PRIFYSGOL
BANGOR
UNIVERSITY

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

McIntyre, Robert; Zurawlew, Michael; Mee, Jessica; Walsh, Neil; Oliver, Sam

American Journal of Physiology - Regulatory Integrative and Comparative Physiology

Peer reviewed version

Cyswllt i'r cyhoeddiad / Link to publication

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

5 **Authors**

6 Robert D. McIntyre[1], Michael J. Zurawlew[2], Jessica A. Mee[3], Neil P. Walsh[2] and Samuel J.

7 Oliver[1]

8

9 **Institution and Affiliations**

10 [1] School of Human and Behavioural Sciences, Bangor University, Bangor, United Kingdom.

11 [2] School of Sport and Exercise Sciences, Liverpool John Moores University, United Kingdom.

12 [3] School of Sport and Exercise Science, University of Worcester, Worcester, United Kingdom.

13

14 Corresponding author: Samuel J. Oliver, Institute for Applied Human Physiology, School of

15 Human and Behavioural Sciences, Bangor University, George Building, Normal Site,

16 Gwynedd, LL57 2PZ, s.j.oliver@bangor.ac.uk, ORCID: 0000-0002-9971-9546

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 ± 0.15°C, HWI -0.41 ± 0.15°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 ± 0.14°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

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.

Methods

Experimental approaches

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

Participant recruitment and randomization

Part 1 participant flow and attrition before protocol completion, and biochemical and statistical analyses are summarized in Fig. 2. Participants were matched for $\dot{V}O_{2peak}$ in groups of three and randomly assigned to either HWI, EHA, or CON (randomizer.org). Participants were 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; $\dot{V}O_{2peak}$, 58 ± 8 mL·kg^{-1}·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). $\dot{V}O_{2peak}$ 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. $\dot{V}O_{2peak}$ was determined as

148   the highest oxygen uptake attained over a 30-s period. The average values of breath-by-breath

149     $\dot{V}O_2$ and $\dot{V}CO_2$ 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% $\dot{V}O_{2peak}$ 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            $$H \, (\text{W} \cdot \text{m}^{-2}) = [0.23(\text{RER}) + 0.77] \cdot [5.873(\dot{V}O_2)] \cdot (60 \, / \, A_D).$$

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    $\dot{V}O_{2peak}$. 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-cm^2

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 $T_{re} \leq 38.5°C$.

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 ($\leq$ 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 $\leq$ 40-min HWI (40°C) to the neck, as previously

225   described (6). Participants assigned to EHA completed a $\leq$ 60-min treadmill run at a speed

226   equivalent to their 65% $\dot{V}O_{2peak}$ (9.1 ± 1.1 km·h^{-1}) in an environmental chamber (33°C, 40%

227   RH, 0.2 m·s^{-1} wind velocity). Participants assigned to CON completed a daily submaximal

228   treadmill run equivalent to 65% $\dot{V}O_{2peak}$ and work-matched to EHA (8.8 ± 0.9 km·h^{-1}) in

229   temperate conditions (19°C, 45% RH, 0.2 m·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 $T_{re} \leq 38.5$°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 (%ΔPV) 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 pmol·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   Corrected value = Uncorrected value · ((100 + %ΔPV)/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).

Results

Part 1 daily intervention thermal stimulus and external work

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

Part 1 experimental trials

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

Thermal responses at rest in temperate conditions

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

307    $(-0.20 \pm 0.15°C, P = 0.061, d = 1.37)$. 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 $T_{re}-T_{sk}$ 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, $-0.50 \pm 0.19°C$) than CON $(-0.33 \pm 0.13°C; P = 0.049, d = 1.13)$, but not EHA

316    $(-0.37 \pm 0.13°C; P = 0.196, d = 0.88)$; 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, $-0.43 \pm 0.12°C$) than

318   EHA $(-0.22 \pm 0.12°C; P = 0.015, d = 1.75)$ and CON $(-0.16 \pm 0.12°C; P = 0.002, d = 2.27)$.

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, $+0.08 \text{ L·h}^{-1}; P = 0.003, d = 2.13$) and

321   EHA $(+0.06 \text{ L·h}^{-1}; P = 0.013, d = 1.78)$ than CON (-0.06 L·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 $T_{re}-T_{sk}$ gradient, end-exercise heart rate,

329   exercising $\dot{V}O_2$, 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 $T_{re} > 38.5°C$ (HWI, 41 ± 13 min; CON, 7 ± 8; $P < 0.001$), AUC for $T_{re} > 38.5°C$ (HWI,

