572 14. **Zurawlew MJ, Mee JA, and Walsh NP.** Heat acclimation by post-exercise hot water
573 immersion in the morning reduces thermal strain during morning and afternoon exercise-heat-
574 stress. *Int J Sports Physiol Perform* 13: 1281-1286, 2018.
- 575 15. **Buguet A, Gati R, Soubiran G, Straboni JP, Hanniquet AM, Livecchi-Gonnot G,**
576 **and Bittel J.** Seasonal changes in circadian rhythms of body temperatures in humans living in
577 a dry tropical climate. *Eur J Appl Physiol Occup Physiol* 58: 334-339, 1988.
- 578 16. **Romanovsky AA.** Thermoregulation: some concepts have changed. Functional
579 architecture of the thermoregulatory system. *Am J Physiol Regul Integr Comp Physiol* 292:
580 R37-46, 2007.
- 581 17. **Romanovsky AA.** The thermoregulation system and how it works. *Handb Clin Neurol*
582 156: 3-43, 2018.
- 583 18. **Tan CL, Cooke EK, Leib DE, Lin YC, Daly GE, Zimmerman CA, and Knight ZA.**
584 Warm-Sensitive Neurons that Control Body Temperature. *Cell* 167: 47-59 e15, 2016.
- 585 19. **Zhao ZD, Yang WZ, Gao C, Fu X, Zhang W, Zhou Q, Chen W, Ni X, Lin JK,**
586 **Yang J, Xu XH, and Shen WL.** A hypothalamic circuit that controls body temperature. *Proc*
587 *Natl Acad Sci U S A* 114: 2042-2047, 2017.
- 588 20. **Iwen KA, Oelkrug R, and Brabant G.** Effects of thyroid hormones on thermogenesis
589 and energy partitioning. *J Mol Endocrinol* 60: R157-R170, 2018.

- 590 21. **Horowitz M, Peyser YM, and Muhlrud A.** Alterations in cardiac myosin isoenzymes
591 distribution as an adaptation to chronic environmental heat stress in the rat. *J Mol Cell Cardiol*
592 18: 511-515, 1986.
- 593 22. **Mirit E, Gross C, Hasin Y, Palmon A, and Horowitz M.** Changes in cardiac
594 mechanics with heat acclimation: adrenergic signaling and SR-Ca regulatory proteins. *Am J*
595 *Physiol Regul Integr Comp Physiol* 279: R77-85, 2000.
- 596 23. **Maloyan A, and Horowitz M.** beta-Adrenergic signaling and thyroid hormones affect
597 HSP72 expression during heat acclimation. *J Appl Physiol (1985)* 93: 107-115, 2002.
- 598 24. **Yang Y, and Gordon CJ.** Regulated hypothermia in the hypothyroid rat induced by
599 administration of propylthiouracil. *Am J Physiol Regul Integr Comp Physiol* 272: R1390-1395,
600 1997.
- 601 25. **Faul F, Erdfelder E, Lang AG, and Buchner A.** G*Power 3: a flexible statistical
602 power analysis program for the social, behavioral, and biomedical sciences. *Behav Res*
603 *Methods* 39: 175-191, 2007.
- 604 26. **Mee JA, Gibson OR, Doust J, and Maxwell NS.** A comparison of males and females'
605 temporal patterning to short- and long-term heat acclimation. *Scand J Med Sci Sports* 25 Suppl
606 1: 250-258, 2015.
- 607 27. **Gibson OR, Mee JA, Tuttle JA, Taylor L, Watt PW, and Maxwell NS.** Isothermic
608 and fixed intensity heat acclimation methods induce similar heat adaptation following short
609 and long-term timescales. *J Therm Biol* 49-50: 55-65, 2015.

- 610 28. **Shaw AJ, Ingham SA, and Folland JP.** The valid measurement of running economy
611 in runners. *Med Sci Sports Exerc* 46: 1968-1973, 2014.
- 612 29. **Spurway N, and Jones AM.** Lactate testing. In: *Sport and Exercise Physiology Testing*
613 *Guidelines: The British Association of Sport and Exercise Science Guide*, edited by Winter
614 EM, Jones AM, Davison RCR, Bromley PD, and Mercer TH. Abingdon, Oxon: Routledge,
615 2017, p. 112-119.
- 616 30. **Meeusen R, Duclos M, Foster C, Fry A, Gleeson M, Nieman D, Raglin J, Rietjens**
617 **G, Steinacker J, and Urhausen A.** Prevention, diagnosis and treatment of the overtraining
618 syndrome: Joint consensus statement of the European College of Sport Science (ECSS) and
619 the American College of Sports Medicine (ACSM). *Eur J Sport Sci* 13: 1-24, 2013.
- 620 31. **Grove JR, and Prapavessis H.** Preliminary evidence for the reliability and validity of
621 an abbreviated Profile of Mood States. *Int J Sport Psychol* 23: 93-109, 1992.
- 622 32. **Armstrong LE.** Hydration Assessment Techniques. *Nutrition Reviews* 63: 40-54,
623 2005.
- 624 33. **Ramanathan NL.** A new weighting system for mean surface temperature of the human
625 body. *J Appl Physiol* 19: 531-533, 1964.
- 626 34. **Burton AC.** Human Calorimetry: II. The Average Temperature of the Tissues of the
627 Body: Three Figures. *The Journal of Nutrition* 9: 261–280, 1935.

- 628 35. **Du Bois D, and Du Bois EF.** A formula to estimate the approximate surface area if
629 height and weight be known. *Arch Intern Med (Chic)* XVII: 863–871, 1916.
- 630 36. **Gagge AP, and Gonzales RR.** Mechanisms of heat exchange: biophysics and
631 physiology. In: *Handbook of Physiology Section 4, Environmental Physiology*, edited by
632 Fregly MJ, and Blatteis CM. New York: Oxford University Press, 1996, p. 45–85.
- 633 37. **Cheuvront SN, Chinevere TD, Ely BR, Kenefick RW, Goodman DA, McClung JP,**
634 **and Sawka MN.** Serum S-100beta response to exercise-heat strain before and after
635 acclimation. *Med Sci Sports Exerc* 40: 1477-1482, 2008.
- 636 38. **Borg G.** Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med* 2:
637 92-98, 1970.
- 638 39. **Hollies NRS, and Goldman GRF.** Psychological scaling in comfort assessment. In:
639 *Clothing Comfort: Interaction of Thermal, Ventilation, Construction, and Assessment Factors*,
640 edited by Hollies NRS, and Goldman GRF. Ann Arbor: Ann Arbor Science Publishers, 1977,
641 p. 107-120.
- 642 40. **James DVB, Sandals LE, Wood DM, and Jones AM.** Pulmonary gas exchange. In:
643 *Sport and Exercise Physiology Testing Guidelines: The British Association of Sport and*
644 *Exercise Science Guide*, edited by Winter EM, Jones AM, Davidson RCR, Bromley PD, and
645 Mercer TH. Abingdon, Oxon: Routledge, 2017, p. 101-111.

