OPEN

Medicine & Science IN Sports & Exercise

The Official Journal of the American College of Sports Medicine
www.acsm-msse.org

. . . Published ahead of Print

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Accepted for Publication: 1 January 2021

Medicine & Science in Sports & Exercise, Publish Ahead of Print
DOI: 10.1249/MSS.0000000000002612

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This research was supported by both ZONMW and the Dutch Research Council (NWO) with grants held by Hein Daanen. Nicola Gerrett was funded by ZONMW (2019-30601) and Puck Alkemade through the Citius Altius Sanius project (P16-28) from NWO. **Conflict of Interest.** The authors of the study declare that they have no conflicts of interest. The results of the present study do not constitute endorsement by ACSM. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Abstract

Introduction: The aim of this study was to compare the effectiveness of exercise versus hot water immersion heat re-acclimation (HRA) protocols. **Methods**: Twenty-four participants completed a heat stress test (HST (33˚C, 65% RH)), which involved cycling at a power output equivalent to 1.5 $W \cdot kg^{-1}$ for 35 min whereby thermophysiological variables were measured. This was followed by a graded exercise test until exhaustion. HST1 was prior to a 10-day controlled hyperthermia (CH) heat acclimation protocol, and HST2 immediately after. Participants completed HST3 after a 28-day decay period without heat exposure, and were then separated into three groups to complete a 5-day HRA protocol; a control group (CON, n=8), CH-CON, hot water immersion group (CH-HWI, n=8), and a controlled hyperthermia group (CH-CH, n=8). This was followed by HST4. **Results**: Compared to HST1, time to exhaustion and thermal comfort improved, resting rectal temperature (T_{re}), end of exercise T_{re} and mean skin temperature (T_{sk}) were lower and whole body sweat rate (WBSR) greater in HST2, for all groups ($P < 0.05$). After a 28-day decay only WBSR, time to exhaustion and mean T_{sk} returned to pre-HA values. Of these decayed variables, only WBSR was re-instated after HRA; the improvement was observed in both the CH-CH and CH-HWI groups ($P < 0.05$). **Conclusion**: The data suggests HRA protocol may not be necessary for cardiovascular and thermal adaptations within a 28-day decay period, as long as a 10-day CH-HA protocol has successfully induced these physiological adaptations. For sweat adaptations, a 5-day CH or HWI-HRA protocol can reinstate the lost adaptations. **Key words:** Controlled hyperthermia, decay, performance, athletes, adaptation

INTRODUCTION

Despite the well reported benefits of heat acclimation (HA) protocols for athletic performance in hot conditions (1,2), they are logistically and practically difficult to employ alongside other preparation strategies prior to competition. The thermoregulatory benefits of HA can be obtained after approximately 10-14 consecutive days of training in the heat (2) but for many athletes it is not always feasible to incorporate this prolonged and challenging exposure into their training schedule. In recent years, a number of studies have investigated alternative strategies to address these issues; fewer days (3), twice daily sessions to reduce the total number of days (4) and lowering the training load and implementing a post work-out hot water immersion protocol (5). The physiological adaptations that occur from HA, such as a reduced resting core temperature, heart rate and an expanded plasma volume will begin to decay once the stimulus has been removed and the rate of decay varies for each of the phenotypic markers of HA (1). It is thus beneficial for athletes to employ their HA protocol as close to competition as possible to take full advantage of the adaptations acquired. The dilemma for athletes and coaches is how to implement a HA protocol alongside a tapering plan, which usually requires athletes to reduce their training volume 6-21 days prior to competition (6).

A strategy that has not been exploited is that of HA-memory obtained during both HA and the decay period (7). During HA, physiological adaptations occur as a result of altered gene expression and translational processes (8). During the decay period the physiological phenotypes dwindle away to a pre-acclimation state whilst at the molecular level they remain in an altered state, referred to as dormant memory (7). This dormant memory is a key process for heat re-acclimation (HRA), allowing a faster accrual of the adaptations during heat re-acclimation in rats

(7) and a similar phenomenon has been reported in humans (9,10). There is even some evidence to suggest a supercompensation, with lower resting/exercising heart rate after HRA compared to the initial HA (11,12). For 26-30 days away from the heat, just 4 days of HRA are reportedly required to accrue the lost physiological adaptations (9). Ashley et al. (13) suggested that a re-acclimation period of 4 days is recommended after 2 weeks absence from the heat and 5 days for 4 week absence in the heat. Variations exist in the recommended time required for HRA but they consistently point to a shorter HRA duration compared to the initial HA.

HRA strategies that are effective, practical and minimize the interference with any pre-competition preparation and/or tapering are required. Passive heating has been shown to be a practical strategy stimulating health benefits, improved heat tolerance, enhanced exercise performance and inducing hyperthermia (14–16). Whilst evidence suggests that HA adaptations are more complete when the program includes both exercise and heat exposure (2), there is a possibility that if the HA phenotypes have been previously acquired during an effective HA strategy then a HRA protocol with heat alone (passive) may still be effective. This seems plausible given that hot water immersion and exercise both stimulate heat shock protein synthesis, which provides cellular protection against exposure to high temperatures and improves thermal tolerance (16). It is possible then that the thermal stimulus alone from passive heating could be as effective, yet more practical, than an exercising controlled hyperthermia strategy for HRA.

The aim of this study was to assess the effectiveness of HRA on thermophysiological responses and performance during exercise in the heat. We hypothesized that a 5-day HRA, after a 28-day decay period, would result in a similar or even higher (i.e. supercompensation) adaptation response as that of HA. An additional aim was to determine whether HRA, using a practical hot water immersion protocol can be used instead of a controlled hyperthermia (CH) protocol. We hypothesised that hot water immersion would bring about the same physiological adaptations as a controlled hyperthermia HRA protocol.

METHODS

Participants

A power calculation was performed using G*Power software (Heinrich-Heine-Universität Düsseldorf, Germany). A partial eta squared (large effect) of 0.14 for resting T_{re} was determined using previously reported changes following CH in trained groups (9,17). This value, along with an α of 0.05, a ß of 0.80, correlation amongst repeated measures set to 0.6 and non-sphericity correction ε set at 0.34 (1/[repetitions-1]) indicated a minimum total sample size of 21 (7 per group) was required to demonstrate a significant difference. We therefore recruited twenty-four un-acclimated participants who were separated into three groups; control group (CH-CON), active (controlled hyperthermia) heat re-acclimation group (CH-CH) and a passive (hot water immersion) heat re-acclimation group (CH-HWI). Participants between the three groups were matched for age, body surface area and VO_{2peak} and had equal number of male (n=5) and females (n=3) per group (Table 1). According to previous classification guidelines (18,19), most participants were classified in performance levels 2 or 3, but few in levels 1 or 4. Due to

this variation were refer to them collectively as habitually trained; representative of their regular level of engagement with exercise training.

Participants were informed about the study purpose and procedures prior to providing verbal and written consent. The Faculty of Behavioural Movement Science Ethical Committee at Vrije Universiteit Amsterdam approved the study (report no. VCWE-2018-160R1), which conforms to the standards set out by the Declaration of Helsinki. Participants were screened for pre-existing medical conditions and specifically had no history of heat-related illnesses, cardiovascular complications and were non-smokers. Three participants were taking medication; one participant was taking medication (70 mg alendronic acid weekly and 500 mg calci-chew 7.5 mg and mirtazapine daily; one participant was taking Ritalin (ADHD); one participant was taking methotrexate and folic acid). These participants were taking the medication consistently for the duration of the study. Female menstrual cycle was recorded but not controlled for as this would have been unfeasible given the required timings of each part of the protocol (10-day HA, 28-day decay and 5-day HRA consecutively). Six females used the combined pill, one used a hormonal intrauterine device and two reported regular natural menstrual cycles (25-35 days).

Experimental design

After explaining and familiarising participants with the experimental procedures and laboratory area, participants completed a graded exercise test to determine $VO_{2peak.}$ They were then familiarised with the heat stress test (HST). Approximately four- to seven-days after the preliminary visit, participants completed the main experimental trials which are illustrated in Figure 1. Participants completed the first heat stress test (HST1), which was followed by a 10-

(consecutive)-day CH. Forty-eight hours after the final HA session, participants repeated the HST (HST2). All participants then completed a 28-decay period where they were allowed to engage in their typical training regime but were not allowed to be exposed to any hot thermal stimulus (saunas, hot tubs etc). Whilst physical activity data was not recorded during the decay period, participants did provide us with a typical training week. Training mode varied, but were predominately aerobic activities (running, cycling, swimming, rowing) and some (but not all) engaging in strength training once a week. Participants averaged 5.5 ± 2 days of training per week, averaging 181 ± 65 mins of training per week.

Twenty-eight days after completing HST2 they then completed another HST (HST3) to assess the level of decay. The following day, participants began their assigned 5-(consecutive)-day HRA protocol: a control group (CH-CON), an active heat re-acclimation group (CH-CH) or a passive heat re-acclimation group (CH-HWI). Forty-eight hours after completing the final HRA protocol, participants completed the final HST (HST4).

The CH-CON were permitted to train during the allocated 5-day HRA period, but they were not allowed any exposure to a sustained hot thermal stimulus (saunas, hot tubs etc). Testing took place between the months of January and May in the Netherlands; the average outdoor temperature and relative humidity during this period was approximately 8˚C and 78%, respectively.

