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Title

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

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Running head

Post-exercise hot water immersion adaptations and mechanisms

Abstract

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

Keywords

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

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Introduction

It is well established that exercise in hot and hot-humid environments is detrimental to endurance capacity (1, 2) and may expose individuals to the risk of exertional heat illness (3). To reduce these deleterious effects of heat stress, athletes, military personnel, and occupational workers should prepare by completing a period of heat acclimation (4, 5).

Previous research in both recreationally active (6) and endurance-trained individuals (7) demonstrates that taking a hot bath for up to 40 min immediately after submaximal exercise in temperate conditions on six consecutive days reduces resting core body temperature. This reduction in resting core body temperature leads to a subsequent reduction in core body temperature during exercise-heat-stress, a hallmark heat acclimation adaptation. Post-exercise hot water immersion (HWI) also presents a more practical heat acclimation strategy than conventional exercise heat acclimation (EHA), as it eliminates the requirement for access to an environmental chamber and can be more easily incorporated into normal training and an athlete's taper (8). Moreover, McIntyre *et al*. (9) recently demonstrated that despite a similar endogenous thermal stimulus for adaptation, 6 days of HWI elicited larger thermal adaptations than EHA. While the 6-day HWI intervention presents an effective, practical, and time-efficient short-term (< 7 days) heat acclimation strategy, previous literature suggests that medium- (7–14 days) (10, 11) and long-term (> 14-day) (10) interventions provide a more complete state of heat acclimation. It is yet to be determined whether extending the 6-day HWI intervention provides additional thermal benefits. In addition, the true benefit of medium-term conventional exercise-based heat acclimation strategies beyond exercising in temperate conditions is unknown due to the lack of work-matched interventions within the literature and hence further research is warranted. In contrast to the beneficial adaptations of medium-term heat acclimation, the physical demands of prolonged interventions can disrupt training and may trigger overreaching, which has detrimental effects on exercise performance and mood (4, 12). The effects of medium-term heat acclimation on overreaching are currently unknown and, given the applied implications, warrant investigation.

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

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

HWI intervention to 12 days would augment thermal adaptations and that these would confer more complete heat acclimation than EHA. In addition, we expected that compared to CON the high physical demands of daily exercise and heat-stress during HWI and EHA would lead to increased markers of overreaching (i.e., low mood and physical/cognitive performance decrements). Part 2 examined, in a larger cohort of 48 active males, the effect of 6 days of HWI in comparison to CON on plasma thyroid hormone concentrations, and additionally examined the relationship of thyroid hormone changes with hallmark heat acclimation adaptations. We hypothesized that 6 days of HWI would elicit reductions in plasma thyroid hormone concentrations and that these reductions would be associated with heat acclimation thermal 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 participant characteristics of the 21 male participants included in the final analysis are summarized in Table 1. A sample size of 21 (7 participants per group) was estimated (G*Power 3.1.9) (25) as adequate to detect a significant difference in the change in end-exercise rectal core temperature (T_{re}) between heat acclimation and temperate exercise control interventions using a mixed-model analysis of covariance (ANCOVA), standard alpha (0.05) and power (0.80), and a Cohen's F effect size of 0.88. This effect size was calculated from the average reduction in end-exercise T_{re} change after HWI (−0.36°C) (6) and exercise heat acclimation (−0.44°C (26) and −0.49°C (27)) compared to exercise in temperate conditions (0.00°C) (6) and a pooled SD of 0.21°C (control group) (6). Part 2 participants were 48 active males (age, 22 ± 3 years; height, 178 ± 6 cm; body mass, 72 ± 7 kg; $\dot{V}O_{2peak}$, 58 ± 8 mL·kg^{-1}·min^{-1}). Data of fourteen participants (HWI, *n* = 7; CON, *n* = 7) were included in both Parts 1 and 2. All participants in Parts 1 and 2 provided written informed consent and were healthy, non-smokers, free from any known cardiovascular or metabolic diseases, were not taking any medication, and had not been regularly (> once a week) exposed to the heat (including sauna and hot bath use) in the 6 weeks before commencing testing.

Preliminary measurements and familiarization

Participants completed a fitness assessment within a week before their first experimental trial (PRE; Fig. 1). $\dot{V}O_{2peak}$ was assessed using a continuous maximal incremental exercise test performed on a motorized treadmill (HP Cosmos Mercury 4.0, Nussdorf-Traunstein, Germany) in a temperate laboratory (19°C, 45% RH) to volitional exhaustion. $\dot{V}O_{2peak}$ was determined as the highest oxygen uptake attained over a 30-s period. The average values of breath-by-breath $\dot{V}O_2$ and $\dot{V}CO_2$ during the final minute of each submaximal stage were used to calculate running economy, expressed as kilocalories per kilogram per min (28). A running speed that elicited 65% $\dot{V}O_{2peak}$ in temperate conditions was subsequently determined by the interpolation of the running speed–$\dot{V}O_2$ relationship and confirmed via Douglas bag method. All participants ran at a speed below their anaerobic threshold as determined by the onset of blood lactate accumulation (29). Participants were then familiarized with the treadmill running speed, Stroop test, venepuncture, and Profile of Mood States (POMS) questionnaire.