360 23 ± 10 °C·min^{-1}; CON, 1 ± 2 °C·min^{-1}; $P < 0.001$), and end-intervention T_{re} (HWI, $39.3 \pm$

361 $0.2°C$; CON, $38.3 \pm 0.4°C$; $P < 0.001$). 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, $-0.31 \pm 0.32°C$; 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, $-0.33 \pm 0.20°C$; CON, $-0.09 \pm 0.21°C$; $P = 0.001$, 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, $-0.18 \pm 0.24°C$; $P < 0.001$), end-exercise T_{re} (HWI, $-0.42 \pm 0.24°C$; CON,

375 $-0.13 \pm 0.24°C$; $P < 0.001$), T_{re} at sweating onset (HWI, $-0.31 \pm 0.20°C$; CON, $-0.08 \pm 0.19°C$;

376 $P = 0.01$), end-exercise T_{sk} (HWI, $-0.74 \pm 0.54°C$; CON, $-0.30 \pm 0.54°C$; $P < 0.001$), 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 ($P = 0.228$).

17

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    $-0.37 \pm 0.13°C$, 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.

24

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.

25

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.

26

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 Muhlrad 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   **Hausswirth 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:
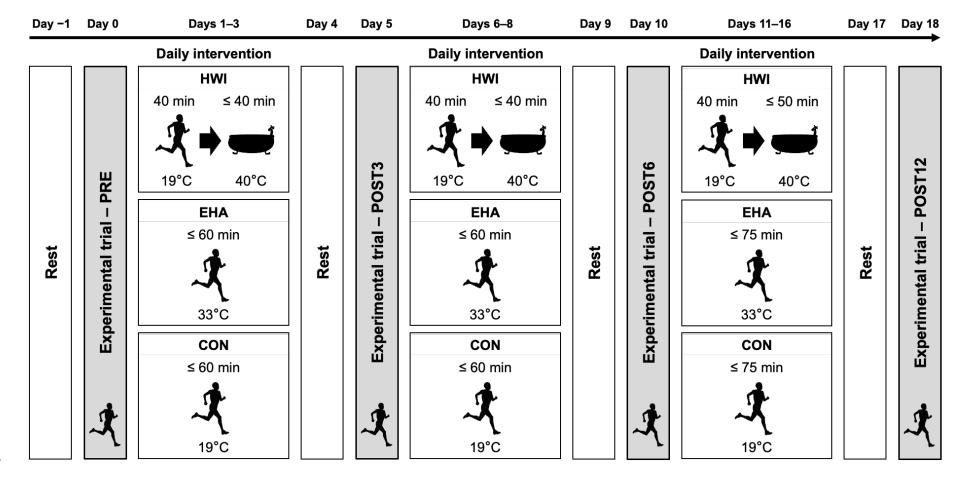
699   190-195, 2018.

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.

703 **Acknowledgments**

704 We would like to thank Dr Jason Edwards and Kevin Williams for their valuable technical

705 assistance with data collection. We are also indebted to the participants for their time and co-

706 operation.

707

708 **Grants**

709 The work was funded by the Chief Scientific Advisers research program, Ministry of Defence

710 UK, through the Defence Science and Technology Laboratory.

711

712 **Disclosures**

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

Figure 1. Schematic of the study design (Part 1). HWI; post-exercise hot water immersion, EHA; exercise heat acclimation and CON; temperate exercise control.
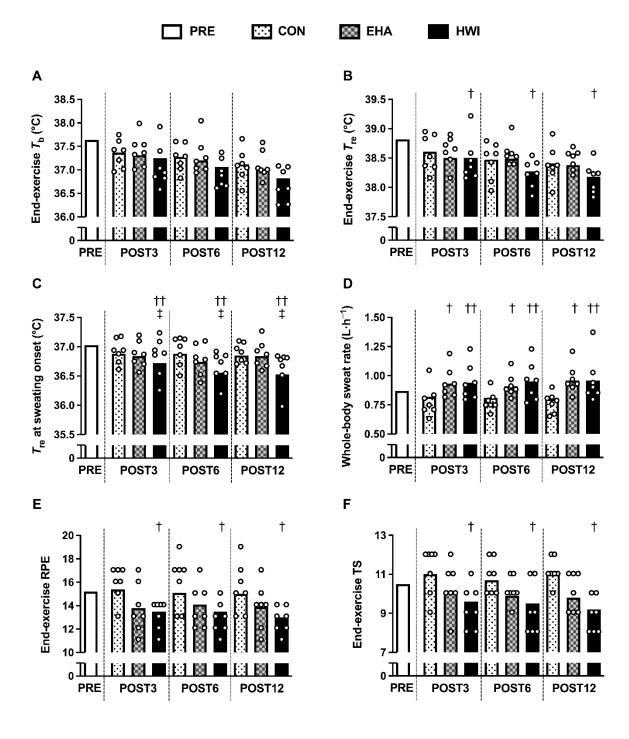
718

Figure 2. Flow diagram indicating the numbers of participants assessed for eligibility, commenced testing, and withdrew, were excluded, or

completed the study protocol (Part 1). HWI; post-exercise hot water immersion, EHA; exercise heat acclimation and CON; temperate exercise

control.