Experimental trials

VO_{2peak}, anthropometric and familiarisation

Participants height (Seca217, Hamburg, Germany) and weight (SATEX 34 SA-1 250, Weegtechniek, Holland B.V., Zeewolde, The Netherlands) were measured initially. All participants then completed a graded exercise test to determine VO_{2peak} on an electrically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) in temperate conditions (22˚C, 32% RH). Participants started cycling at 25W, with the intensity increasing 25W·min^{-1} until volitional exhaustion. Heart rate (Polar Vantage-M, Kempele, Finland) and respiratory gases (Quark CPET, Cosmed, Rome, Italy) were continuously monitored throughout. VO_{2peak} was identified as the highest 15 second moving average over the entire exercise period. After a short break, participants moved to the environmental chamber to be familiarised with the experimental trials (i.e. the HST) in the heat. During this familiarisation session they were also familiarised with the perceptual scores: thermal sensation, thermal comfort and rating of perceived exertion (RPE).

Heat stress test

Testing took place throughout the day, but each participant completed their own respective 4 HSTs at the same time of day. Participants were asked to refrain from consuming caffeine or alcohol and to avoid any strenuous exercise 24 hours preceding all HSTs. In addition, they were instructed to record their food and beverage intake during the preceding 24 hours and asked to replicate this for all tests. They were required to ensure they were euhydrated by consuming 500mL of water the evening before testing and water equal to 10 mL·kg^{-1} of body weight (BW) 0-3 h prior to all HST. Hydration status, as indicated by a USG value ≤1.025 (20)

using handheld refractometer (Atago Co.Ltd, Tokyo, Japan). Despite following the pre-testing hydration guidelines, six participants had USG values slightly above 1.025. After confirming that they had followed the pre-experiment hydration requirements they were allowed to resume with the experiments after consuming a set volume of water (5mL·kg^{-1} of BW).

The HST was conducted in an environmental chamber (b-Cat b.v., Tiel, The Netherlands) set at 33˚C, 65% RH, minimal air movement. Upon entering they sat for 10 min to obtain a baseline sample of all physiological and perceptual data. They then mounted an electrically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) and completed 35 min cycling at an external power output equivalent to 1.5 W·kg^{-1} of BW. This was followed by a 5-minute rest period, where participants could move off the bike, stretch or sit but were required to consume a set volume of water (3mL·kg^{-1} of BW). They returned to the bike to complete a graded exercise test to exhaustion (GXT) as a performance measure, whereby the load was increased by 25W·min^{-1}, starting from 1.5 W·kg^{-1} of BW, until volitional exhaustion. No feedback or encouragement was provided. To assess the physiological responses to exercise in the heat, heart rate, rectal temperature (T_{re}) and mean skin temperature (T_{sk}) were continuously monitored and perceptual scores (thermal sensation, thermal comfort and RPE) were recorded at 5 min intervals and at the end of the GXT.

Controlled Hyperthermia

Participants completed 10 HA sessions at approximately the same time of day (±3h) in the same conditions as the HST (33˚C, 65%RH). A controlled hyperthermia HA protocol was followed where the aim was to increase T_{re} to 38.5˚C (referred to as 'thermal drive') within

approximately 35 min and then hold it slightly above 38.5˚C for 1 hour (referred to as 'thermal maintenance'). Thermal maintenance was regulated by adjusting the external power output or where necessary resting inside the chamber. Participants were free to drink water ad libitum during each HA session and the volume consumed was recorded. For the CH-CH group this protocol was followed for another 5 days during the HRA. Prior to each session a urine sample was collected to monitor hydration status during the course of the 10 days HA. Nude BW was measured pre and post each HA session, alongside fluid volume to calculate whole body sweat rate (WBSR). Heart rate, T_{re} and mean T_{sk} were continuously monitored and perceptual scores (thermal sensation, thermal comfort and RPE) were recorded at 5 min intervals.

Hot water immersion

Passive heating, using hot water (40˚C) immersion for 40 mins was selected based on its high practical value; baths are accessible to most and the protocol will have minimal impact on training schedules. The water temperature of 40˚C is below the thermal pain threshold and pilot testing confirmed that the proposed protocol was challenging but tolerable. This seemed a good balance between providing a strong enough stimulus, whilst still achievable for all to complete.

Participants sat in a neutral room for 5-10 min whilst a 5 min baseline value of physiological and perceptual responses were collected. They then entered the bath (Lay-Z-Spa BW54113 Monaco 2018 Model, Shanghai, China) which was set at 40˚C, for 40 min. Water temperature was recorded using a PT100 sensor (GMH3750-SET1, Greisinger electronic GmbH, Germany) every 5 min. Participants were required to keep their shoulders under the water, however this protocol is challenging and the risk of syncope is high. Participants were allowed

'relief' breaks for 2 min, every 10 min, whereby they could sit on a stool, with the lower body still submerged to provide some relief from the oppressive bath temperature. If T_{re} rose >39˚C, participants were required to sit on a stool with the water to their waist. Whilst immersed, participants were free to drink water ad libitum and the volume consumed recorded. During immersion, T_{re} and heart rate were monitored continuously and thermal sensation and thermal comfort reported every 5 min. Immersion ended after 40 min unless the participants withdrew themselves or T_{re} exceeded 39.5˚C. Following immersion, participants lay supine for a minimum of 10 min and sat upright for a minimum of 5 min to ensure T_{re} began to decline and blood pressure appeared well regulated.

Measurement and calculations

For all sessions (HA, HST and HRA), T_{re} was measured as an indicator of core body temperature. Prior to all experimental trials, participants self-inserted a rectal thermometer (MSR, Seuzach, Switzerland or Yellow Springs Instruments, Ohio, USA) at least 10 cm past the anal sphincter. During all experimental trials, except the immersion trials, T_{sk} was measured using iButtons (DS1922, Maxim Integrated Products, USA) attached to the skin using tape (Fixomull Stretch ADH, BSN Medical GmbH, Germany) from four sites (chest, forearm, thigh and calf) and a weighted mean skin temperature calculated (21). Heart rate was continuously monitored in all trials using a heart rate monitor (Polar Vantage-M, Kempele, Finland). Thermal sensation was rated using an adapted scale with intermediary values ranging from +10 (extremely hot) to −10 (extremely cold) with 0 indicating thermal neutrality (22). Thermal comfort was rated on an adapted 6-point Likert scale with intermediary values; 0=comfortable, 2=slightly uncomfortable, 4=uncomfortable, 6=very uncomfortable (23). RPE was recorded using the 6- to 20-point Borg

Scale (24). Thermal sensation, thermal comfort and RPE were assessed in all trials at 5 min intervals with the exception of the GXT, where they were recalled at the end of the test shortly after exercise termination. Prior to and after all sessions, nude body weight was measured using platform scales (SATEX 34 SA-1 250, Weegtechniek, Holland B.V., Zeewolde, The Netherlands) and WBSR calculated after correcting for duration and water volume consumption. All physiological data were averaged over 5 min. Exercise performance from the GXT was expressed in absolute terms, indicated as the total exercising time, i.e. time to exhaustion (TTE, in seconds). For further assessment of exercise performance, the data was also expressed as a percentage change, relative to performance from HST1.

To compare the two HRA protocols, the thermal impulse per session was quantified from rectal temperature data, using the following equation (25):

$$\text{thermal impulse} = \int (T_{re-i} - T_{re-0})\, dt_1 \ [^{\circ}\text{C} \cdot \text{min}]$$

where $T_{\text{re-i}}$ = rectal temperature at time i (˚C), $T_{\text{re-0}}$ = initial (time zero) rectal temperature, and dt_{n} = duration of each stimulus (min).

Data analysis

All data was synced and formatted using MATLAB (R2019a, The MathWorks Inc., Natick, USA), figures were produced in GraphPad Prism (version 7, GraphPad Software, La Jolla, CA) and data analysed using SPSS version 25 (IBM SPSS Statistics 20, NY, USA). Descriptive data are reported as mean and standard deviations (±SD). Significance was set at $P < 0.05$. The

Shapiro-Wilk test was used to check if the data were normally distributed. To examine performance and physiological responses to the HST, we used a mixed ANOVA, with time (HST1-4) as within subject factors and HRA protocol (CH-CH, CH-HWI, CH-CON) as between subject factors. To estimate the magnitude of the effects, partial Eta squared (η^2) for ANOVA effects were calculated with $\eta^2 > 0.06$ representing a moderate effect and $\eta^2 > 0.14$ a large effect. Specific post hoc analyses were conducted to answer specific questions related to our hypotheses, with the alpha adjusted accordingly. To assess whether the CH-HA was successful, comparisons were made between HST1 and HST2. To confirm whether decay had occurred HST2 and HST3 were compared. To confirm whether decay was complete, HST3 and 1 were compared. To assess whether HRA was successful, HST3 and 4 were compared and finally to determine whether a supercompensation had occurred HST2 and 4 were compared. The effect size of each pairwise comparison was calculated and reported as Hedges' *g* with 90% confidence intervals (CI); <0.19 is classified as 'trivial', 0.2–0.49 as 'small', 0.5–079 as 'moderate' and >0.8 as a 'large' effect.