Experimental trials

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

On the day of the experimental trials, participants arrived at the laboratory at 0730 h and were provided with a standardized breakfast (2091 kilojoules, 71 g carbohydrate, 18 g fat, 17 g protein) and a bolus of water (7 $mL \cdot kg^{-1}$ of nude body mass). At 0800 h, dressed in a t-shirt, shorts, socks and trainers, participants rested for 20 min in temperate conditions (19°C, 45% RH). Following the seated rest, participants completed the abbreviated POMS questionnaire (31) to determine total mood disturbance and energy index (vigor−fatigue) as markers of overreaching (30). A venous blood sample was then taken without stasis for the determination of plasma volume and plasma concentrations of free T3, free T4, total T3, and total T4. A urine sample was then analyzed to confirm urine specific gravity was <1.03 (32) and a flexible, sterile, single-use rectal thermistor (Henleys Medical Supplies Ltd., Herts, UK) was self-inserted 10 cm beyond the anal sphincter to measure T_{re}. A pre-exercise nude body mass was recorded using a digital platform scale (Model 703; Seca, Hamburg, Germany) and skin thermistors were attached on the right side of the body for the determination of mean skin temperature (T_{sk}), as previously described (33). Mean body temperature (T_b) was estimated using the formula: $T_b = 0.64 \cdot T_{re} + 0.36 \cdot T_{sk}$ (34). Following instrumentation, participants rested for a further 30 min in temperate conditions (19°C, 45% RH) to establish baseline measures. Body surface area (A_D) by the Du Bois equation (35), and $\dot{V}O_2$ and respiratory exchange ratio (RER) from a 60-s expired gas collection by Douglas bag method between 29–30 min of seated rest were used to estimate resting metabolic heat production (*H*) as follows (36):

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

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

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

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

Daily intervention

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

Participants assigned to HWI completed a 40-min treadmill run dressed in shorts, socks, and trainers at a speed equivalent to their 65% $\dot{V}O_{2peak}$ (9.1 ± 1.6 $km \cdot h^{-1}$) in temperate conditions (19°C, 45% RH, 0.2 $m \cdot s^{-1}$ wind velocity). Following exercise (2–3 min transition), dressed in shorts, participants began a semi-recumbent ≤ 40-min HWI (40°C) to the neck, as previously described (6). Participants assigned to EHA completed a ≤ 60-min treadmill run at a speed equivalent to their 65% $\dot{V}O_{2peak}$ (9.1 ± 1.1 km·h^{-1}) in an environmental chamber (33°C, 40% RH, 0.2 m·s^{-1} wind velocity). Participants assigned to CON completed a daily submaximal treadmill run equivalent to 65% $\dot{V}O_{2peak}$ and work-matched to EHA (8.8 ± 0.9 km·h^{-1}) in temperate conditions (19°C, 45% RH, 0.2 m·s^{-1} wind velocity). Owing to the nature of these interventions, it was not possible to blind the participants. In Part 1, to maintain the endogenous thermal stimulus for adaptation after the first six intervention sessions (Days 1–3 and Days 6–8, Fig. 1), maximum immersion (HWI) and exercise duration (EHA and CON) increased by 25%, as of the seventh intervention session (Days 11–16, intervention sessions 7–12), to ≤ 50 min and ≤ 75 min, respectively. All intervention sessions were terminated if the maximal immersion/exercise duration was reached, at the participant's volition, or if T_{re} exceeded 39.5°C. Upon removal from the hot water/environmental chamber, participants rested in a seated position for 5 min in a temperate laboratory, were provided with a bolus of water, and were free to leave the laboratory when $T_{re} \leq 38.5$°C.

Blood sample collection and analysis

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

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

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

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

Statistical analysis

Data were analyzed using SPSS version 27 (IBM Corporation, NY, USA) or GraphPad Prism Version 9.1 (GraphPad Software Inc. La Jolla, USA). All data were checked for normality and sphericity; plasma free T4 data was reciprocal transformed to address statistical assumptions of sphericity. Data are presented as untransformed mean and SD unless otherwise stated, and statistical significance was accepted at $P < 0.05$. In Part 1, the mean daily endogenous thermal stimulus and external work during HWI, EHA, and CON were compared using a two-way mixed model ANOVA. A two-way mixed model ANCOVA, with baseline (PRE) as the covariate, was used to detect differences in heat acclimation adaptations, endurance capacity, overreaching markers, and plasma thyroid hormone concentrations after 3, 6, and 12 days of HWI, EHA, or CON. Bonferroni-adjusted pairwise comparisons were used where appropriate to determine where differences occurred. The size of the between-intervention differences was calculated using Cohen's *d* effect size with values greater than 0.2, 0.5, and 0.8 representing small, medium, and large effects, respectively (46). In Part 2, the mean daily endogenous thermal stimulus and external work during HWI and CON were compared using *t*-tests and a one-way ANCOVA was used to detect differences in heat acclimation adaptations and plasma thyroid hormone concentrations after 6 days of HWI or CON. Bonferroni-adjusted pairwise comparisons were used where appropriate to determine where differences occurred. Pearson's correlations determined the strength of the relationship between the endogenous thermal stimulus, changes in resting T_{re} and plasma thyroid hormone concentrations after 12 days of heat acclimation by HWI and EHA. Pearson correlation coefficients of 0.00–0.19 were regarded as very weak, 0.20–0.39 as weak, 0.40–0.59 as moderate, and 0.60–0.79 as strong 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