- 646 41. **Dupuy O, Lussier M, Fraser S, Bherer L, Audiffren M, and Bosquet L.** Effect of
647 overreaching on cognitive performance and related cardiac autonomic control. *Scand J Med*
648 *Sci Sports* 24: 234-242, 2014.
- 649 42. **Le Meur Y, Hausswirth C, Natta F, Couturier A, Bignet F, and Vidal PP.** A
650 multidisciplinary approach to overreaching detection in endurance trained athletes. *J Appl*
651 *Physiol (1985)* 114: 411-420, 2013.
- 652 43. **Schaal K, Meur YLE, Louis J, Filliard JR, Hellard P, Casazza G, and Hausswirth**
653 **C.** Whole-Body Cryostimulation Limits Overreaching in Elite Synchronized Swimmers. *Med*
654 *Sci Sports Exerc* 47: 1416-1425, 2015.
- 655 44. **Dill DB, and Costill DL.** Calculation of percentage changes in volumes of blood,
656 plasma, and red cells in dehydration. *J Appl Physiol* 37: 247-248, 1974.
- 657 45. **Sherk VD, Chrisman C, Smith J, Young KC, Singh H, Bemben MG, and Bemben**
658 **DA.** Acute bone marker responses to whole-body vibration and resistance exercise in young
659 women. *J Clin Densitom* 16: 104-109, 2013.
- 660 46. **Cohen J.** *Statistical Power Analysis for the Behavioral Sciences.* Hillsdale, NJ:
661 Lawrence Erlbaum Associates, 1988.
- 662 47. **Schober P, Boer C, and Schwarte LA.** Correlation Coefficients: Appropriate Use and
663 Interpretation. *Anesth Analg* 126: 1763-1768, 2018.

- 664 48. **Karlsen A, Nybo L, Norgaard SJ, Jensen MV, Bonne T, and Racinais S.** Time
665 course of natural heat acclimatization in well-trained cyclists during a 2-week training camp in
666 the heat. *Scand J Med Sci Sports* 25 Suppl 1: 240-249, 2015.
- 667 49. **Daanen HA, Jonkman AG, Layden JD, Linnane DM, and Weller AS.** Optimising
668 the acquisition and retention of heat acclimation. *Int J Sports Med* 32: 822-828, 2011.
- 669 50. **Racinais S, Periard JD, Karlsen A, and Nybo L.** Effect of heat and heat
670 acclimatization on cycling time trial performance and pacing. *Med Sci Sports Exerc* 47: 601-
671 606, 2015.
- 672 51. **Pandolf KB, Burse RL, and Goldman RF.** Role of physical fitness in heat
673 acclimatisation, decay and reinduction. *Ergonomics* 20: 399-408, 1977.
- 674 52. **Regan JM, Macfarlane DJ, and Taylor NAS.** An evaluation of the role of skin
675 temperature during heat adaptation. *Acta Physiol Scand* 158: 365-375, 1996.
- 676 53. **Racinais S, Alonso JM, Coutts AJ, Flouris AD, Girard O, González-Alonso J,**
677 **Hauswirth C, Jay O, Lee JK, Mitchell N, Nassis GP, Nybo L, Pluim BM, Roelands B,**
678 **Sawka MN, Wingo JE, and Périard JD.** Consensus recommendations on training and
679 competing in the heat. *Br J Sports Med* 49: 1164-1173, 2015.
- 680 54. **Shvartz E, Shapiro Y, Magazanik A, Meroz A, Birnfeld H, Mechtinger A, and**
681 **Shibolet S.** Heat acclimation, physical fitness, and responses to exercise in temperate and hot
682 environments. *J Appl Physiol Respir Environ Exerc Physiol* 43: 678-683, 1977.

- 683 55. **Kampmann B, Brode P, Schutte M, and Griefahn B.** Lowering of resting core
684 temperature during acclimation is influenced by exercise stimulus. *Eur J Appl Physiol* 104:
685 321-327, 2008.
- 686 56. **Ravanelli N, Gagnon D, Imbeault P, and Jay O.** A retrospective analysis to determine
687 if exercise training-induced thermoregulatory adaptations are mediated by increased fitness or
688 heat acclimation. *Exp Physiol* 106: 282-289, 2020.
- 689 57. **Avellini BA, Shapiro Y, Fortney SM, Wenger CB, and Pandolf KB.** Effects on heat
690 tolerance of physical training in water and on land. *J Appl Physiol Respir Environ Exerc*
691 *Physiol* 53: 1291-1298, 1982.
- 692 58. **Gerrett N, Alkemade P, and Daanen H.** Heat Reacclimation Using Exercise or Hot
693 Water Immersion. *Med Sci Sports Exerc* 53: 1517-1528, 2021.
- 694 59. **Waldock KAM, Gibson OR, Relf RL, Eichhorn G, Hayes M, Watt PW, and**
695 **Maxwell NS.** Exercise heat acclimation and post-exercise hot water immersion improve resting
696 and exercise responses to heat stress in the elderly. *J Sci Med Sport* 24: 774-780, 2021.
- 697 60. **Mee JA, Peters S, Doust JH, and Maxwell NS.** Sauna exposure immediately prior to
698 short-term heat acclimation accelerates phenotypic adaptation in females. *J Sci Med Sport* 21:
699 190-195, 2018.
- 700 61. **Foster J, Hodder SG, Lloyd AB, and Havenith G.** Individual Responses to Heat
701 Stress: Implications for Hyperthermia and Physical Work Capacity. *Front Physiol* 11: 541483,
702 2020.