Figure 3. Influence of 3 (POST3), 6 (POST6), and 12 days (POST12) of a temperate exercise control (CON, $n = 7$), exercise heat acclimation (EHA, $n = 7$), or post-exercise hot water immersion (HWI, $n = 7$) on resting mean body temperature (T_b, A), rectal core temperature (T_{re}, B), mean skin temperature (T_{sk}, C), and metabolic heat production (H, D) in temperate conditions (19°C, 45% RH). Bars represent baseline-adjusted means; circles represent individual participant responses. [†]denotes HWI lower than CON, $P < 0.05$; [††]denotes HWI lower than CON, $P < 0.01$; [‡]denotes HWI lower than EHA, $P < 0.05$.
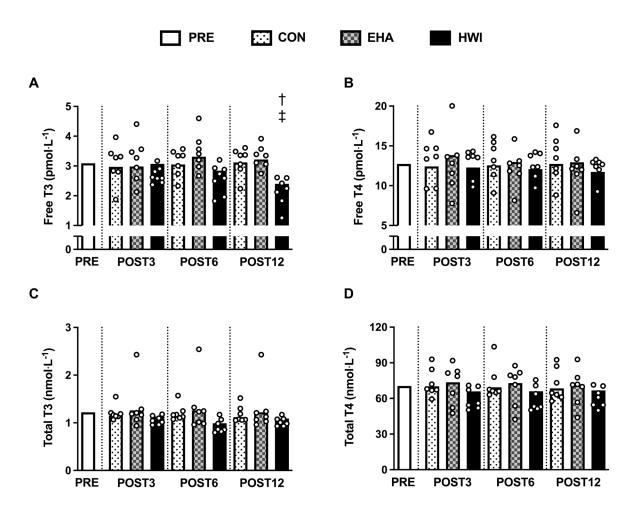
Figure 4. Influence of 3 (POST3), 6 (POST6), and 12 days (POST12) of a temperate exercise control (CON, $n = 7$), exercise heat acclimation (EHA, $n = 7$), or post-exercise hot water immersion (HWI, $n = 7$) on end-exercise mean body temperature (T_b, A), end-exercise rectal core temperature (T_{re}, B), T_{re} at sweating onset (C), whole-body sweat rate (D), end-exercise rating of perceived exertion (RPE, E), and end-exercise thermal sensation (TS, F) in the heat (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, $P < 0.05$.
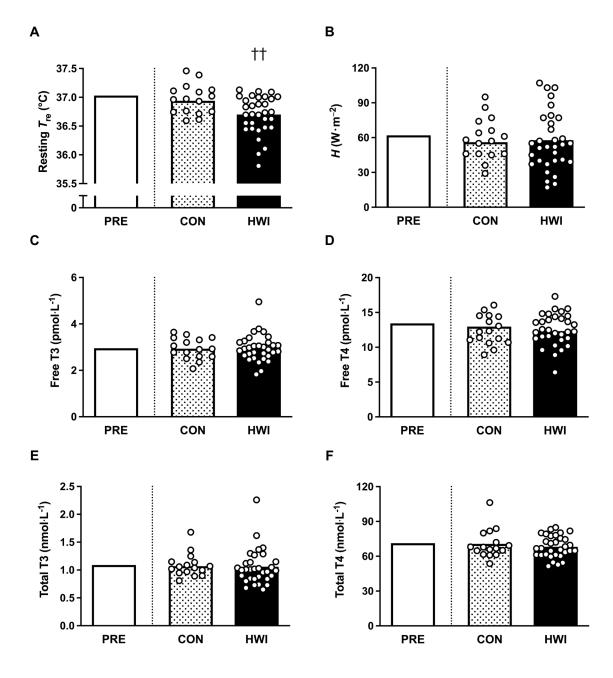
743

744
745
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, $P < 0.05$.