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

Thermal and perceptual responses to exercise in the heat

Thermal and perceptual responses to submaximal exercise in the heat were different between the interventions after 12 days. End-exercise T_{re} following exercise-heat-stress was lower after HWI (Fig. 4B, −0.50 ± 0.19°C) than CON (−0.33 ± 0.13°C; P = 0.049, d = 1.13), but not EHA (−0.37 ± 0.13°C; P = 0.196, d = 0.88); no difference was observed between EHA and CON (P = 1.000, d = 0.30). T_{re} at sweating onset was lower after HWI (Fig. 4C, −0.43 ± 0.12°C) than EHA (−0.22 ± 0.12°C; P = 0.015, d = 1.75) and CON (−0.16 ± 0.12°C; P = 0.002, d = 2.27). Conversely, EHA did not reduce T_{re} at sweating onset compared to CON (P = 1.000, d = 0.52). Whole-body sweat rate was greater after HWI (Fig. 4D, +0.08 $L \cdot h^{-1}$; P = 0.003, d = 2.13) and EHA (+0.06 $L \cdot h^{-1}$; P = 0.013, d = 1.78) than CON (−0.06 $L \cdot h^{-1}$), but no difference was detected between HWI and EHA (P = 1.000, d = 0.35). In accordance with thermal adaptations, perceptual responses to exercise-heat-stress were lower after HWI (RPE Fig. 4E, −2 ± 1; TS Fig. 4F, −1 ± 1) than CON (RPE, 0 ± 1, P = 0.036, d = 1.57; TS, 0 ± 1, P = 0.047, d = 1.55) but not EHA (RPE, −1 ± 1, P = 0.951, d = 0.54; TS, −1 ± 1, P = 1.000, d = 0.55); no differences were observed between EHA and CON ($P \geq 0.157$, d = 1.07). There were no differences between interventions for the change in T_{re} during the 40-min treadmill run in the heat, end-exercise T_b (Fig. 4A), end-exercise T_{sk}, end-exercise T_{re}−T_{sk} gradient, end-exercise heart rate, exercising $\dot{V}O_2$, or exercising RER (Table 3; all $P \geq 0.059$). The rate of thermal and perceptual adaptations was not different between HWI, EHA or CON from POST3 to POST12, as indicated by no interaction effects (all $P \geq 0.087$). There were also no main effects of time (all $P \geq 0.148$).

Overreaching markers and endurance capacity

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

Thyroid hormones

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

Part 2 daily intervention thermal stimulus and external work

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

Part 2 experimental trials

Thermal responses at rest in temperate conditions

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

Thermal and perceptual responses to exercise in the heat

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

Thyroid hormones

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

Discussion

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

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

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

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

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

Previous research has shown that intensified training during exercise heat acclimation can trigger markers of overreaching including increased perceived fatigue and decreased performance (12). In contrast we observed no evidence of overreaching after EHA or HWI; a discrepancy that might be explained by the lower exercise intensity and the inclusion of three rest days in our study compared with previous research. More participants did however withdraw with lower limb discomfort (i.e., knee/ankle pain, etc.) in EHA (25%) and CON (25%) than in HWI (7%; Fig. 2); a finding that might be explained by the ~35% greater external work during EHA and CON interventions than HWI. This finding provides insight into the practical feasibility of these interventions but is difficult to compare with previous research as heat acclimation studies do not often report participant flow and attrition. Based on our findings, athletes and coaches may be more inclined to choose HWI in the knowledge it carries less injury risk than EHA. Although this is a reasonable hypothesis, future studies with adequate sample sizes are required to specifically evaluate the injury and illness risks of heat acclimation.

Athletes and coaches should consider HWI rather than EHA before traveling to hot climates as it leads to a more complete state of heat acclimation, can be incorporated into the post-exercise washing routine, and eliminates the requirement for an increased training load or access to an environmental chamber. These benefits reduce the disruption to normal training compared with conventional exercise-based strategies, which is especially important during tapering in the lead-up to sporting events. Whilst adverse events after HWI, including syncope, have not been observed by us (6, 7, 13, 14), or reported by others (58, 59), practitioners should follow protocol guidelines carefully. In particular, hot water immersions should be terminated at the participant's volition or if T_{re} exceeds 39.5°C rather than attempting to complete 40 min. In our study this led to a gradual daily increase in hot water immersion duration up to a maximum of 40 min for the first six (Days 1–3, 33 ± 4 min; Days 6–8, 35 ± 5 min), and then 50 min for the seventh to twelfth immersions (Days 11–16, 39 ± 5 min). The current and previous studies demonstrate the effectiveness of HWI to prepare young, healthy, active males (6, 7, 9, 13, 14) and elderly males and females for heat stress (59). Further research is required to confirm that HWI is effective to cause beneficial thermal, perceptual and performance adaptations in pediatric, female, and older athletic populations. We hypothesize that HWI will be an effective strategy in these populations as Mee et al. (60) demonstrated that combining both active and passive heat acclimation strategies can accelerate thermal adaptations in females. The large dual thermal stimulus from 6 days of HWI should be sufficient to initiate heat acclimation adaptations in these populations as they typically have smaller body masses than adult males and consequently gain heat more quickly (61). Due to the smaller body masses these future investigations might require shorter maximum HWI durations to cause the beneficial thermal adaptations.