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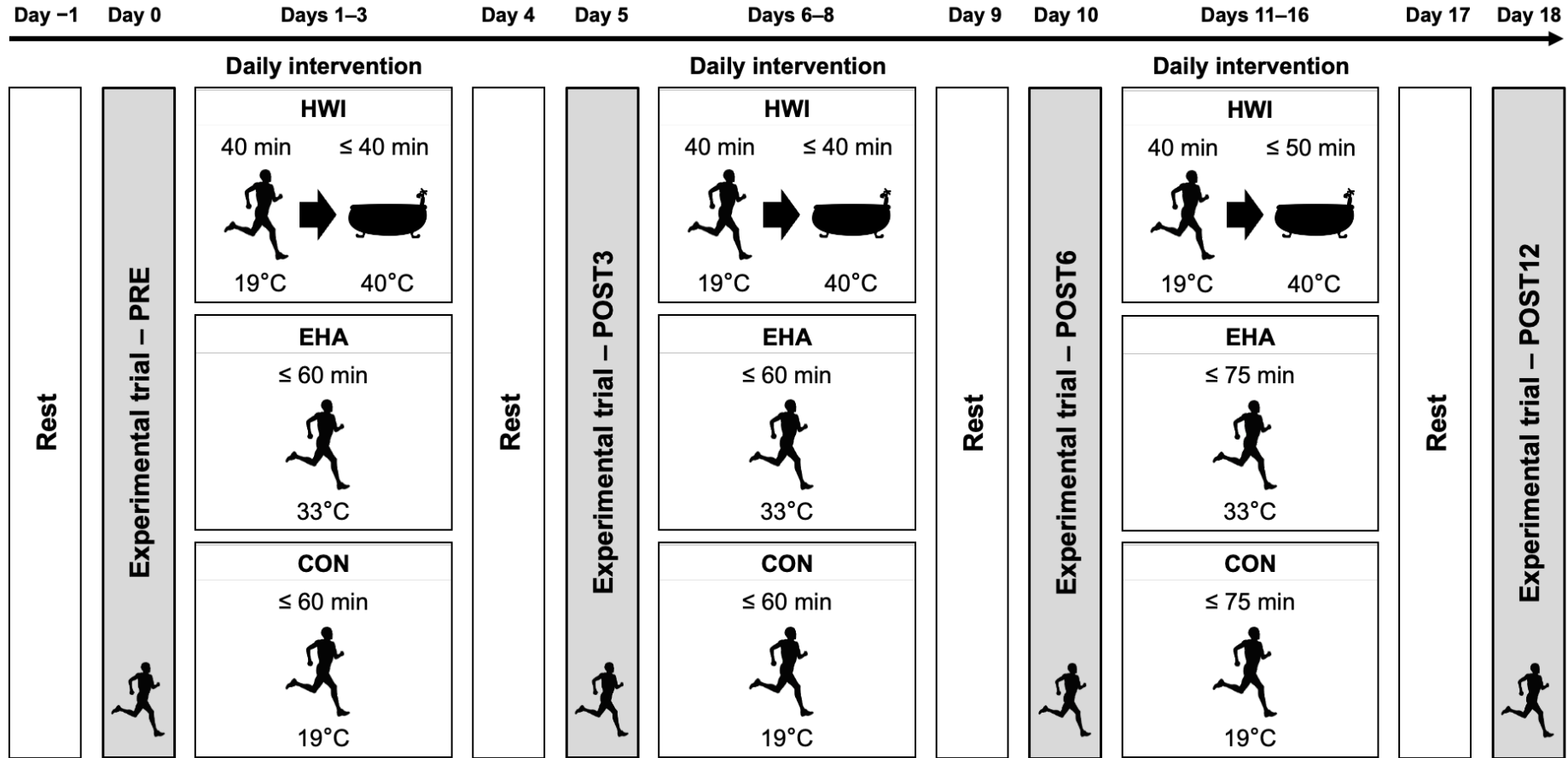
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711

712 **Disclosures**

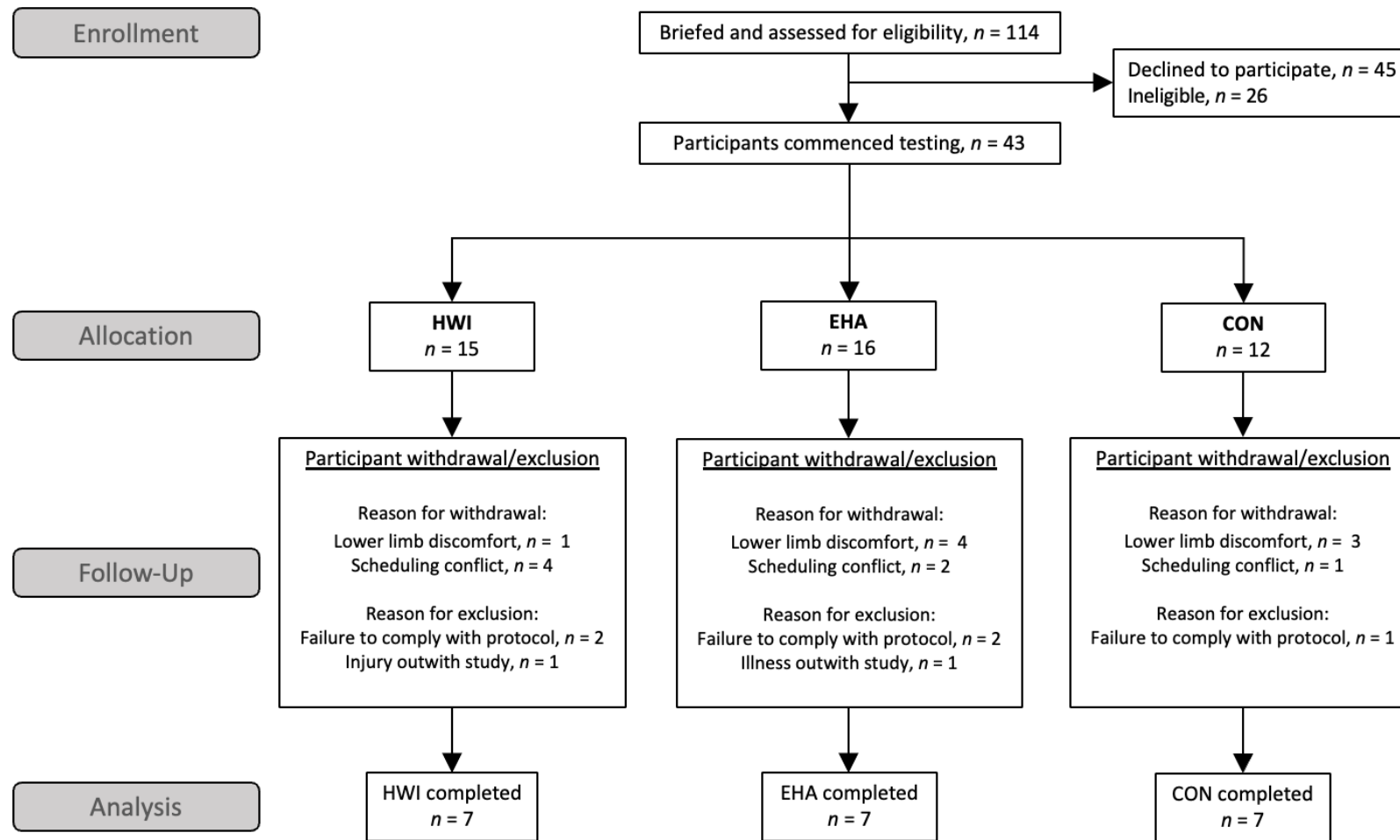
713 The authors declare that they have no conflicts of interest concerning this article.