The same statistical approach was used to examine physiological responses to the HA and HRA sessions, with day (HA days 1-10 and HRA days 1-5) as within subject factors and HRA group (CH-CH, CH-HWI, CH-CON) as between subject factors. For HA and HRA, post hoc analysis were made relative to HA1 or HRA1, with the alpha adjusted accordingly. Violations of sphericity were corrected for using the Greenhouse-Geisser adjustment. Non-parametric analysis of the perceptual responses (thermal sensation, thermal comfort and RPE) were conducted using permutation tests.

RESULTS

Heat acclimation and heat re-acclimation programs

All participants completed 10 days of HA using a controlled hyperthermia technique and the daily physiological responses of each group are summarised in Table 2. T_{re} increased to 38.5˚C in 37 ± 7 min, which did not differ over HA days, nor were they different between groups ($P > 0.05$, $\eta^2 < 0.08$). Heart rate during the thermal drive phase and thermal maintenance phase of controlled hyperthermia did not differ over the number of HA days and were also similar between groups ($P > 0.05$, $\eta^2 < 0.07$). Whilst daily WBSR were not different between groups (Table 2, $P = 0.57$, $\eta^2 = 0.09$), there was a main effect of time for WBSR, with the improvements occurring from HA4-HA10 compared to HA1 ($P > 0.05$, $g > 0.60$). There was no interaction (group x time) effect for WBSR ($P = 0.62$, $\eta^2 = 0.05$).

All participants completed their respective HRA protocols. Table 3 summarises the daily physiological responses of the CH-HWI and CH-CH group. For the CH-CH group, T_{re} increased to 38.5˚C in 40 ± 7 min. Heart rate during the thermal drive phase was 141 ± 20 bpm and 133 ± 20 bpm during the thermal maintenance phase of controlled hyperthermia. The lower heart rate during thermal maintenance compared to the thermal drive, is indicative of maintaining the elevated yet stable T_{re} with rest periods and low intensity exercise. For the CH-HWI group, the water temperature was 39.7 ± 0.86˚C and all participants completed the required 40 min. Resting T_{re} was 37.42 ± 0.24˚C and increased to 38.86 ± 0.34˚C at the end of HWI. Resting heart rate was 72 ± 12 bpm and increased to 102 ± 14 bpm at the end of HWI.

Daily WBSR during HRA for CH-CH and CH-HWI were not different between groups (Table 3, P = 0.61, η^2 = 0.05) but there was a main effect of time, with the improvements occurring from HRA2-5 compared to HRA1 (P > 0.05, g = 0.17-0.38). There was no interaction effect for WBSR (P = 0.43, η^2 = 0.29). Heart rate and thermal impulse for T_{re} were both significantly higher for the CH-CH group (P < 0.001, η^2 > 0.75) but the data were not different over time and no interaction effects were observed (P > 0.05, η^2 < 0.15).

Heat stress test - Exercise Performance

During HST3, one participant from the CH-HWI group felt unwell and did not complete the GXT. GXT data of this participant were removed before analysis. Figure 2 illustrates the GXT data and the associated ES with 90% CI are illustrated in Figure 3. Analysis revealed main effects of time (P < 0.001, η^2 =0.28), with the GXT during HST2 being longer than HST1 (P = 0.04, g = 0.37), and both HST3 and 4 being shorter than HST2 (P < 0.002, g = 0.25 and 0.35, respectively), whilst no other differences were observed. There was no effect of HRA group (P = 0.94, η^2 =0.006), nor an interaction effect (P = 0.22, η^2 =0.12). A main effect of time (P = 0.05, η^2 =0.22) and HRA group (P = 0.036, η^2 =0.27), but no interaction effect was found for performance as a percentage change from HST1 (P = 0.43 η^2 =0.03). HST3 was significantly lower than HST2 (P = 0.004) but HST 2 and 3 were similar to HST 4 (P > 0.08, g = 0.24 and -0.64, respectively).

Physiological responses to exercise

For the following data, the effect size and 90% CI are presented in Figure 3. Figure 4 illustrates T_{re} at rest and at the end of the 35 min cycling protocol (1.5 $W \cdot kg^{-1}$ of BW). For resting T_{re}, there was a main effect of time ($P < 0.001$, $\eta^2 = 0.47$) with HST 2 and 3 being significantly lower than HST1 ($P < 0.009$, g = -0.51 and -1.06), but no other time effects were found ($P > 0.05$, g > -0.33). There were no differences between HRA groups ($P = 0.768$, $\eta^2 = 0.03$), nor an interaction effect ($P = 0.135$, $\eta^2 = 0.15$). A main effect of time was also observed for end of exercise T_{re} and post hoc analysis indicated that T_{re} was significantly lower after 10 days HA (HST2 vs. HST1, $P = 0.046$, g = -0.46). T_{re} did not return to pre-HA values, as HST2 and 3 were similar ($P = 0.0001$, g = -0.28), but T_{re} was lower than HST1 after the decay (HST3, $P = 0.001$, g = -0.76). There were no differences in end of exercise T_{re} between HST2, 3 and 4 ($P < 0.05$, g = -0.01 to -0.31). There were no differences between HRA groups ($P = 0.72$, $\eta^2 = 0.03$), nor an interaction effect ($P = 0.28$, $\eta^2 = 0.11$).

Figure 5 (left panels) illustrates WBSR, end of exercise mean T_{sk} and heart rate. For WBSR there was a main effect of time ($P = 0.0001$, $\eta^2 = 0.41$) with an elevated WBSR during HST2 compared to HST1 ($P = 0.0001$, g = 0.60). After the decay period WBSR decreased, evidenced by a significant reduction during HST3 compared to HST2 ($P = 0.001$, g = -0.51) and decay was complete (HST 1 and 3, $P = 0.99$, g = 0.09). After HRA, WBSR increased with HST4 being greater than HST3 ($P = 0.008$, g = 0.35) but this was not greater than HST2 ($P = 0.285$, g = -0.17). There was no main effect of group (P = 0.31, $\eta^2 = 0.11$) but an interaction effect did exist for WBSR ($P = 0.050$, $\eta^2 = 0.184$). During HST4, the WBSR for the CH-CH and CH-HWI groups were significantly higher than the CH-CON group ($P < 0.05$, g = 0.5 to 1.1).

For mean T_{sk} there was a main effect of time (P=0.001, η^2 =0.32), no effect of HRA group and no interaction effect (P>0.05, η^2 <0.29). Mean T_{sk} during HST2 was lower than HST1 (*P* = 0.004, *g* = -0.78) and HST3 was higher than HST2 (*P* = 0.003, *g* = -0.75) Decay was complete as there no differences were observed between HST1 and 3. No differences were observed between HST2 or 3 with 4 (*P* > 0.05, *g* < 0.41).

For resting heart rate there were no main effects for time, group or interactions (*P* > 0.05, η^2 <0.13). For heart rate at the end of exercise, there was a main effect of time (*P* = 0.001, η^2 <0.38), no effect of group and no interaction effect (*P* < 0.05, η^2 <0.12). Heart rate at the end of exercise was lower during HST2 and HST3 compared to HST1 (*P* < 0.001, *g* = -0.43 to -0.57). There were no other differences observed.

Thermal sensation, thermal comfort and RPE

Figure 5 (right panels) illustrates the perceptual measures at the end of the 35 min cycling (1.5 $W \cdot kg^{-1}$ of BW). For thermal sensation, there was no effect of group, time or an interaction effect (*P* > 0.3, η^2 <0.12). For thermal comfort, there was a main effect of time with all HST2 and 3 being lower than HST1 (*P* < 0.001, *g* < 0.70), but no group or an interaction effect (*P* > 0.5). For RPE there was no group, time or interaction effect (*P* > 0.2).

DISCUSSION

The present study aimed to assess the effectiveness of HRA on thermophysiological responses and performance during exercise in the heat. In summary, the main findings indicated that following a successful controlled hyperthermia HA protocol, most of the adaptations were

retained during the 28-day decay period. Of the phenotypes to decay, the only variable to improve after HRA was WBSR. Additional exposure to the heat, via active (CH-CH) or passive (CH-HWI) exposure did increase WBSR to similar extent; both greater than no additional heat exposure (CH-CON). The data suggests that in habitually trained individuals, HRA may not be necessary within a 28-day decay period, as long as a 10-day CH-HA protocol has been successful in bringing about these physiological adaptations.

Heat acclimation

All participants completed the initial 10-day CH-HA protocol which resulted in a similar stimulus between groups, evidenced by the lack of any group effects (Table 2). To address our research questions, it was important that the HA phenotypes were observed in all groups. Compared to HST1, time to exhaustion improved, resting and end of exercise T_{re}, end of exercise mean T_{sk} and thermal comfort were lower and WBSR greater in HST2, for all groups. The effect sizes (Figure 3) indicated that these adaptations were all more than trivial effects, but the large CI reflect the uncertainty within the data. Thermal sensation nor RPE were lowered after completing the HA protocol, although the effect sizes were moderate. Overall, most of the adaptations were acquired and there were no differences between groups. The adaptations were comparable with other studies. Weller et al. (9) who used a mixed fixed load and CH protocol, reported a reduction in resting T_{re} of -0.26˚C, and the average of our group was -0.20˚C. However, they reported greater improvements after 60 min exercise in T_{re} (-0.50˚C) compared to our moderate (g = -0.46) improvements after HA (-0.17˚C). The attenuation in T_{re} at the end of exercise was most likely associated with the parallel decrease in resting T_{re} rather than a reduced heat storage during exercise (26). This was unexpected, given the increased sweat production

and lower heart rate but our shorter cycling protocol (35 min) may have masked the thermophysiological adaptations during exercise. End of exercise heart rate was 10 bpm lower for all groups and WBSR increased by 0.3 $L \cdot hr^{-1}$ which is comparable to other studies (9,27).