Perspectives and Significance

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

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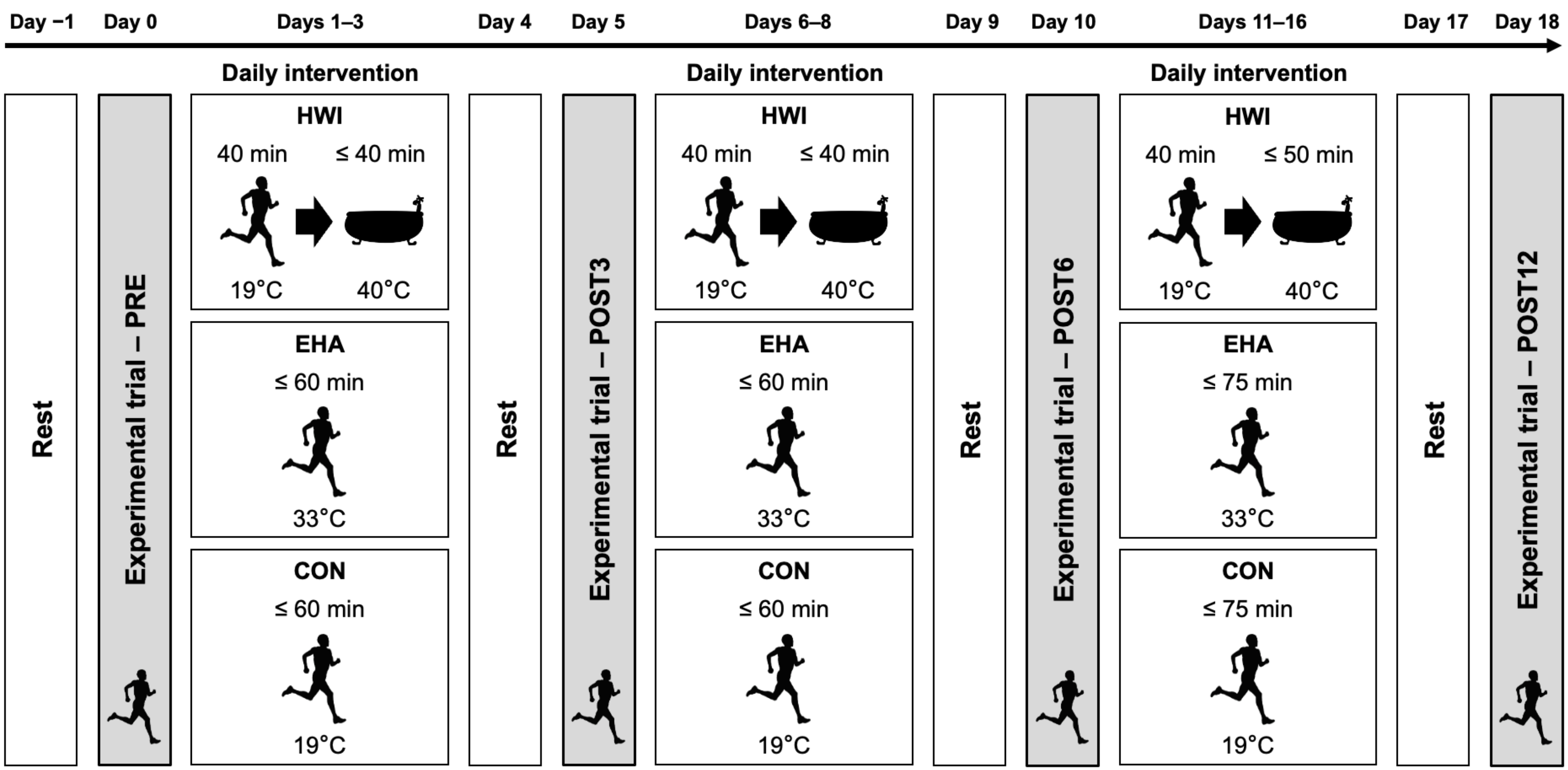

Figure 1. Schematic of the study design (Part 1). HWI; post-exercise hot water immersion, EHA; exercise heat acclimation and CON; temperate exercise control.