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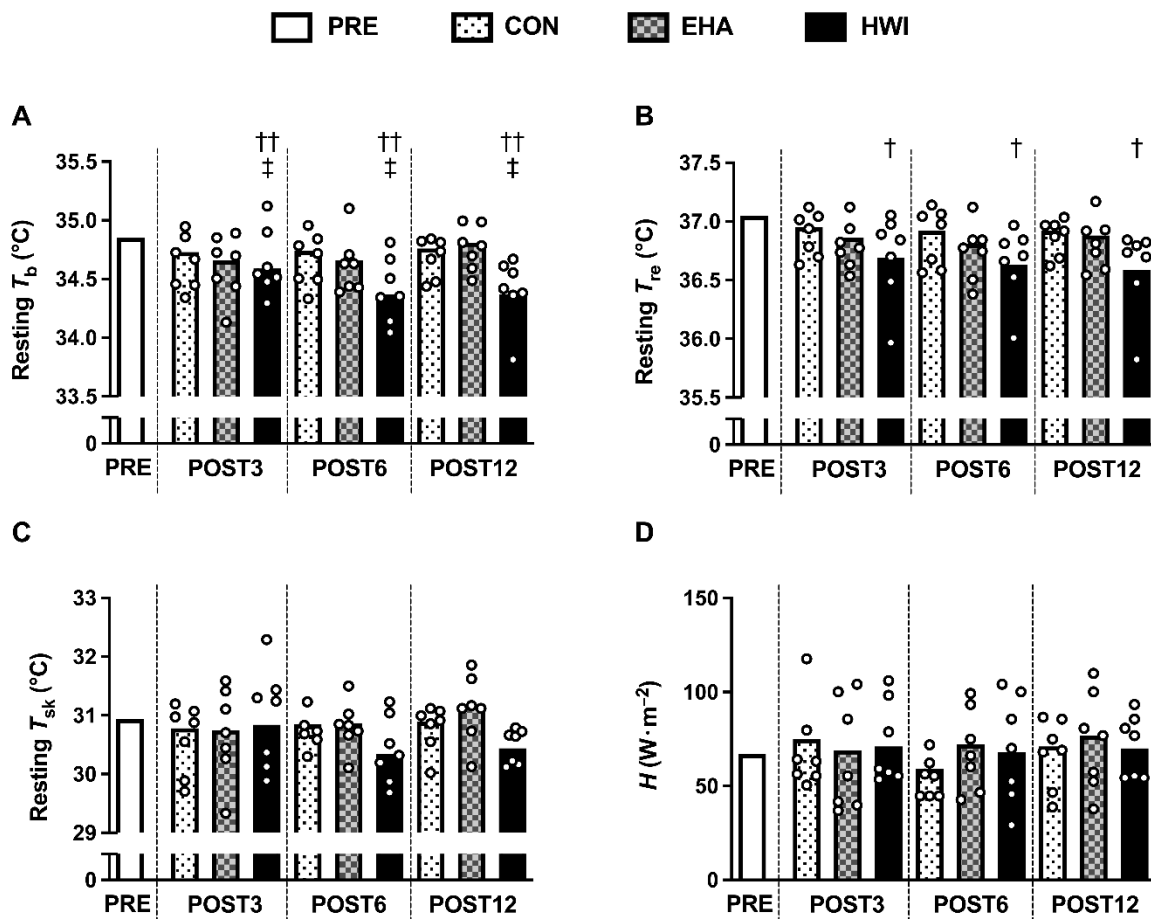
715 **Figure 1.** Schematic of the study design (Part 1). HWI; post-exercise hot water immersion, EHA; exercise heat acclimation and CON; temperate
 716 exercise control.

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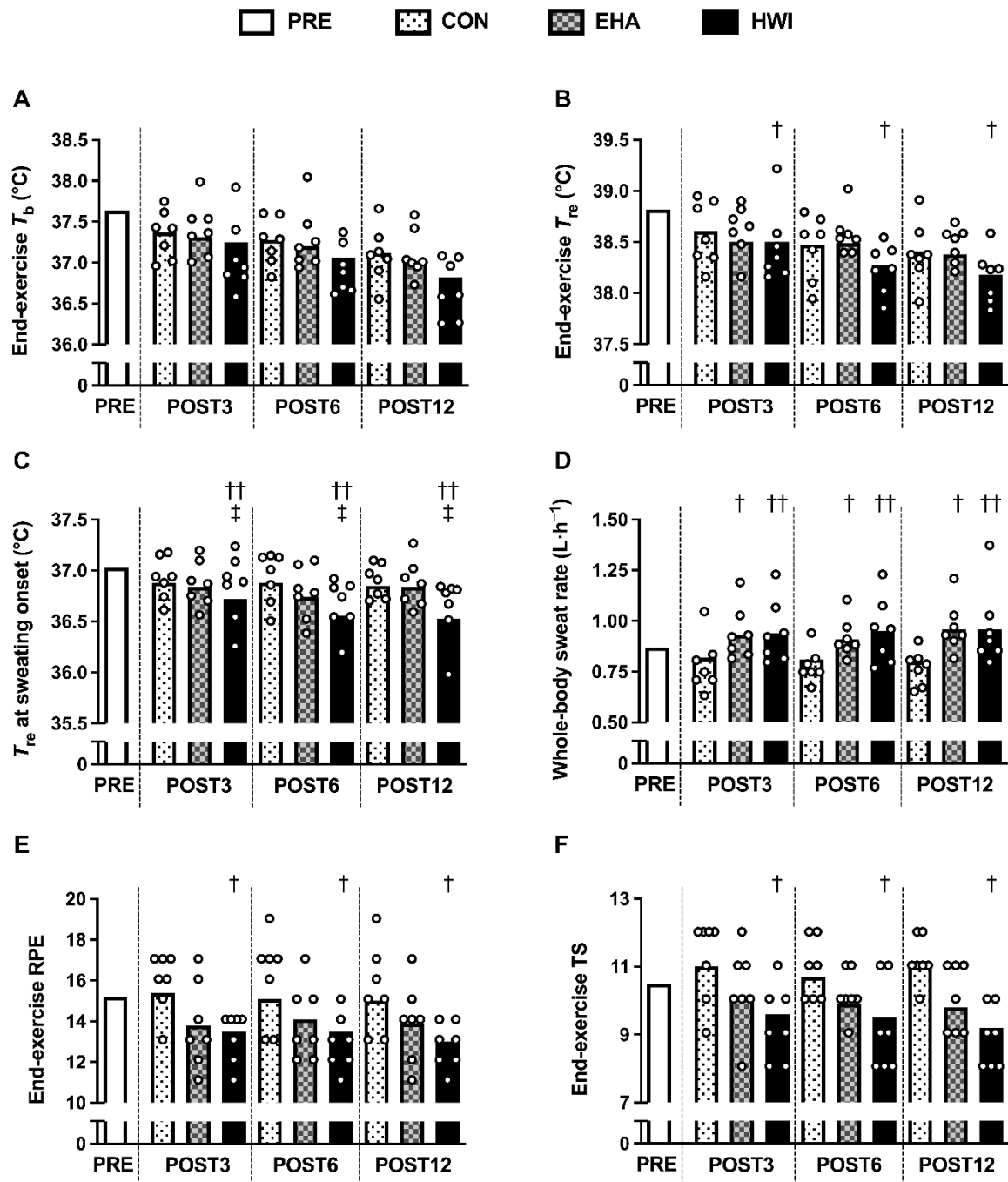
719 **Figure 2.** Flow diagram indicating the numbers of participants assessed for eligibility, commenced testing, and withdrew, were excluded, or
720 completed the study protocol (Part 1). HWI; post-exercise hot water immersion, EHA; exercise heat acclimation and CON; temperate exercise
721 control.