An interesting observation during the initial 10-day CH-HA protocol was the attainment of an increased WBSR after just 4 days of CH, whilst a meta-analysis showed that sweat rate adaptations are greatest after at least 8 days of HA (2,28,29). The aforementioned time course for HA phenotypes is generally based on studies utilising fixed load protocols. Controlled hyperthermia is becoming increasingly common but the time course and magnitude of the adaptations are difficult to evaluate due to the limited number of studies (29). Neal et al. (17) used CH HA protocol and also noted elevated sweat output after just 4 days. It is unclear why we both observed a faster adaptation response; perhaps the training status of our participants allowed for a faster sweat rate adaptation. This warrants more research.

Decay

We employed a 28-day decay period, with the expectation that most of the adaptations would be lost, or at least lower than post-acclimation values. Exercise performance (time to exhaustion, WBSR and mean T_{sk} were the only HA phenotypes to decay; the non-significant differences between HST1 and 3 and the trivial effect size, suggest that this decay was complete. The decay observed for mean T_{sk} may be associated with the concomitant reduction in WBSR, which would result in reduced evaporative cooling at the skin. However, it is unclear why performance (time to exhaustion) decayed, despite a lowered T_{re} and improved thermal comfort even after the decay period.

It has previously been suggested that the time course for HA and decay vary for different phenotypes (2). It has been suggested that sudomotor adaptations have the longest accrual time (>7 days), but along with cardiovascular adaptations, are also quickly lost (30). Whilst we have already questioned the delayed attainment of the sudomotor adaptations following CH-HA, we did observe a sudomotor decay that is consistent with the literature (9,12,31,32). Since local sweat production is fundamental for sweat gland adaptations (33) it stands to reason that the removal of the heat stimulus resulted in a sweat gland detraining response. This may account for the fast sudomotor decay observed here and by others.

It has been suggested that one day of HA is lost for every two days away from the heat (34), but accumulating evidence, from this study and others (9,10) seems to suggest that decay occurs less quickly than originally thought. There are some studies showing very little decay after short periods (7-days) away from the heat after both CH and fixed load HA protocols. Only a few studies have employed longer decay periods and the findings are inconclusive. A consistent finding amongst many studies is that heart rate declines at a faster rate than core body temperature. Our findings are at odds with this, as neither T_{re} or heart rate decayed. The cardiorespiratory fitness status of our participants may have accounted for the minimal decay observed in our study (10). Furthermore, their habitual training schedule, which predominately included endurance-based training sessions, may have occasionally elevated core body temperature and thus attenuated decay.

An interesting observation from our study was the continued adaptation (i.e. gains) in T_{re} during the so called 'decay' period. Daanen et al. (30) also found that the adaptations for T_{re} were more pronounced after a 3-day decay period than they were immediately post HA. Weller et al. (9), showed small gains in resting and end of exercise T_{re} after 26-day decay period. Why T_{re} is able to maintain this adaptation throughout a decay period, whilst sudomotor and cardiovascular responses are lost more quickly is unclear. Daanen et al. (30) suggested that the short recovery time (<24 h) between heat exposures was insufficient for the adaptations to manifest. This may have resulted in a latency period before this particular HA phenotype became evident.

Heat re-acclimation

Whilst the HA phenotypes disappear after prolonged non-heat exposure, it has been suggested that at the molecular level they remain in an altered state (7,8). It was our aim to see whether this dormant memory could be harnessed to off-set the practical and logistical challenges for athletes when trying to combine a successful HA protocol into the tapering phase of training. This memory would allow athletes to adopt a HA protocol several weeks prior to competition, employ a decay period alongside their tapering phase and in a few days prior to competition acclimate faster. For these reasons, passive heating, using hot water immersion, was investigated as a potential HRA protocol as it would expose athletes to a heat stimulus which may be sufficient to trigger the dormant memory and bring about the re-induction. The protocol used was adapted from previous research (5) to ensure compliance. Hot water immersion is accompanied by a high risk of syncope and completing the full duration was challenging for some of our participants. To alleviate the risk and decrease discomfort, participants were allowed

'relief' breaks whereby they sat partially submerged for 2 min, every 10 min. All participants of the CH-HWI group successfully completed the protocol.

A number of studies suggest HRA is faster than HA, but no study has been able to conclusively confirm this as decay for all adaptations were incomplete (9–12,32,35–37). In the present study, exercise performance (time to exhaustion), WBSR and mean T_{sk} were the only variables that significantly decayed and this decay appears complete. However, following HRA the only variable to improve was WBSR. The shorter period (5 vs. 10 days) suggests a faster HRA for sudomotor adaptations. This is supported by the faster WBSR increase during HRA (1 day) compared to HA (4 days). Furthermore, this improved sudomotor response was observed in both the CH and HWI HRA groups. Whilst it is generally accepted that exercise in the heat is more effective than passive heat exposure for developing HA phenotypes (2), our findings suggest this does not hold true for sweat rate, which improved during HST4 in both the CH-HWI and CH-CH groups. WBSR during the two HRA protocols were similar, which suggests that as long as the exposure results in a WBSR of 1.1-1.5 $L \cdot hr^{-}$ then this is enough to stimulate the dormant memory and bring about the re-adaptations faster. This value may even exceed the required sweat rate, as Taylor et al. (38) suggested that the minimum WBSR for sudomotor adaptation is approximately 0.4-0.8 $L \cdot hr^{-1}$.

Compared to CH-CH, the duration of exposure was considerably shorter for the CH-HWI group (~90 vs 40 min, respectively), and the heart rate and thermal impulse for T_{re} considerably lower. Despite this, CH-CH was not superior in reinstating the lost HA adaptations, nor promoting a super compensation. Most HA protocols focus on elevating core body temperature

as this is a key stimulus for HA, but Regan et al. (39) highlighted the importance of an elevated skin temperature on HA. Skin temperature was not measured during the HWI, but we can assume it was similar to the water temperature (~40˚C) and fluctuated slightly during the 'relief' periods. Mean T_{sk} during CH-HRA (not reported) was 35.9 ± 0.97˚C. Whilst the daily duration was shorter and the thermal impulse for the CH-HWI group lower, the elevated skin temperature during the hot water immersion was evidently sufficient to promote high sweat rates to initiate sudomotor adaptations. Sweating relies on central and cutaneous thermal afferents; the higher thermal impulse for the CH-CH group predominantly stimulated central afferents but the dual stimulus (core and skin temperature) from HWI stimulated both central and cutaneous thermal afferents to bring about the adaptations.

Exercise performance (time to exhaustion) and mean T_{sk} also decayed but showed no further adaptations to HRA, regardless of HRA strategy. Although we hypothesised that the CH-HWI group would initiate similar adaptations to CH-CH, it is also plausible the combination of exercise and heat would be superior in improving performance compared to passive heat alone. However, HRA, whether by HWI or CH did not improve the time to exhaustion and it is not clear why this did not occur in the presence of a lower T_{re} and improved WBSR. Previous HRA studies have focused predominately on strategies for the benefit of occupational workers as opposed to athletic performance. As such, this is the first study to investigate the influence of HRA on a performance variable and whilst time to exhaustion lacks ecologically validity for some sports, it is an important first step in discerning the effect of HRA on exercise performance.

The concept of supercompensation occurring with HRA was alluded to by Weller et al. (9) who found that after HRA, heart rate and T_{re} tended to decrease below the values at the end of HA. They were not able to confirm whether this truly exists as decay had been minimal or non-existent. In our study we could test this hypothesis for WBSR, mean T_{sk} and performance (time to exhaustion), all of which decayed completely. Our data indicates three important findings. Firstly, in the presence of decay, not all of the HA phenotypes will regain the adaptations with a 5-day HRA protocol. Secondly, sudomotor adaptations that are lost during a decay period can be reinstated to a similar post HA/pre-decay values but supercompensation did not occur. Thirdly, when decay has not occurred, super compensation does not exist with HRA, regardless of passive or active HRA protocols. The possibility that longer HRA protocols could result in supercompensation is unlikely given that we employed a 5-day HRA protocol and others have reported gains after as little as 1 day of HRA. Pandolf et al (10) suggested that fitter individuals acclimate fast, decay slow and rapidly re-acclimate.

The success of HRA is most likely influenced by the success of the HA protocol and the duration of the decay period. It may be the case that when full HA has been achieved, the rate of decay is slower. And when decay is large, more HRA days are needed to return the loss, but this might vary for the different HA phenotypes. In the present study, T_{re} did not change at all during decay and no additional improvements were observed with HRA. Whereas WBSR decayed, and the adaptation was re-instated in both HRA protocols. We have been unable to elucidate any mechanisms accounting for decay and re-induction because all studies to date, including this one, have not been able to examine the effect of HRA when decay has been 100% complete. This is difficult to control for and it may be that future studies employ a > 2-month decay period to

ensure all adaptations are lost. Given that the two HRA protocols used in the present study did not show any differential superiority, it may be suggested that hot water immersion could still be a successful HRA strategy in the presence of molecular dormant memory (i.e. when decay is complete) from a successful HA protocol; more research is required.