Figure 6. Influence of 6 days of a temperate exercise control (CON, $n = 16$) or post-exercise hot water immersion (HWI, $n = 32$) on resting rectal core temperature (T_{re}; A), resting metabolic heat production (H, B), and resting plasma concentrations of free triiodothyronine (T3; C), free thyroxine (T4; D), total T3 (E), and total T4 (F). Bars represent baseline-adjusted means; circles represent individual participant responses. [††]denotes HWI lower than CON, $P < 0.01$.
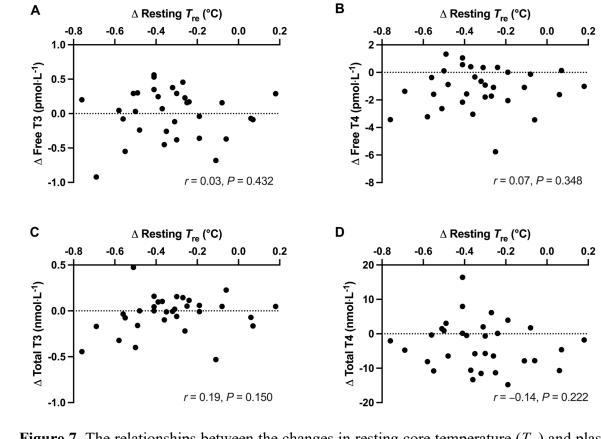
Figure 7. The relationships between the changes in resting core temperature (T_{re}) and plasma concentrations of thyroid hormones free triiodothyronine (T3; A), free thyroxine (T4; B), total T3 (C), and total T4 (D) after 6 days of post-exercise hot water immersion ($n = 32$).

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
$\dot{V}O_{2peak}$ (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.

765

42

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 $T_{re} \geq 38.5°C$ (min)	7 ± 12	35 ± 14 [††]	36 ± 5 [††]	8 ± 12	38 ± 11 [††]	38 ± 6 [††]	7 ± 10	38 ± 13 [††]	39 ± 8 [††]
AUC ($°C·min^{-1}$)	1 ± 3	17 ± 10 [††]	17 ± 5 [††]	2 ± 4	16 ± 8 [††]	18 ± 4 [††]	1 ± 1	12 ± 6 [††]	20 ± 6 [††]
End-intervention T_{re} (°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 $T_{re} > 38.5°C$. 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).