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724 **Figure 3.** Influence of 3 (POST3), 6 (POST6), and 12 days (POST12) of a temperate exercise
725 control (CON, $n = 7$), exercise heat acclimation (EHA, $n = 7$), or post-exercise hot water
726 immersion (HWI, $n = 7$) on resting mean body temperature (T_b , A), rectal core temperature
727 (T_{re} , B), mean skin temperature (T_{sk} , C), and metabolic heat production (H , D) in temperate
728 conditions (19°C , 45% RH). Bars represent baseline-adjusted means; circles represent
729 individual participant responses. †denotes HWI lower than CON, $P < 0.05$; ††denotes HWI
730 lower than CON, $P < 0.01$; ‡denotes HWI lower than EHA, $P < 0.05$.

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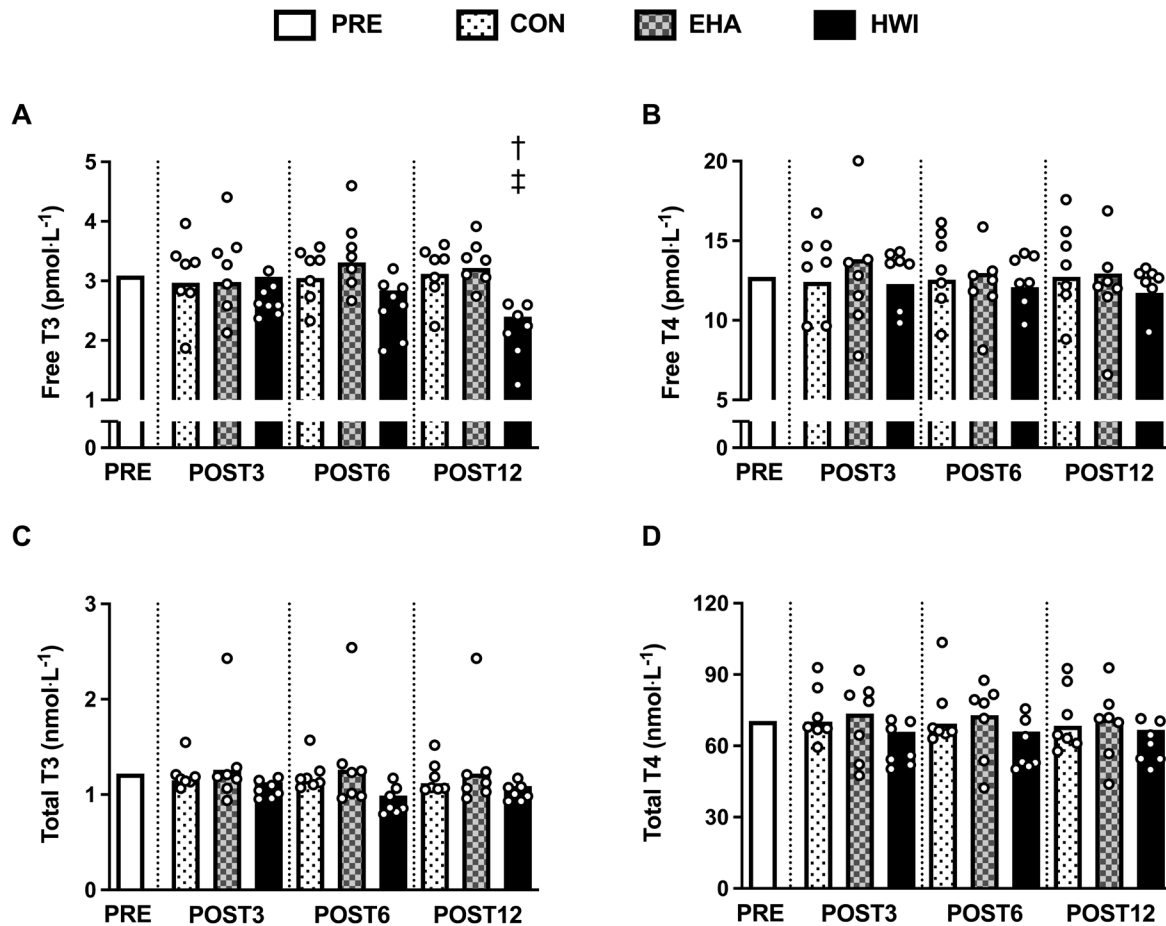


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734 **Figure 4.** Influence of 3 (POST3), 6 (POST6), and 12 days (POST12) of a temperate exercise
 735 control (CON, $n = 7$), exercise heat acclimation (EHA, $n = 7$), or post-exercise hot water
 736 immersion (HWI, $n = 7$) on end-exercise mean body temperature (T_b , A), end-exercise rectal
 737 core temperature (T_{re} , B), T_{re} at sweating onset (C), whole-body sweat rate (D), end-exercise
 738 rating of perceived exertion (RPE, E), and end-exercise thermal sensation (TS, F) in the heat
 739 (33°C, 40% RH). Bars represent baseline-adjusted means; circles represent individual

740 participant responses. circles represent individual participant responses. †denotes group
741 difference to CON, $P < 0.05$; ††denotes group difference to CON, $P < 0.01$; ‡denotes HWI
742 lower than EHA, $P < 0.05$.