Limitations

We were unable to control for the menstrual cycle phase due to the pre-determined timings of the protocol. As a result, females were only tested at a similar menstrual phase during HST 2 and 3. Core body temperature is usually higher during the luteal phase (40,41) and we did observe an elevated resting T_{re} during at least one HST in 3 (out of 9) participants, which corresponded with their (self-reported) luteal phase. Whilst an elevated core temperature has been reported to impair incremental exercise performance in the heat (41), we did not observe any trends for this when tests were completed in the luteal phase. There is limited evidence for local or whole body sweat rate differences between menstrual cycle phase in hot and humid conditions (40,41). As such, we doubt that the observed WBSR adaptations were influenced by menstrual cycle phases during the 4 HSTs. This certainly warrants further investigation; although the challenge to align HA, decay and HRA protocols with the menstrual cycle remains.

CONCLUSION

In habitually trained individuals, most of the physiological adaptations acquired during an initial 10-day controlled hyperthermia heat acclimation protocol, were not lost during a 28-day decay period. However, we did observe that sudomotor adaptations were lost during a 28-day decay period but they can be reinstated to similar post-HA/pre-decay values. Most importantly,

the sudomotor adaptations can be re-instated with either an active or passive 5-day heat acclimation strategy, such as controlled hyperthermia or hot water immersion. It was clear that a super compensation does not exist in all the heat acclimation phenotypes measured, whether decay occurred or not, and regardless of a passive or active heat re-acclimation protocol. Collectively the data suggests HRA protocol may not be necessary within a 28-day decay period, as long as a 10-day controlled hyperthermia heat acclimation protocol has been successful in bringing about the appropriate physiological adaptations. However, if there is reason to believe that the sweat adaptation has started to decay, then a 5-day passive or active heat re-acclimation protocol can reinstate the lost adaptations.

Acknowledgements

This research was supported by both ZONMW and the Dutch Research Council (NWO) with grants held by Hein Daanen. Nicola Gerrett was funded by ZONMW (2019-30601) and Puck Alkemade through the Citius Altius Sanius project (P16-28) from NWO. The authors thank the following people for their assistance during the data collection: Lisa Klous, Mireille Folkerts, Guilio Tan, Romin Gaalman, Mae El Hamoud, Iris Dijkstra, Amber Berlijn, Michelle van Delden and Emma Wiedenmann. Special thanks are given to the participants for their dedication and commitment to this long and challenging study.

Conflict of Interest

The authors of the study declare that they have no conflicts of interest. The results of the present study do not constitute endorsement by ACSM. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

REFERENCES

1. Daanen HAM, Racinais S, Périard JD. Heat Acclimation Decay and Re-Induction: A Systematic Review and Meta-Analysis. Sport Med. 2018.48(2):409–30.
2. Périard JD, Racinais S, Sawka MN. Adaptations and mechanisms of human heat acclimation: Applications for competitive athletes and sports. Scand J Med Sci Sports. 2015.25(S1):20–38.
3. Garrett AT, Creasy R, Rehrer NJ, Patterson MJ, Cotter JD. Effectiveness of short-term heat acclimation for highly trained athletes. Eur J Appl Physiol. 2012.112(5):1827–37.
4. Willmott AGB, Gibson OR, Hayes M, Maxwell NS. The effects of single versus twice daily short term heat acclimation on heat strain and 3000 m running performance in hot, humid conditions. J Therm Biol. 2016.56:59–67.
5. Zurawlew MJ, Walsh NP, Fortes MB, Potter C. Post-exercise hot water immersion induces heat acclimation and improves endurance exercise performance in the heat. Scand J Med Sci Sports. 2016.26(7):745–54.
6. Houmard JA. Impact of Reduced Training on Performance in Endurance Athletes. Sport Med. 1991.12(6):380–93.
7. Tetievsky A, Assayag M, Ben-Hamo R, et al. Heat acclimation memory: Do the kinetics of the deacclimated transcriptome predispose to rapid reacclimation and cytoprotection? J Appl Physiol. 2014.117(11):1262–77.
8. Horowitz M. Heat acclimation and cross-tolerance against novel stressors: genomic-physiological linkage. Prog Brain Res. 2007.162:373–92.
9. Weller AS, Linnane DM, Jonkman AG, Daanen HAM. Quantification of the decay and re-induction of heat acclimation in dry-heat following 12 and 26 days without exposure to

heat stress. Eur J Appl PhysioI. 2007.102(1):57–66.

10. Pandolf KB, Burse RL, Goldman RF. Role of physical fitness in heat acclimatisation, decay and reinduction. Ergonomics. 1977.20(4):399–408.

11. Lind AR, Bass DE. Optimal exposure time for development of acclimatization to heat. J Appl Physiol. 1963.22:704–8.

12. Saat M, Sirisinghe RG, Singh R, Tochihara Y. Decay of heat acclimation during exercise in cold and exposure to cold environment. Eur J Appl Physiol. 2005.95(4):313–20.

13. Ashley CD, Ferron J, Bernard TE. Loss of Heat Acclimation and Time to Re-establish Acclimation. J Occup Environ Hyg. 2015.12(5):302–8.

14. Brunt VE, Howard MJ, Francisco MA, Ely BR, Minson CT. Passive heat therapy improves endothelial function, arterial stiffness and blood pressure in sedentary humans. J Physiol C 2016 Authors J Physiol C. 2016.594(18):5329–42.

15. Racinais S, Wilson MG, Périard JD. Passive heat acclimation improves skeletal muscle contractility in humans. Am J Physiol Integr Comp Physiol. 2017.312(1):R101–7.

16. Faulkner SH, Jackson S, Fatania G, Leicht CA. The effect of passive heating on heat shock protein 70 and interleukin-6: A possible treatment tool for metabolic diseases? Temperature. 2017.4(3):292–304.

17. Neal RA, Massey HC, Tipton MJ, Young JS, Corbett J. Effect of permissive dehydration on induction and decay of heat acclimation, and temperate exercise performance. Front Physiol. 2016.7(564):1–11.

18. De Pauw K, Roelands B, Cheung SS, et al. Guidelines to Classify Subject Groups in Sport-Science Research [Internet]. Vol. 8, International Journal of Sports Physiology and Performance. 2013 [cited 2020 Oct 26]. Available from: www.IJSPP-Journal.com

19. Decroix L, De Pauw K, Foster C, Meeusen R. Guidelines to classify female subject groups in sport-science research. Int J Sports Physiol Perform. 2016.11(2):204–13.

20. Kenefick RW, Cheuvront SN. Hydration for recreational sport and physical activity. Nutr Rev. 2012.70:S137–42.

21. Ramanathan NL. A new weighting system for mean surface temperature of the human body A new weighting system for mean surface temperature of the human body1. J Appl Physiol. 1964.19(3):531–3.

22. ASHRAE. Ashare Handbook Fundamentals I-P Edition. Atlanta, GA: ASHRAE; 2005.

23. Gagge AP, Stolwijk JAJ, Hardy JD. Comfort and thermal sensations and associated physiological responses at various ambient temperatures. Environ Res. 1967.1(1):1–20.

24. Borg GAV. Psychophysical bases of perceived exertion. Plast Reconstr Surg. 1954.14(5):377–81.

25. Taylor NAS, Cotter JD. Heat adaptation : guidelines for the optimisation of human performance : review article. Int Sport Med J. 2006.7(1):33–57.

26. Buono MJ, Heaney JH, Canine KM. Acclimation to humid heat lowers resting core temperature. Reg Int Comp Physiol. 1998.274(5):R1295–9.

27. Neal RA, Corbett J, Massey HC, Tipton MJ. Effect of short-term heat acclimation with permissive dehydration on thermoregulation and temperate exercise performance. Scand J Med Sci Sport. 2016.26(8):875–84.

28. Cotter JD, Patterson MJ, Taylor NAS. Sweat distribution before and after repeated heat exposure. Eur J Appl Physiol. 1997.76(2):181–6.

29. Tyler CJ, Reeve T, Hodges GJ, Cheung SS. The Effects of Heat Adaptation on Physiology, Perception and Exercise Performance in the Heat: A Meta-Analysis. Sport Med [Internet].

2016 [cited 2019 Jun 28].46:1699–724. Available from: https://link.springer.com/content/pdf/10.1007%2Fs40279-016-0538-5.pdf

30. Daanen HAM, Jonkman AG, Layden JD, Linnane DM, Weller AS. Optimising the Acquisition and Retention of Heat Acclimation. Int J Sports Med. 2011.32(11):822–8.

31. Williams CG, Wyndham CH, Morrison JF. Rate of loss of acclimatization in summer and winter. J Appl Physiol. 1967.22(1):21–6.

32. Pichan G, Sridharan K, Swamy Y V, Joseph S, Gautam RK. Physiological acclimatization to heat after a spell of cold conditioning in tropical subjects. Aviat Space Environ Med. 1985.56(5):436–40.

33. Buono MJ, Numan TR, Claros RM, Brodine SK, Kolkhorst FW. Is active sweating during heat acclimation required for improvements in peripheral sweat gland function? Am J Physiol Integr Comp Physiol. 2009.297(4):R1082–5.

34. Givoni B, Goldman RF. Predicting effects of heat acclimatization on heart rate and rectal temperature. J Appl Physiol. 1973.35(6):875–9.

35. Ashley CD, Ferron J, Bernard TE. Loss of Heat Acclimation and Time to Re-establish Acclimation. J Occup Environ Hyg. 2015.12(5):302–8.