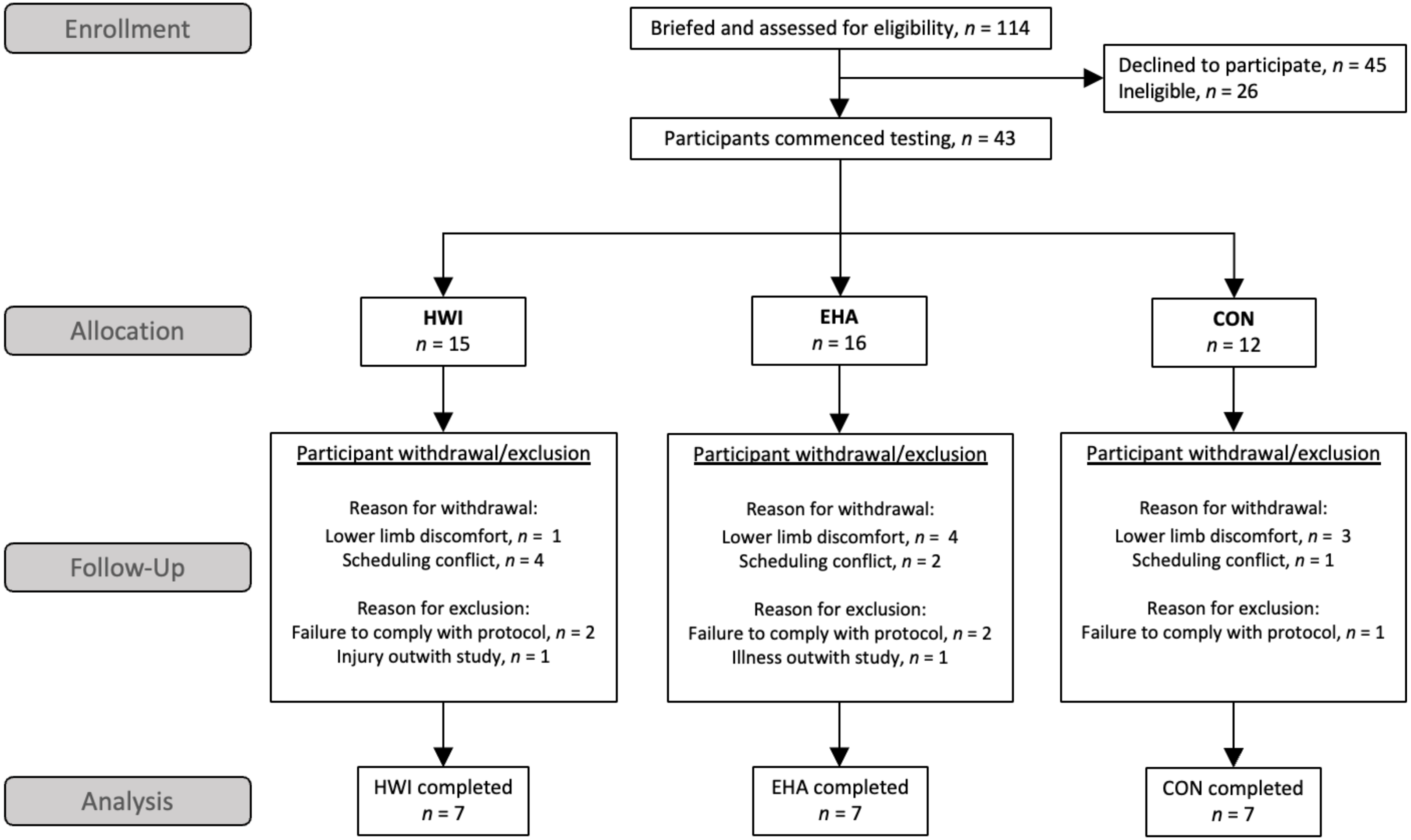

Figure 2. Flow diagram indicating the numbers of participants assessed for eligibility, commenced testing, and withdrew, were excluded, or completed the study protocol (Part 1). HWI; post-exercise hot water immersion, EHA; exercise heat acclimation and CON; temperate exercise control.

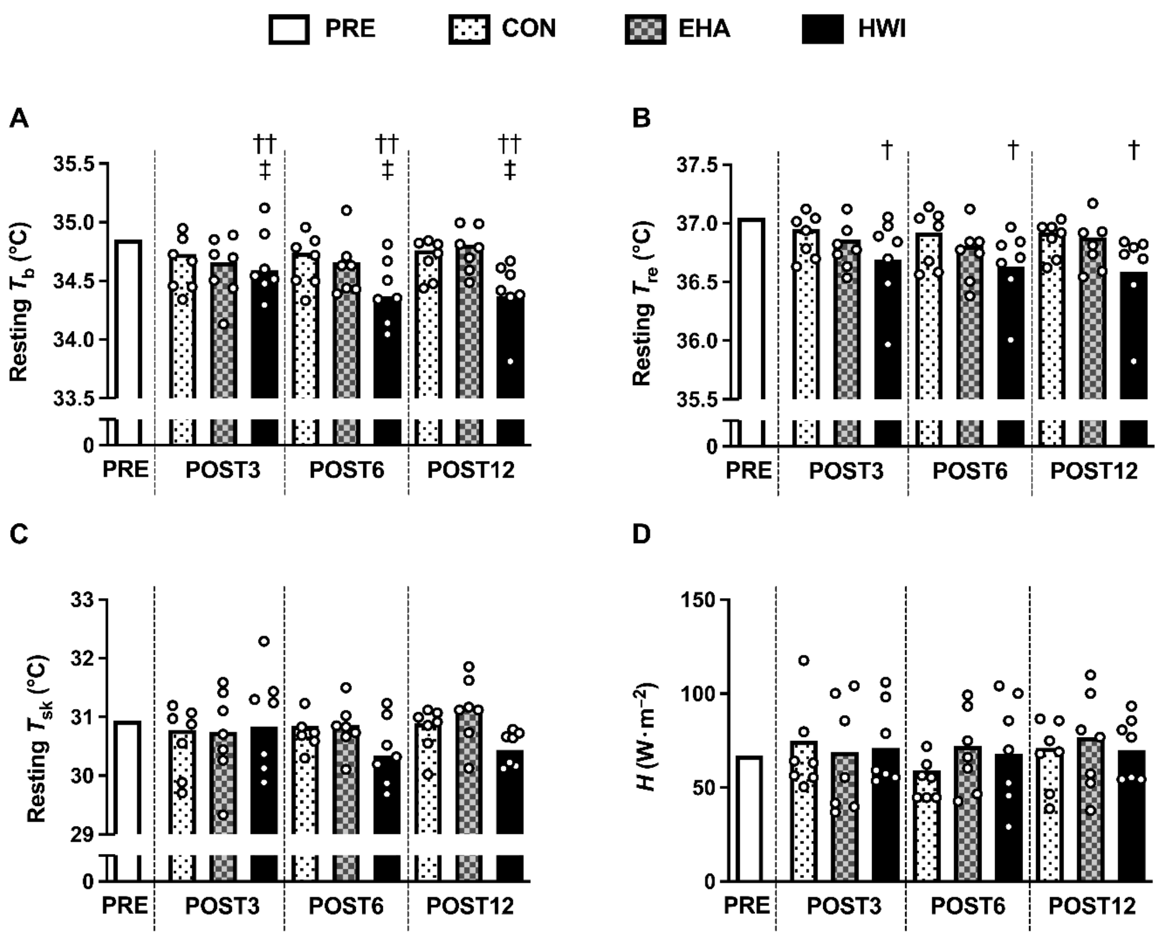

Figure 3. Influence of 3 (POST3), 6 (POST6), and 12 days (POST12) of a temperate exercise control (CON, n = 7), exercise heat acclimation (EHA, n = 7), or post-exercise hot water immersion (HWI, n = 7) on resting mean body temperature (T_b, A), rectal core temperature (T_{re}, B), mean skin temperature (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, $P < 0.05$.