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746 **Figure 5.** Influence of 3 (POST3), 6 (POST6), and 12 days (POST12) of a temperate exercise

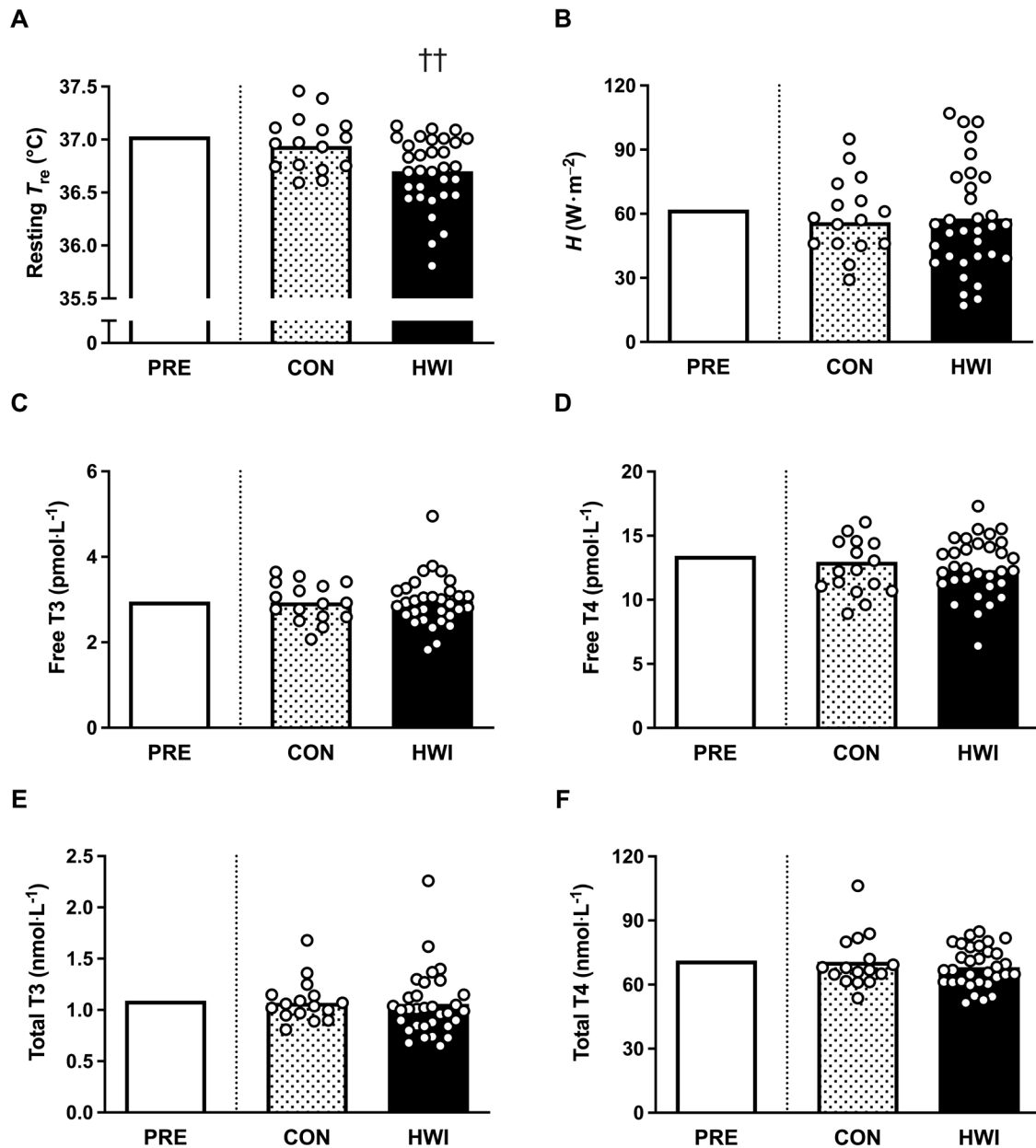
747 control (CON, $n = 7$), exercise heat acclimation (EHA, $n = 7$), or post-exercise hot water

748 immersion (HWI, $n = 7$) on plasma concentrations of free triiodothyronine (T3; A), free

749 thyroxine (T4; B), total T3 (C), and total T4 (D). Bars represent baseline-adjusted means;

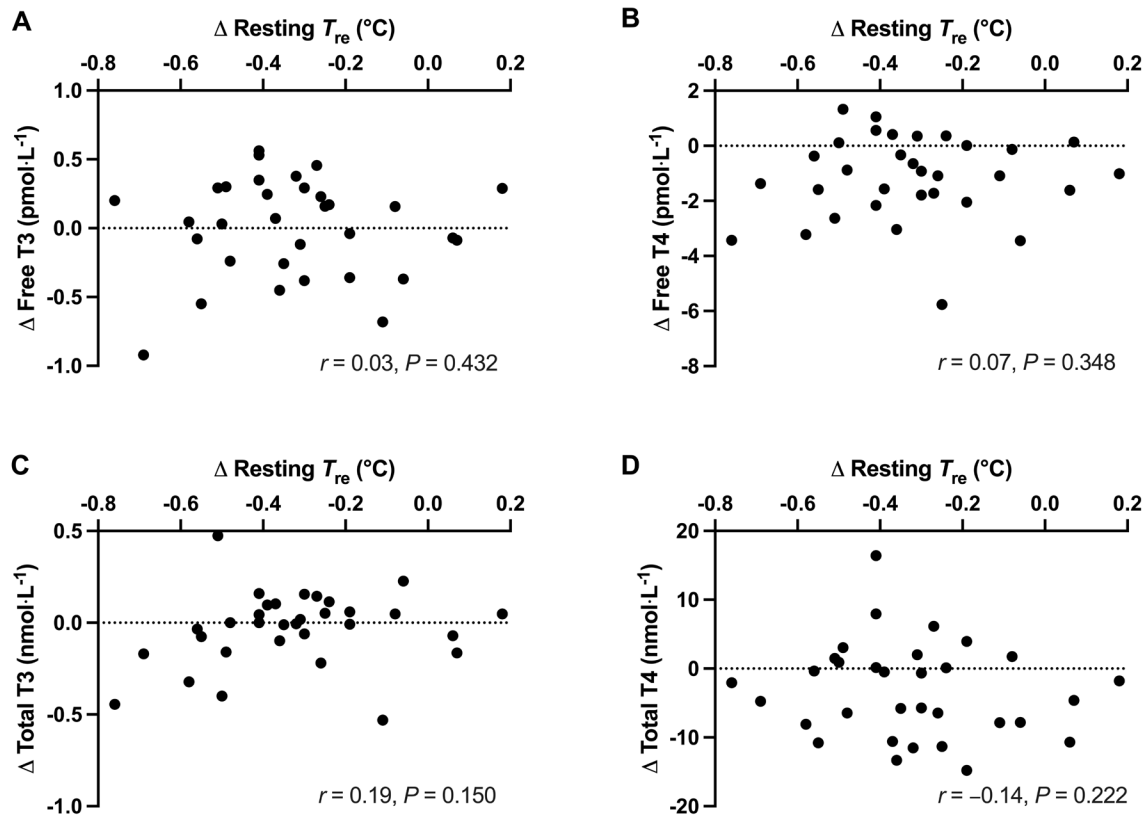
750 circles represent individual participant responses. †denotes HWI lower than CON, $P < 0.05$;

751 ‡denotes HWI lower than EHA, $P < 0.05$.