36. Henschel A, Longstreet Taylor H, Keys A. The persistence of heat acclimatization in man. Am J Physiol. 1943.140(3):321–5.

37. Wyndham CH, Jacobs GE. Loss of Acclimatization After Six Days of Work in Cool Conditions on the Surface of a Mine. J Appl Physiol. 1957.(11):197–8.

38. Taylor NAS, Patterson MJ, Regan JM, Amos D. Heat Acclimation Procedures: Preparation for Humid Heat Exposure. Aeronautical and Maritime Research Laboratoy. 1997.

39. Regan JM, Macfarlane DJ, Taylor NAS. An evaluation of the role of skin temperature during heat adaptation. Acta Physiol Scand. 1996.158(4):365–75.

40. Lei T-H, Stannard SR, Perry BG, et al. Influence of menstrual phase and arid *vs* . humid heat stress on autonomic and behavioural thermoregulation during exercise in trained but unacclimated women. J Physiol [Internet]. 2017 [cited 2018 Nov 19].595(9):2823–37. Available from: http://doi.wiley.com/10.1113/JP273176

41. Janse De Jonge X a K, Thompson MW, Chuter VH, Silk LN, Thom JM. Exercise performance over the menstrual cycle in temperate and hot, humid conditions. Med Sci Sports Exerc [Internet]. 2012 [cited 2014 Jun 23].44(11):2190–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22776870

Figures titles

Figure 1: A schematic diagram illustrating the experimental protocol to examine the effects of three different HRA protocols on the adaptive responses to exercise in the heat. HST: heat stress test, CH: controlled hyperthermia, CH-CH, heat re-acclimation with controlled hyperthermia; CH-HWI, heat re-acclimation with hot water immersion; CH-CON, control group.

Figure 2: Top panel shows the time to exhaustion (TTE) during the GXT test. The bottom panel shows exercise performance as a percentage change from HST1 for CH-CON (n=8), CH-HWI group (n=7) and CH-CH group (n=8) during HST1, HST2, HST3 and HST4. Grey plots illustrate individual responses, whilst solid black line illustrates the mean (±SD). * indicates main effects of time between HST1 and 2 ($P < 0.05$), ∞ indicates main effects of time between HST2 and 3 ($P < 0.05$).

Figure 3: Effect size and 90% confidence interval of the physiological and perceptual responses to heat stress test and graded exercise performance test (GXT) (n=24). Skin temperature (T_{sk}), heart rate (HR), thermal sensation (TS), thermal comfort (TC), and rating of perceived exertion (RPE) are the end of exercise response. The symbols (‘+’ ‘-‘ ‘=’) to the right of the graph indicate the expected (effect) direction of each dependent variable for each pairwise comparisons: HST 1 and 2 indicates heat acclimation adaptation; HST 2 and 3 indicate decay; HST 1 and 3 indicate complete decay; HST 3 and 4, indicate heat re-acclimation response; HST 2 and 4, indicate supercompensation. The shaded vertical bars denote effect size ranges of trivial (<0.19, white), small (0.2-0.49, lighter grey), moderate (0.5-0.79 grey) and large (>0.8, darker grey) effects. T_{re}: rectal temperature, WBSR: whole body sweat rate.

Figure 4: T_{re} at rest (top panel) and at the end of exercise (bottom panel) for CH-CON (n=8), CH-HWI group (n=8) and CH-CH group (n=8) during HST1, HST2, HST3 and HST4. Grey plots illustrate individual responses, whilst solid black line illustrates the mean (±SD). * and ** indicate main effects of time between HST1 and 2 ($P < 0.05$ and $P < 0.001$, respectively), ## indicate main effects of time between HST1 and 3 ($P < 0.001$).

Figure 5: Whole body sweat rate (WBSR), end of exercise mean T_{sk}, heart rate, thermal comfort, thermal sensation and rating of perceived exertion (RPE) for CH-CON (n=8), CH-HWI group (n=8) and CH-CH group (n=8) during HST1, HST2, HST3 and HST4. Grey plots illustrate individual responses, whilst solid black line illustrates the mean (±SD). * and ** indicates main effects of time between HST1 and 2 ($P < 0.05$ and $P < 0.001$, respectively), ## indicate main effects of time between HST1 and 3 ($P < 0.001$),), ∞ and ∞ ∞ indicates main effects of time between HST2 and 3 ($P < 0.05$ and $P < 0.001$, respectively), ## indicates main effects of time between HST1 and 3 ($P < 0.001$), ϕ and ϕϕ indicate main effects of time between HST3 and 4 ($P < 0.05$ and $P < 0.001$, respectively), ∞ and ∞ ∞ indicate main effects of time between HST2 and 3 ($P < 0.05$ and $P < 0.001$, respectively).