766

Table 3. Change (mean ± 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 ± 0.20	−0.11 ± 0.22	−0.09 ± 0.20	−0.19 ± 0.20	−0.18 ± 0.21	−0.03 ± 0.20	−0.26 ± 0.20 [††, ‡‡]	−0.48 ± 0.22 [††, ‡‡]	−0.48 ± 0.20 [††, ‡‡]
Resting T_{re} (°C)	−0.10 ± 0.19	−0.13 ± 0.18	−0.13 ± 0.19	−0.19 ± 0.19	−0.24 ± 0.18	−0.17 ± 0.19	−0.35 ± 0.19 [††]	−0.41 ± 0.18 [††]	−0.46 ± 0.19 [††]
Resting T_{sk} (°C)	−0.17 ± 0.63	−0.09 ± 0.36	−0.05 ± 0.42	−0.19 ± 0.62	−0.07 ± 0.36	−0.23 ± 0.41	−0.10 ± 0.63	−0.60 ± 0.36	−0.50 ± 0.42
Resting H (W·m⁻²)	7 ± 20	−9 ± 15	4 ± 17	2 ± 20	4 ± 15	9 ± 17	3 ± 21	0 ± 15	3 ± 17
Plasma volume (%)	3 ± 7	3 ± 5	2 ± 7	3 ± 7	6 ± 5	5 ± 7	1 ± 7	4 ± 5	3 ± 7
Submaximal exercise									
End-exercise T_b (°C)	−0.27 ± 0.24	−0.36 ± 0.24	−0.52 ± 0.25	−0.33 ± 0.25	−0.44 ± 0.25	−0.62 ± 0.26	−0.39 ± 0.25	−0.58 ± 0.25	−0.83 ± 0.26
End-exercise T_{re} (°C)	−0.21 ± 0.23	−0.36 ± 0.21	−0.41 ± 0.20	−0.32 ± 0.24	−0.33 ± 0.21	−0.44 ± 0.21	−0.32 ± 0.23 [†]	−0.56 ± 0.21 [†]	−0.64 ± 0.20 [†]
Δ T_{re} during exercise (°C)	−0.10 ± 0.26	−0.22 ± 0.29	−0.28 ± 0.29	−0.16 ± 0.28	−0.09 ± 0.30	−0.29 ± 0.30	−0.06 ± 0.27	−0.15 ± 0.30	−0.18 ± 0.30
T_{re} at sweating onset (°C)	−0.15 ± 0.16	−0.15 ± 0.19	−0.18 ± 0.15	−0.19 ± 0.17	−0.29 ± 0.19	−0.19 ± 0.15	−0.30 ± 0.17 [††, ‡]	−0.47 ± 0.19 [††, ‡]	−0.50 ± 0.15 [††, ‡]
Whole-body sweat rate (L·h⁻¹)	−0.05 ± 0.09	−0.05 ± 0.06	0.07 ± 0.11	0.06 ± 0.09 [†]	0.04 ± 0.06 [†]	0.09 ± 0.10 [†]	0.08 ± 0.09 [††]	0.08 ± 0.06 [††]	0.10 ± 0.10 [††]
End-exercise T_{sk} (°C)	−0.38 ± 0.49	−0.38 ± 0.46	−0.73 ± 0.54	−0.39 ± 0.50	−0.66 ± 0.47	−0.95 ± 0.55	−0.50 ± 0.52	−0.60 ± 0.48	−1.15 ± 0.57
End-exercise heart rate (beats·min⁻¹)	−8 ± 5	−12 ± 7	−14 ± 8	−12 ± 5	−15 ± 7	−20 ± 8	−11 ± 5	−17 ± 7	−20 ± 8
Mean $\dot{V}O_2$ (L·min⁻¹)	−0.10 ± 0.13	−0.10 ± 0.15	−0.16 ± 0.13	−0.01 ± 0.13	0.00 ± 0.15	−0.06 ± 0.13	−0.04 ± 0.13	−0.04 ± 0.14	−0.06 ± 0.12
Mean RER	−0.01 ± 0.04	−0.02 ± 0.03	−0.03 ± 0.04	−0.02 ± 0.04	−0.02 ± 0.03	−0.01 ± 0.04	−0.02 ± 0.04	−0.02 ± 0.03	−0.02 ± 0.04
End-exercise RPE (6–20 scale)	0 ± 2	0 ± 1	0 ± 2	−1 ± 2	−1 ± 1	−1 ± 2	−2 ± 2 [†]	−2 ± 1 [†]	−2 ± 2 [†]
End-exercise TS (1–13 scale)	0 ± 1	0 ± 1	0 ± 1	0 ± 1	−1 ± 1	−1 ± 1	−1 ± 1 [†]	−1 ± 1 [†]	−1 ± 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 ± 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 ± 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 ± 10	2 ± 12	2 ± 10	5 ± 10	7 ± 12	2 ± 10	4 ± 10	4 ± 12	2 ± 10
Energy index	−3 ± 4	−2 ± 6	−3 ± 5	−3 ± 4	−5 ± 6	−3 ± 5	−2 ± 4	−4 ± 6	−3 ± 5
Stroop reaction time (ms)	−29 ± 58	−25 ± 40	−11 ± 64	−13 ± 58	−32 ± 40	−15 ± 63	−16 ± 62	−18 ± 43	−28 ± 68
Stroop accuracy (%)	0 ± 2	−1 ± 3	1 ± 4	−1 ± 3	−1 ± 3	−2 ± 4	2 ± 3	1 ± 3	0 ± 4
Sleep duration (h)	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1
Sleep efficiency (%)	0 ± 9	−2 ± 7	−1 ± 8	−6 ± 9	−5 ± 7	1 ± 8	−2 ± 9	2 ± 7	−2 ± 8
Endurance capacity									
TTE (s)	−27 ± 676	75 ± 808	212 ± 991	101 ± 627	539 ± 749	323 ± 919	321 ± 743	686 ± 888	1030 ± 1089
End TTE T_{re} (°C)	−0.14 ± 0.30	−0.24 ± 0.34	−0.32 ± 0.47	−0.20 ± 0.31	−0.06 ± 0.34	−0.29 ± 0.47	−0.20 ± 0.31	−0.03 ± 0.34	−0.25 ± 0.47
End-TTE heart rate (beats·min⁻¹)	−8 ± 8	−10 ± 7	−16 ± 10	−10 ± 8	−12 ± 7	−20 ± 10	−8 ± 8	−10 ± 7	−14 ± 10
End-TTE blood lactate (beats·min⁻¹)	0.2 ± 1.4	−0.1 ± 0.7	0.2 ± 0.6	0.5 ± 1.3	−0.2 ± 0.7	−0.9 ± 0.6	−0.2 ± 1.3	−0.1 ± 0.7	0.2 ± 0.6

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

767