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754 **Figure 6.** Influence of 6 days of a temperate exercise control (CON, $n = 16$) or post-exercise
755 hot water immersion (HWI, $n = 32$) on resting rectal core temperature (T_{re} ; A), resting
756 metabolic heat production (H , B), and resting plasma concentrations of free triiodothyronine
757 (T3; C), free thyroxine (T4; D), total T3 (E), and total T4 (F). Bars represent baseline-adjusted
758 means; circles represent individual participant responses. †† denotes HWI lower than CON, $P <$
759 0.01.



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762 **Figure 7.** The relationships between the changes in resting core temperature (T_{re}) and plasma
763 concentrations of thyroid hormones free triiodothyronine (T3; A), free thyroxine (T4; B), total
764 T3 (C), and total T4 (D) after 6 days of post-exercise hot water immersion ($n = 32$).

Table 1. Part 1 participant characteristics of post-exercise hot water immersion (HWI), exercise heat acclimation (EHA), and temperate exercise control (CON).

	HWI	EHA	CON
Age (years)	22 ± 3	21 ± 2	22 ± 2
Height (cm)	176 ± 4	183 ± 5	177 ± 6
Body mass (kg)	70 ± 6	75 ± 6	70 ± 7
$\dot{V}O_{2\text{peak}}$ (mL·kg ⁻¹ ·min ⁻¹)	53 ± 7	54 ± 3	53 ± 4
Running economy (Kcal·kg ⁻¹ ·min ⁻¹)	3.3 ± 0.1	3.6 ± 0.4	3.5 ± 0.3

Data are displayed as mean ± SD; n = 7, each group.

Table 2. The daily endogenous thermal stimulus and external work during temperate exercise control (CON), exercise heat acclimation (EHA), and post-exercise hot water immersion (HWI) interventions.

	Days 1–3			Days 6–8			Days 11–16		
	CON	EHA	HWI	CON	EHA	HWI	CON	EHA	HWI
Duration $T_{re} \geq 38.5^\circ\text{C}$ (min)	7 ± 12	35 ± 14 ^{††}	36 ± 5 ^{††}	8 ± 12	38 ± 11 ^{††}	38 ± 6 ^{††}	7 ± 10	38 ± 13 ^{††}	39 ± 8 ^{††}
AUC ($^\circ\text{C}\cdot\text{min}^{-1}$)	1 ± 3	17 ± 10 ^{††}	17 ± 5 ^{††}	2 ± 4	16 ± 8 ^{††}	18 ± 4 ^{††}	1 ± 1	12 ± 6 ^{††}	20 ± 6 ^{††}
End-intervention T_{re} ($^\circ\text{C}$)	38.24 ± 0.34	39.17 ± 0.28 ^{††}	39.24 ± 0.16 ^{††}	38.22 ± 0.46	39.11 ± 0.22 ^{††}	39.27 ± 0.14 ^{††}	38.23 ± 0.22	38.99 ± 0.25 ^{††}	39.31 ± 0.18 ^{††}
External work (km)	7.4 ± 1.1	7.7 ± 1.6	6.1 ± 1.1 [‡]	7.6 ± 1.7	8.1 ± 1.7	6.1 ± 1.1 [‡]	8.7 ± 1.7	9.0 ± 1.5	6.1 ± 1.1 [‡]

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, Days 6–8 and Days 11–16. ^{††}denotes a group difference to CON, $P < 0.01$; [‡]denotes a group difference to EHA, $P < 0.05$. $n = 21$ (Part 1).

Table 3. Change (mean \pm SD) from baseline in heat acclimation adaptations at rest (19°C, 45% RH) and during 40-min submaximal exercise in the heat (33°C, 40% RH) after 3 (POST3), 6 (POST6), and 12 days (POST12) of a temperate exercise control (CON), exercise heat acclimation (EHA), or post-exercise hot water immersion (HWI).