Figure 1

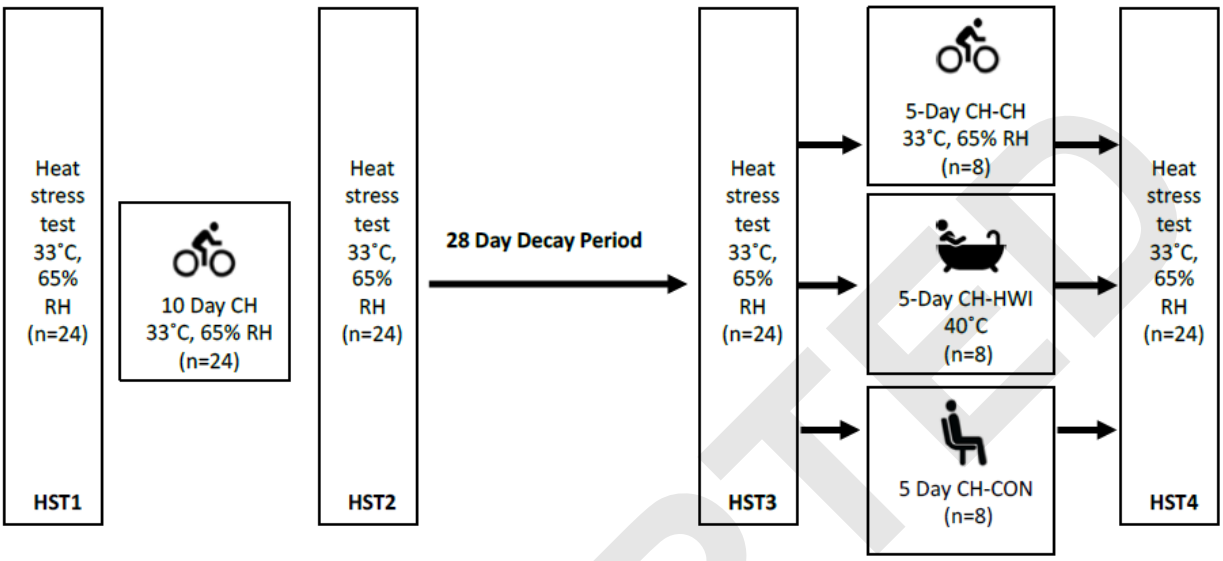

Figure 2

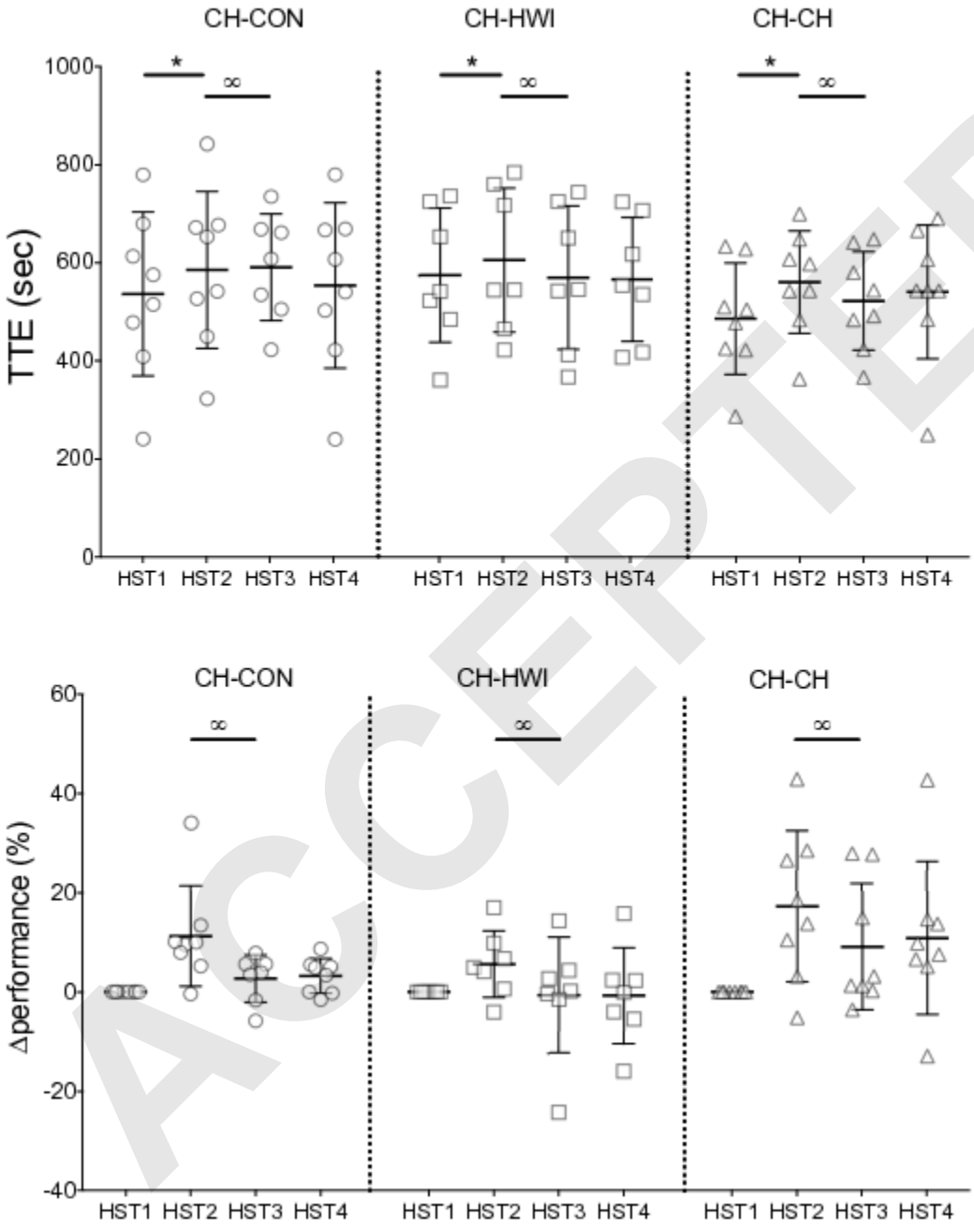

Figure 3

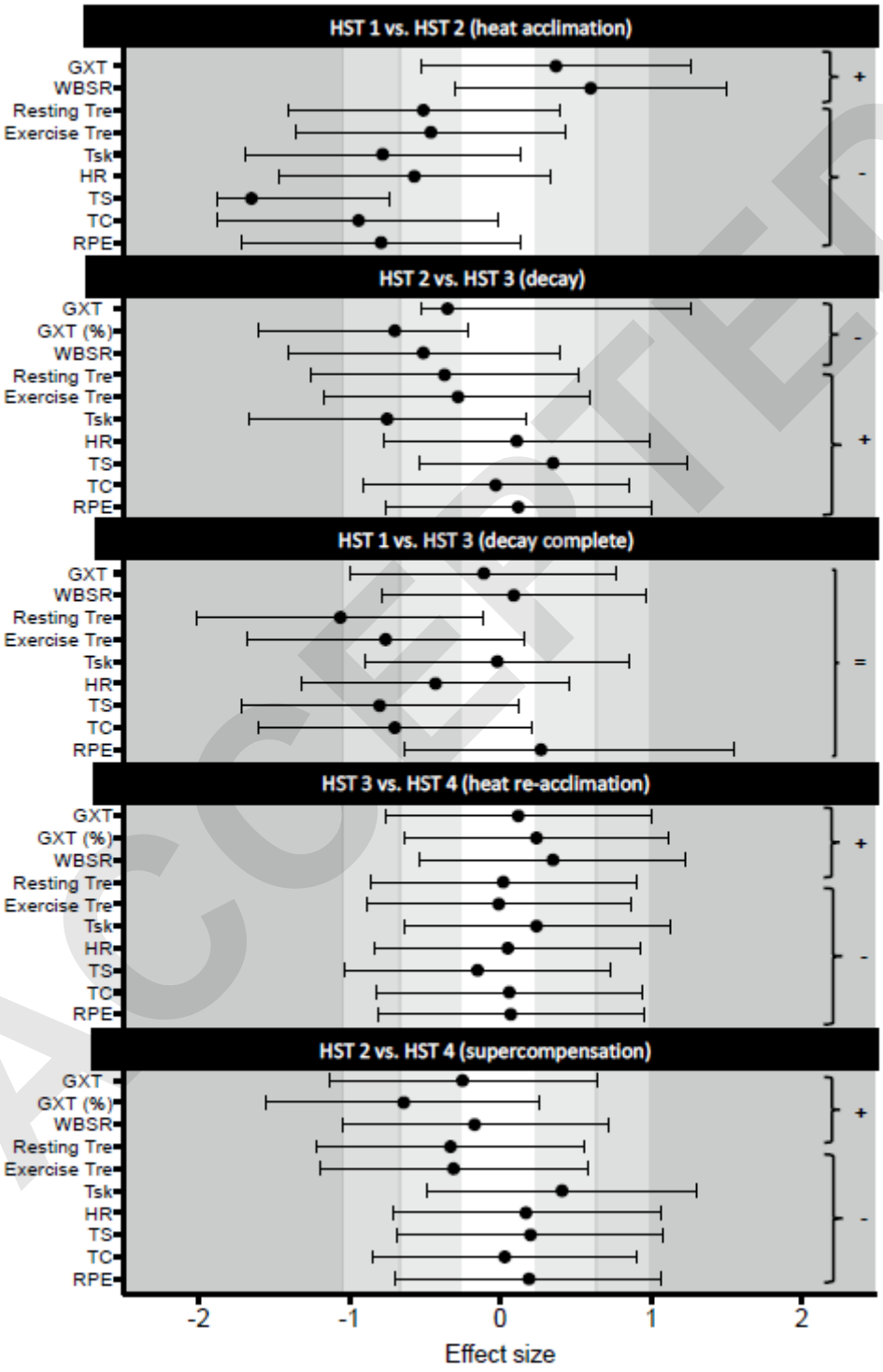

Figure 4

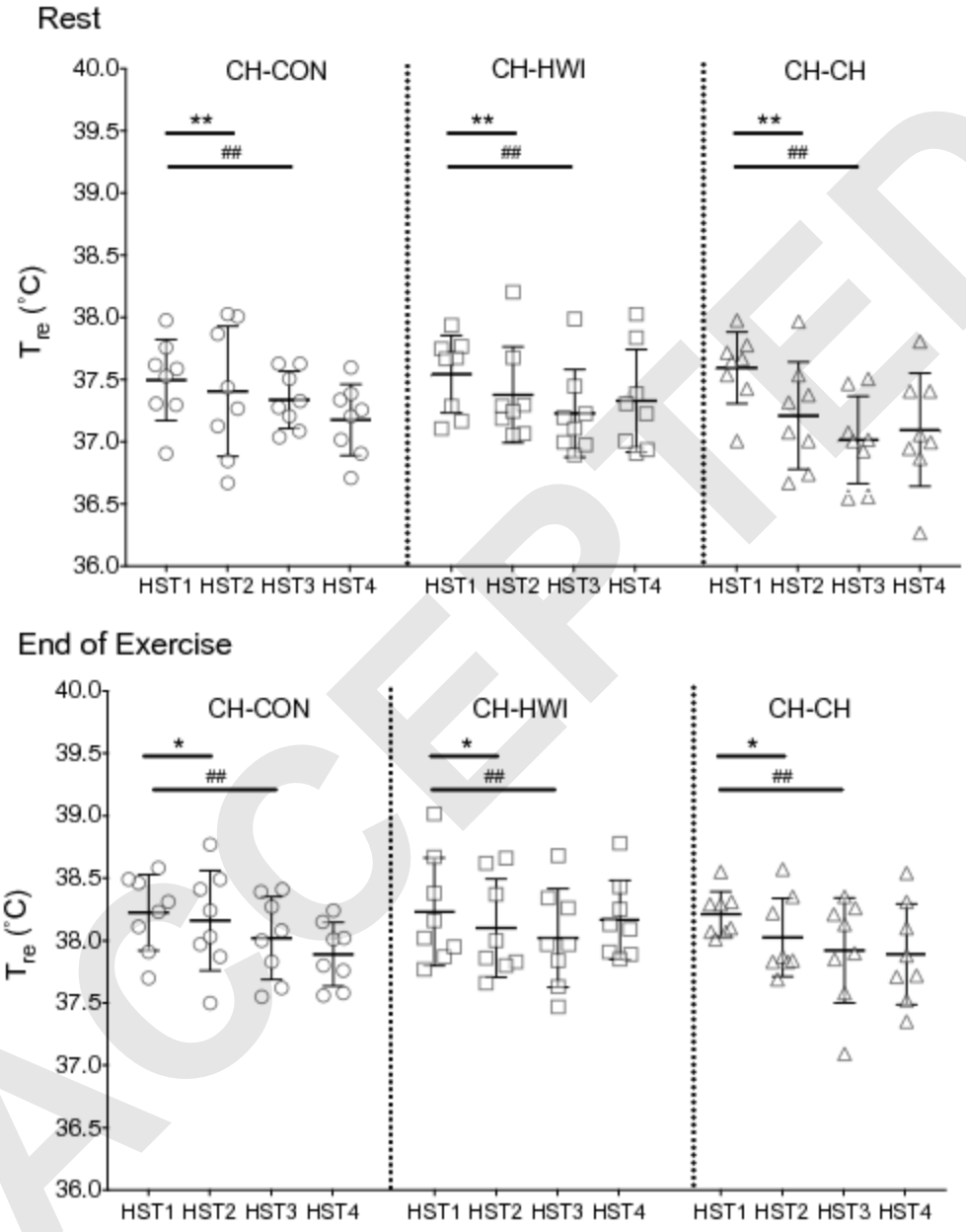

Figure 5

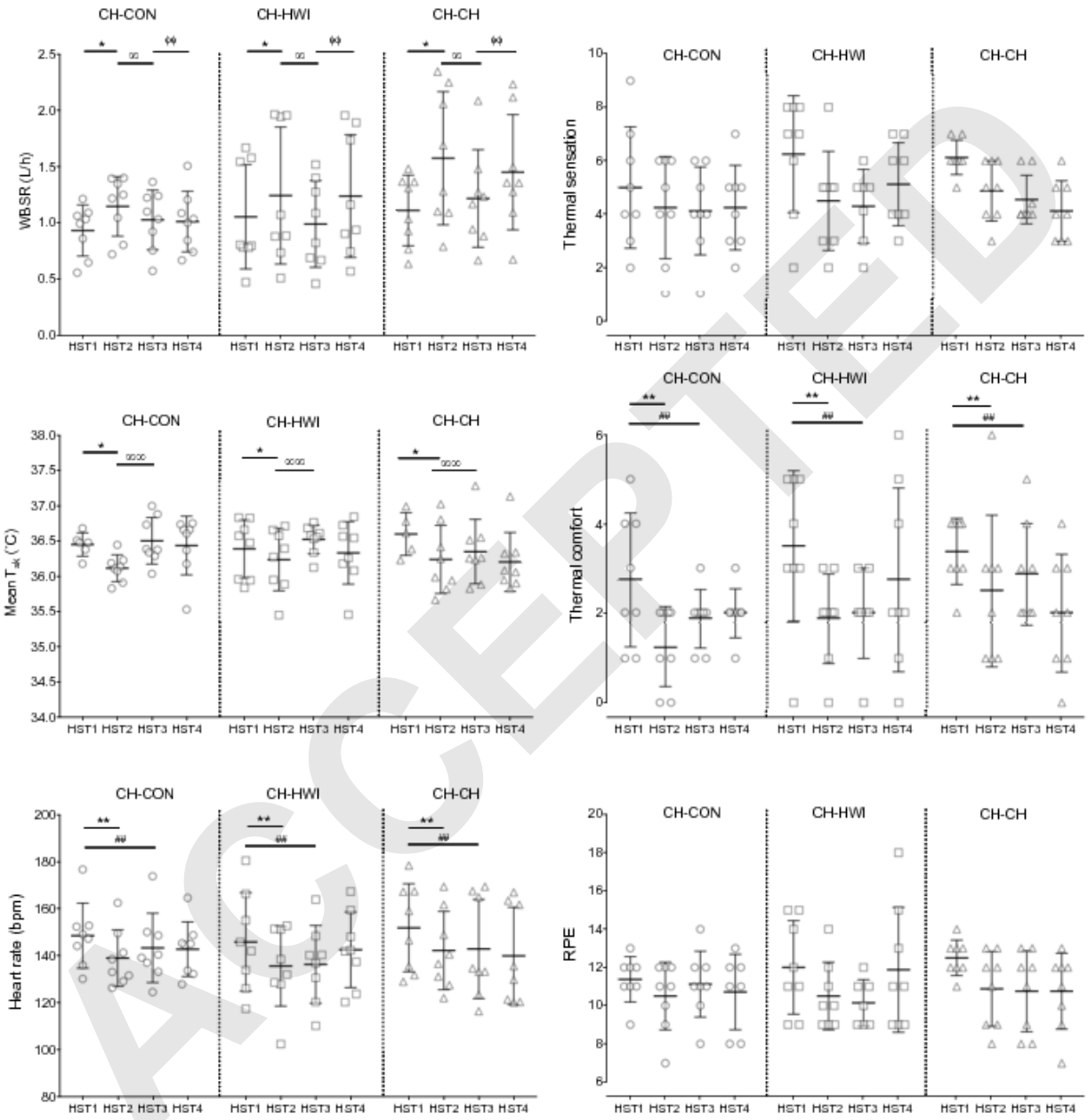

Table 1: Mean (±SD) participants characteristics of the three experimental groups. No differences were observed between any of the variables listed ($p>0.05$).

	CH-CON (n=8, 5M, 3F)	CH-CH (n=8, 5M, 3F)	CH-HWI (n=8, 5M, 3F)
Age (yr)	30 ± 8	34 ± 8	30 ± 8
Height (cm)	182.5 ± 10	181.2 ± 6.2	183.6 ± 9.6
Weight (kg)	74.8 ± 11	77.0 ± 7.2	75.2 ± 13.9
BSA (m^2)	1.96 ± 0.2	1.97 ± 0.1	1.97 ± 0.2
VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$)	52.8 ± 8.9	49.8 ± 7.8	53.0 ± 10.4

M, males; F, females; BSA, body surface area; CH-CH, heat re-acclimation with controlled hyperthermia; CH-HWI, heat re-acclimation with hot water immersion; CH-CON, control group.

Table 2: Mean (±SD) daily physiological response to the initial 10-day controlled hyperthermia (CH) heat acclimation (HA) protocol completed by all three groups; control group (CH-CON, n=8), hot water bathing group (CH-HWI, n=8), and the controlled hyperthermia group (CH-CH, n=8).

		HA1	HA2	HA3	HA4	HA5	HA6	HA7	HA8	HA9	HA10
Time to 38.5˚C T_{re} (min)	CH-CON	37 ± 8	37 ± 7	36 ± 7	36 ± 6	38 ± 8	36 ± 5	38 ± 8	37 ± 5	35 ± 5	38 ± 5
	CH-HWI	37 ± 4	38 ± 9	34 ± 6	37 ± 5	36 ± 5	36 ± 6	37 ± 6	35 ± 5	35 ± 6	40 ± 10
	CH-CH	36 ± 8	38 ± 8	38 ± 8	41 ± 10	40 ± 6	42 ± 7	42 ± 9	38 ± 5	38 ± 5	41 ± 6
WBSR* ($L \cdot hr^{-1}$)	CH-CON	1.00 ± 0.32	1.16 ± 0.32	1.27 ± 0.44	1.28 ± 0.40	1.24 ± 0.33	1.38 ± 0.42	1.42 ± 0.41	1.43 ± 0.46	1.53 ± 0.36	1.43 ± 0.40
	CH-HWI	1.06 ± 0.28	1.13 ± 0.26	1.23 ± 0.33	1.23 ± 0.25	1.14 ± 0.28	1.31 ± 0.31	1.34 ± 0.30	1.41 ± 0.28	1.43 ± 0.37	1.43 ± 0.34
	CH-CH	1.01 ± 0.53	1.02 ± 0.47	0.99 ± 0.51	1.07 ± 0.53	1.18 ± 0.57	1.13 ± 0.63	1.17 ± 0.61	1.25 ± 0.66	1.33 ± 0.66	1.30 ± 0.72