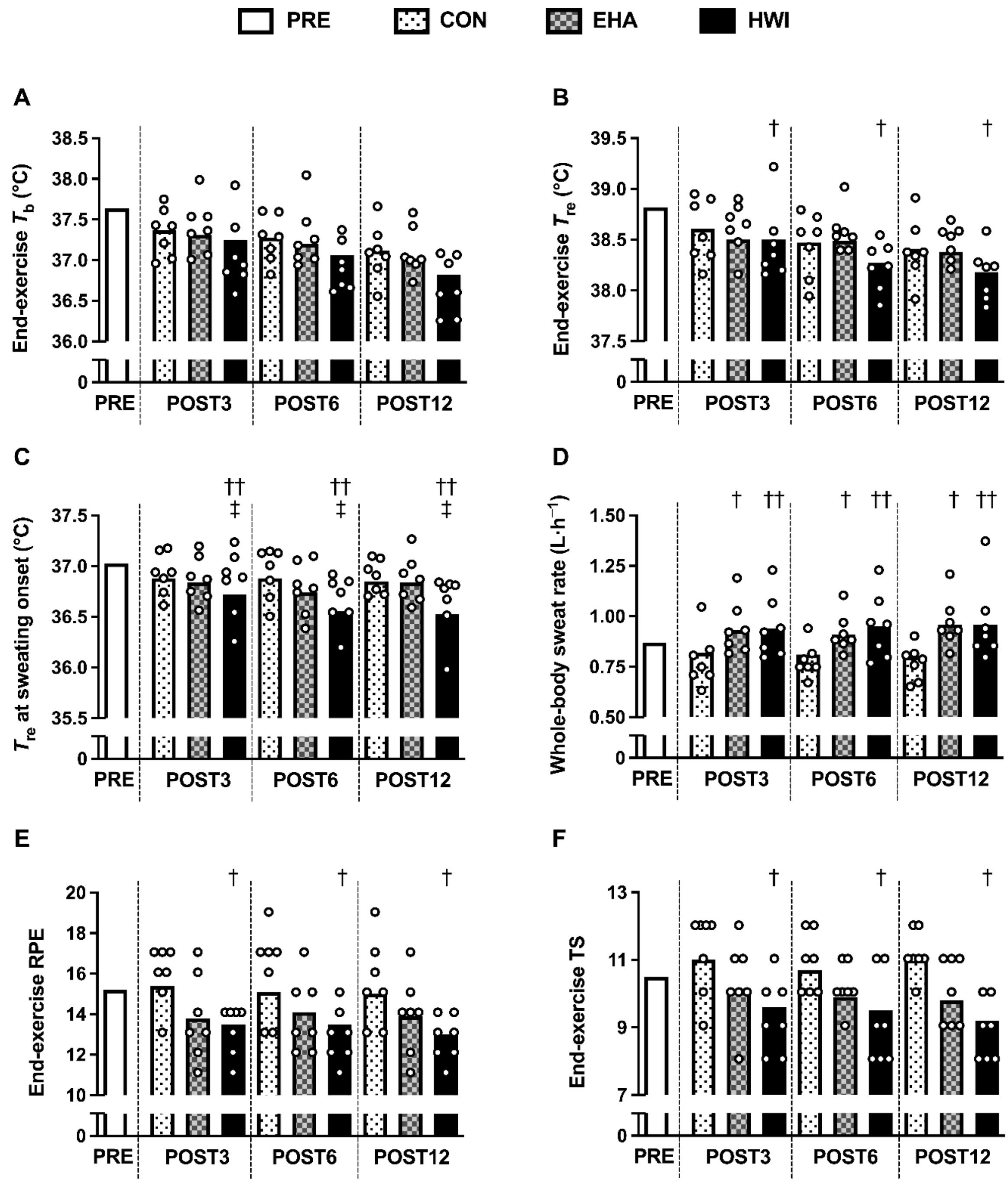

Figure 4. Influence of 3 (POST3), 6 (POST6), and 12 days (POST12) of a temperate exercise control (CON, n = 7), exercise heat acclimation (EHA, n = 7), or post-exercise hot water immersion (HWI, n = 7) on end-exercise mean body temperature (T_b, A), end-exercise rectal core temperature (T_{re}, B), T_{re} 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