	CON			EHA			HWI		
	POST3	POST6	POST12	POST3	POST6	POST12	POST3	POST6	POST12
Rest									
Resting T_b (°C)	-0.12 \pm 0.20	-0.11 \pm 0.22	-0.09 \pm 0.20	-0.19 \pm 0.20	-0.18 \pm 0.21	-0.03 \pm 0.20	-0.26 \pm 0.20 ^{††, ‡‡}	-0.48 \pm 0.22 ^{††, ‡‡}	-0.48 \pm 0.20 ^{††, ‡‡}
Resting T_{re} (°C)	-0.10 \pm 0.19	-0.13 \pm 0.18	-0.13 \pm 0.19	-0.19 \pm 0.19	-0.24 \pm 0.18	-0.17 \pm 0.19	-0.35 \pm 0.19 ^{††}	-0.41 \pm 0.18 ^{††}	-0.46 \pm 0.19 ^{††}
Resting T_{sk} (°C)	-0.17 \pm 0.63	-0.09 \pm 0.36	-0.05 \pm 0.42	-0.19 \pm 0.62	-0.07 \pm 0.36	-0.23 \pm 0.41	-0.10 \pm 0.63	-0.60 \pm 0.36	-0.50 \pm 0.42
Resting H (W·m ⁻²)	7 \pm 20	-9 \pm 15	4 \pm 17	2 \pm 20	4 \pm 15	9 \pm 17	3 \pm 21	0 \pm 15	3 \pm 17
Plasma volume (%)	3 \pm 7	3 \pm 5	2 \pm 7	3 \pm 7	6 \pm 5	5 \pm 7	1 \pm 7	4 \pm 5	3 \pm 7
Submaximal exercise									
End-exercise T_b (°C)	-0.27 \pm 0.24	-0.36 \pm 0.24	-0.52 \pm 0.25	-0.33 \pm 0.25	-0.44 \pm 0.25	-0.62 \pm 0.26	-0.39 \pm 0.25	-0.58 \pm 0.25	-0.83 \pm 0.26
End-exercise T_{re} (°C)	-0.21 \pm 0.23	-0.36 \pm 0.21	-0.41 \pm 0.20	-0.32 \pm 0.24	-0.33 \pm 0.21	-0.44 \pm 0.21	-0.32 \pm 0.23 [†]	-0.56 \pm 0.21 [†]	-0.64 \pm 0.20 [†]
ΔT_{re} during exercise (°C)	-0.10 \pm 0.26	-0.22 \pm 0.29	-0.28 \pm 0.29	-0.16 \pm 0.28	-0.09 \pm 0.30	-0.29 \pm 0.30	-0.06 \pm 0.27	-0.15 \pm 0.30	-0.18 \pm 0.30
T_{re} at sweating onset (°C)	-0.15 \pm 0.16	-0.15 \pm 0.19	-0.18 \pm 0.15	-0.19 \pm 0.17	-0.29 \pm 0.19	-0.19 \pm 0.15	-0.30 \pm 0.17 ^{††, ‡}	-0.47 \pm 0.19 ^{††, ‡}	-0.50 \pm 0.15 ^{††, ‡}
Whole-body sweat rate (L·h ⁻¹)	-0.05 \pm 0.09	-0.05 \pm 0.06	0.07 \pm 0.11	0.06 \pm 0.09 [†]	0.04 \pm 0.06 [†]	0.09 \pm 0.10 [†]	0.08 \pm 0.09 ^{††}	0.08 \pm 0.06 ^{††}	0.10 \pm 0.10 ^{††}
End-exercise T_{sk} (°C)	-0.38 \pm 0.49	-0.38 \pm 0.46	-0.73 \pm 0.54	-0.39 \pm 0.50	-0.66 \pm 0.47	-0.95 \pm 0.55	-0.50 \pm 0.52	-0.60 \pm 0.48	-1.15 \pm 0.57
End-exercise heart rate (beats·min ⁻¹)	-8 \pm 5	-12 \pm 7	-14 \pm 8	-12 \pm 5	-15 \pm 7	-20 \pm 8	-11 \pm 5	-17 \pm 7	-20 \pm 8
Mean $\dot{V}O_2$ (L·min ⁻¹)	-0.10 \pm 0.13	-0.10 \pm 0.15	-0.16 \pm 0.13	-0.01 \pm 0.13	0.00 \pm 0.15	-0.06 \pm 0.13	-0.04 \pm 0.13	-0.04 \pm 0.14	-0.06 \pm 0.12
Mean RER	-0.01 \pm 0.04	-0.02 \pm 0.03	-0.03 \pm 0.04	-0.02 \pm 0.04	-0.02 \pm 0.03	-0.01 \pm 0.04	-0.02 \pm 0.04	-0.02 \pm 0.03	-0.02 \pm 0.04
End-exercise RPE (6–20 scale)	0 \pm 2	0 \pm 1	0 \pm 2	-1 \pm 2	-1 \pm 1	-1 \pm 2	-2 \pm 2 [†]	-2 \pm 1 [†]	-2 \pm 2 [†]
End-exercise TS (1–13 scale)	0 \pm 1	0 \pm 1	0 \pm 1	0 \pm 1	-1 \pm 1	-1 \pm 1	-1 \pm 1 [†]	-1 \pm 1 [†]	-1 \pm 1 [†]

T_b , mean body temperature; T_{re} , rectal core temperature; T_{sk} , mean skin temperature; H , metabolic heat production; RER, respiratory exchange ratio; RPE, rating of perceived exertion; TS, thermal sensation. Data are baseline-adjusted mean change \pm SD change at POST3, POST6, and POST12. [†]denotes a group difference to CON, $P < 0.05$; ^{††}denotes a group difference to CON, $P < 0.01$; [‡]denotes a group difference to EHA, $P < 0.05$. $n = 21$ (Part 1).

Table 4. Change (mean \pm SD) from baseline in markers of overreaching and endurance capacity in the heat (33°C, 40% RH) after 3 (POST3), 6 (POST6), and 12 days (POST12) of a temperate exercise control (CON), exercise heat acclimation (EHA), or post-exercise hot water immersion (HWI).

	CON			EHA			HWI		
	POST3	POST6	POST12	POST3	POST6	POST12	POST3	POST6	POST12
Markers of overreaching									
Total mood disturbance	5 \pm 10	2 \pm 12	2 \pm 10	5 \pm 10	7 \pm 12	2 \pm 10	4 \pm 10	4 \pm 12	2 \pm 10
Energy index	-3 \pm 4	-2 \pm 6	-3 \pm 5	-3 \pm 4	-5 \pm 6	-3 \pm 5	-2 \pm 4	-4 \pm 6	-3 \pm 5
Stroop reaction time (ms)	-29 \pm 58	-25 \pm 40	-11 \pm 64	-13 \pm 58	-32 \pm 40	-15 \pm 63	-16 \pm 62	-18 \pm 43	-28 \pm 68
Stroop accuracy (%)	0 \pm 2	-1 \pm 3	1 \pm 4	-1 \pm 3	-1 \pm 3	-2 \pm 4	2 \pm 3	1 \pm 3	0 \pm 4
Sleep duration (h)	6 \pm 1	6 \pm 1	6 \pm 1	6 \pm 1	6 \pm 1	6 \pm 1	6 \pm 1	6 \pm 1	6 \pm 1
Sleep efficiency (%)	0 \pm 9	-2 \pm 7	-1 \pm 8	-6 \pm 9	-5 \pm 7	1 \pm 8	-2 \pm 9	2 \pm 7	-2 \pm 8
Endurance capacity									
TTE (s)	-27 \pm 676	75 \pm 808	212 \pm 991	101 \pm 627	539 \pm 749	323 \pm 919	321 \pm 743	686 \pm 888	1030 \pm 1089
End TTE T_{re} (°C)	-0.14 \pm 0.30	-0.24 \pm 0.34	-0.32 \pm 0.47	-0.20 \pm 0.31	-0.06 \pm 0.34	-0.29 \pm 0.47	-0.20 \pm 0.31	-0.03 \pm 0.34	-0.25 \pm 0.47
End-TTE heart rate (beats·min ⁻¹)	-8 \pm 8	-10 \pm 7	-16 \pm 10	-10 \pm 8	-12 \pm 7	-20 \pm 10	-8 \pm 8	-10 \pm 7	-14 \pm 10
End-TTE blood lactate (beats·min ⁻¹)	0.2 \pm 1.4	-0.1 \pm 0.7	0.2 \pm 0.6	0.5 \pm 1.3	-0.2 \pm 0.7	-0.9 \pm 0.6	-0.2 \pm 1.3	-0.1 \pm 0.7	0.2 \pm 0.6

T_{re} , rectal core temperature; TTE, time to exhaustion. Data are baseline-adjusted mean change \pm SD change at POST3, POST6, and POST12. $n = 21$, except $n = 16$ for TTE (Part 1).