Average	CH-CON	137 ± 20	141 ±22	140 ± 20	139 ± 22	137 ± 21	137 ± 19	135 ± 20	137 ± 20	138 ± 21	137± 23

heart rate (bpm) - thermal drive	CH-HWI	139 ± 23	136± 21	139 ± 21	136 ± 17	137 ± 17	140 ± 17	140 ±18	138 ± 16	140 ± 18	138 ± 15
	CH-CH	137 ± 18	141 ± 20	138 ± 20	139 ± 21	136 ± 19	135 ± 20	138 ± 17	139 ± 19	137 ± 17	136 ± 17
Average heart rate (bpm) - thermal maintenance	CH-CON	128 ± 21	128 ± 19	124 ± 19	125 ± 20	123 ± 16	122 ± 18	135 ± 17	123 ± 18	127 ± 14	127± 17
	CH-HWI	133 ± 25	129 ± 20	128 ± 20	126 ± 15	130 ± 19	130 ± 16	140 ± 18	132 ± 16	130 ± 16	130 ± 16
	CH-CH	130 ± 22	135 ± 21	129 ± 21	132 ± 20	128 ± 16	130 ± 18	138 ± 17	129 ± 18	131 ± 14	135 ± 17

T_{re}; rectal temperature, whole body sweat rate; WBSR, heart rate

* A main effect of time indicated that WBSR was significantly highly than HA1, from day 4-10

Table 3: Mean (±SD) daily physiological response to the 5-day heat re-acclimation (HRA) protocol completed by either the hot water immersion (CH-HWI, n=8) or the controlled hyperthermia (CH-CH, n=8). Thermal impulse, whole body sweat rate (WBSR) and the average heart rate of the entire HRA protocol data is presented.

		HRA1	HRA2	HRA3	HRA4	HRA5
Thermal impulse	CH-HWI	1325 ± 14	1324 ± 24	1311 ± 26	1320 ± 28	1322 ± 27
(˚C·min)	CH-CH	3517 ± 244 #	3493 ± 249 #	3470 ± 234 #	3480 ± 317 #	3517 ± 298 #
Heart rate	CH-HWI	95 ± 5	93 ± 7	88 ± 3	119 ± 3	114 ± 11
(bpm)	CH-CH	135 ± 17	138 ± 16	139 ± 14	135 ± 14	134 ± 12
WBSR*	CH-HWI	1.1 ± 0.6	1.3 ± 0.6	1.3 ± 0.7	1.4 ± 0.7	1.4 ± 0.7
($L \cdot hr^{-1}$)	CH-CH	1.3 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.5	1.5 ± 0.5

T_{re}; rectal temperature, whole body sweat rate; WBSR.* A main effect of time indicated that WBSR was significantly higher than HRA1, from day 2-5. # A main effect of HRA protocol with CH-CH being systematically higher than CH-HWI