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

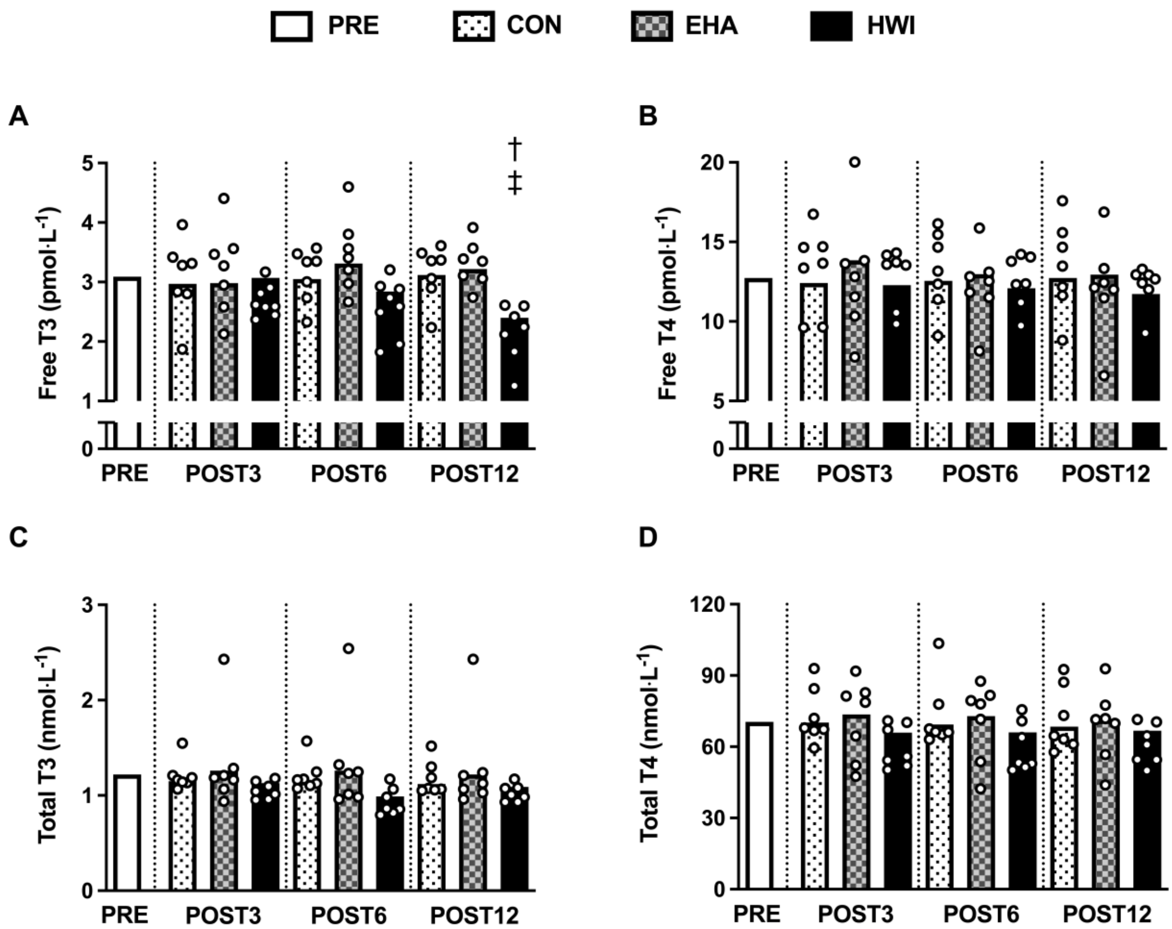

Figure 5. Influence of 3 (POST3), 6 (POST6), and 12 days (POST12) of a temperate exercise control (CON, *n* = 7), exercise heat acclimation (EHA, *n* = 7), or post-exercise hot water immersion (HWI, *n* = 7) on plasma concentrations of free triiodothyronine (T3; A), free thyroxine (T4; B), total T3 (C), and total T4 (D). Bars represent baseline-adjusted means; circles represent individual participant responses. †denotes HWI lower than CON, *P* < 0.05; ‡denotes HWI lower than EHA, *P* < 0.05.

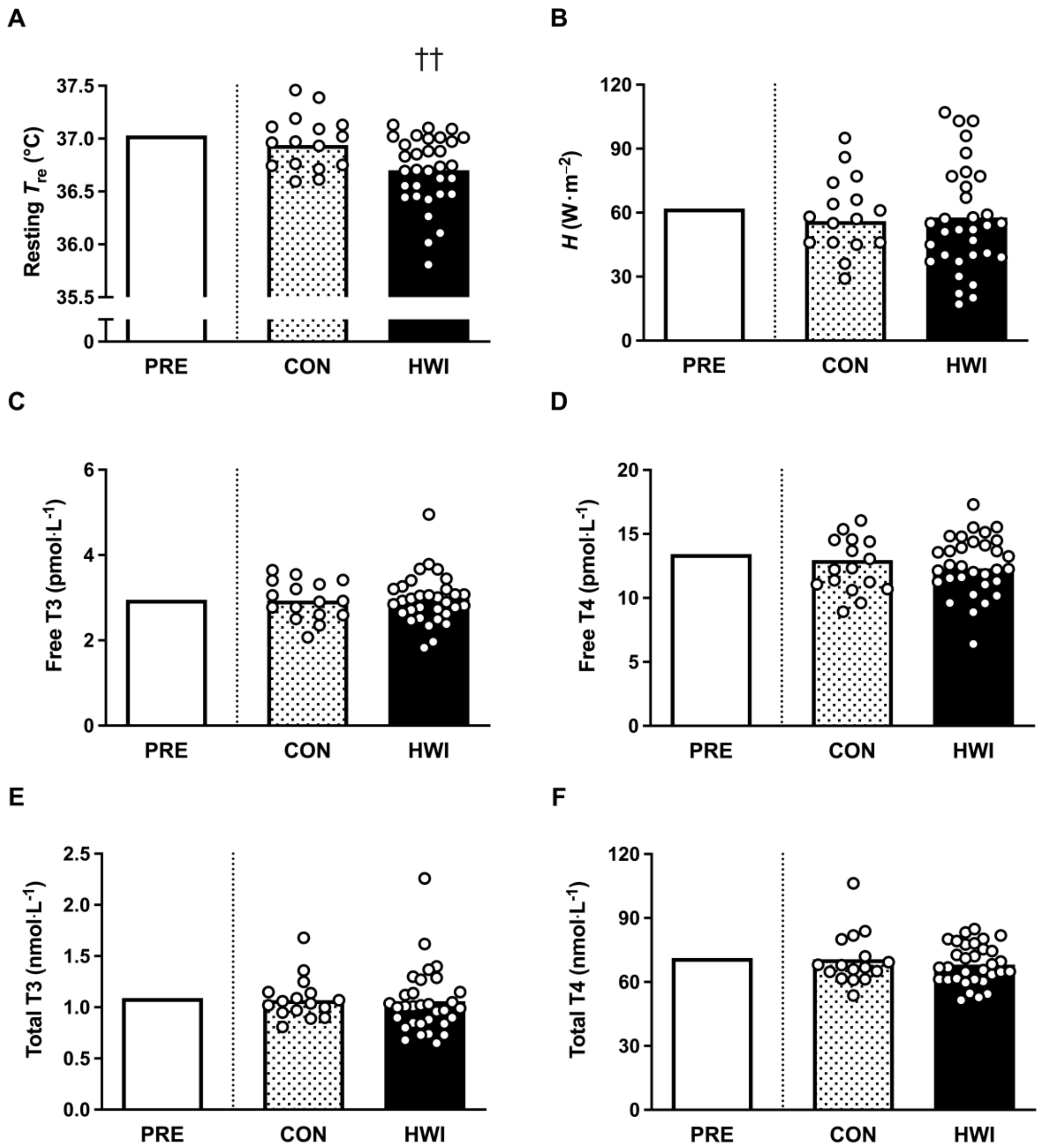

Figure 6. Influence of 6 days of a temperate exercise control (CON, n = 16) or post-exercise hot water immersion (HWI, n = 32) on resting rectal core temperature (T_{re}; A), resting metabolic heat production (H, B), and resting plasma concentrations of free triiodothyronine (T3; C), free thyroxine (T4; 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.

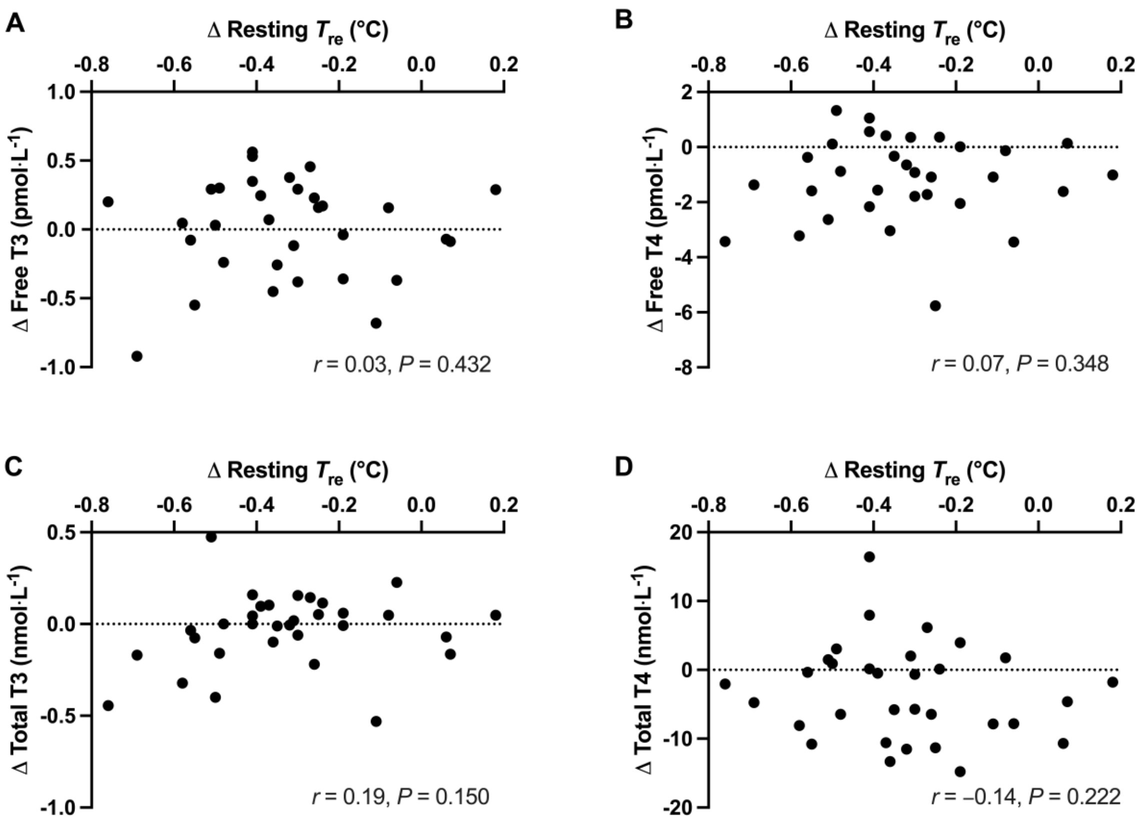

Figure 7. The relationships between the changes in resting core temperature (T_{re}) and plasma concentrations of thyroid hormones free triiodothyronine (T3; A), free thyroxine (T4; B), total T3 (C), and total T4 (D) after 6 days of post-exercise hot water immersion (n = 32).

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.

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} ≥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

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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 ($W \cdot m^{-2}$)	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 ($L \cdot h^{-1}$)	−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 ($beats \cdot min^{-1}$)	−8 ± 5	−12 ± 7	−14 ± 8	−12 ± 5	−15 ± 7	−20 ± 8	−11 ± 5	−17 ± 7	−20 ± 8
Mean $\dot{V}O_2$ ($L \cdot min^{-1}$)	−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