Effectiveness of Short-Term Heat Acclimation on Intermittent Sprint Performance With Moderately Trained Females Controlling for Menstrual Cycle Phase

Garrett, A., Dodd, E., Biddlecombe, V., Gleadall-Siddall, D., Burke, R., Shaw, J., Bray, J., Jones, H., Abt, G. & Gritt, J.

Published PDF deposited in Coventry University’s Repository

Original citation:
Garrett, A, Dodd, E, Biddlecombe, V, Gleadall-Siddall, D, Burke, R, Shaw, J, Bray, J, Jones, H, Abt, G & Gritt, J 2019, 'Effectiveness of Short-Term Heat Acclimation on Intermittent Sprint Performance With Moderately Trained Females Controlling for Menstrual Cycle Phase', Frontiers in Physiology, vol. 10, 1458.
https://dx.doi.org/10.3389/fphys.2019.01458

DOI 10.3389/fphys.2019.01458
ESSN 1664-042X

Publisher: Frontiers Media

Copyright © 2019 Garrett, Dodd, Biddlecombe, Gleadall-Siddall, Burke, Shaw, Bray, Jones, Abt and Gritt. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Copyright © and Moral Rights are retained by the author(s) and/ or other copyright owners. A copy can be downloaded for personal non-commercial research or study, without prior permission or charge. This item cannot be reproduced or quoted extensively from without first obtaining permission in writing from the copyright holder(s). The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the copyright holders.
Effectiveness of Short-Term Heat Acclimation on Intermittent Sprint Performance With Moderately Trained Females Controlling for Menstrual Cycle Phase

Andrew T. Garrett1*, Edward Dodd1, Victoria Biddlecombe1, Damien Gleadall-Siddall1, Rachel Burke1, Jake Shaw1, James Bray1, Huw Jones2, Grant Abt1 and Jarrod Gritt1

1 Department of Sport, Health and Exercise Science, Faculty of Health Science, University of Hull, Hull, United Kingdom, 2 Mathematics and Physical Science, Faculty of Science and Engineering, University of Hull, Hull, United Kingdom

Introduction: Investigate the effectiveness of short-term heat acclimation (STHA), over 5-days (permissive dehydration), on an intermittent sprint exercise protocol (HST) with females. Controlling for menstrual cycle phase.

Materials and Methods: Ten, moderately trained, females (Mean [SD]; age 22.6 [2.7] y; stature 165.3 [6.2] cm; body mass 61.5 [8.7] kg; \( \dot{V}O_2 \) peak 43.9 [8.6] mL·kg\(^{-1}\)·min\(^{-1}\)) participated. The HST (31.0°C; 50%RH) was 9 × 5 min (45-min) of intermittent exercise, based on exercise intensities of female soccer players, using a motorized treadmill and Wattbike. Participants completed HST1 vs. HST2 as a control (C) trial. Followed by 90 min, STHA (no fluid intake), for five consecutive days in 39.5°C; 60%RH, using controlled-hyperthermia (\( \sim \) rectal temperature \( T_{re} \) 38.5°C). The HST3 occurred within 1 week after STHA. The HST2 vs HST3 trials were in the luteal phase, using self-reported menstrual questionnaire and plasma 17\( \beta \)-estradiol.

Results: Pre (HST2) vs post (HST3) STHA there was a reduction at 45-min in \( T_{re} \) by 0.20°C (95%CI \(-0.30\) to \(-0.10°C\); \( d = 0.77 \)); \( T_{sk} \) (\(-0.50\); \(-0.90\) to \(-0.10°C\); \( d = 0.80 \)); and \( T_{b} \) (\(-0.25\); \(-0.35\) to \(-0.15°C\); \( d = 0.92 \)). Cardiac frequency reduced at 45-min (\(-8\); \(-16\) to \(-1\) b·min\(^{-1}\)); \( d = 1.11 \)) and %PV increased (7.0; \(-0.4\) to 14.5%; \( d = 1.27 \)). Mean power output increased across all nine maximal sprints by 56W (\(-26\) to 139W; \( d = 0.69 \); \( n = 9 \)). There was limited difference (\( P > 0.05 \)) for these measures in HST1 vs HST2 C trial.

Discussion: Short-term heat acclimation (5-days) using controlled-hyperthermia, leads to physiological adaptation during intermittent exercise in the heat, in moderately trained females when controlling for menstrual cycle phase.

Keywords: female, menstrual cycle, dehydration, fluid-regulation, plasma volume
INTRODUCTION

The worldwide popularity of football results in competitive matches being held under whole-body environmental conditions,some of which can be extremely hot.18°C with high levels of RH
(Ozgunen et al., 2010). The 2016 Olympic Games in Brazil and the future Olympic Games in Tokyo, Japan (Gerrett et al., 2019) are examples of this.

It has been reported that the menstrual cycle plays a significant role in athletic performance and health (Avellini et al., 1979; Tenaglia et al., 1999; Stachenfeld et al., 2008). However, research is limited and recent evidence has been contradictory. It has demonstrated little effect of the menstrual cycle (Sunderland and Nevill, 2003) (Stachenfeld et al., 2008). However, some possible variations in endurance performance during exercise stress have been reported (Avellini et al., 1979; Tenaglia et al., 1999; Stachenfeld et al., 2008). Particularly, in those taking the OCP (Charkoudian and Johnson, 1997; Rogers and Baker, 1997) which can increase by 0.2–0.6°C in the early follicular phase (Stephenson and Kolka, 1985; Kolka and Stephenson, 1989; Grucza et al., 1993; Hessemer et al., 1986). In contrast, there has been evidence to demonstrate no thermoregulatory effect during exercise (Taylor and Cotter, 2006). The controlled hyperthermia technique has been postulated to provide greater heat adaptation than the constant work-rate or self-regulated work-rate methodologies. The results of this study support the idea that core temperature elevation is a key consideration for successful heat acclimation associated with high skin temperature and sweating response.

In our previous work using a male cohort (Garrett et al., 2014), the use of a permissive dehydration stimulus, that is restricting fluid intake during acclimation has received recent attention (Hessmer et al., 1986). In contrast, there has been evidence to demonstrate no thermoregulatory effect during exercise (Taylor and Cotter, 2006). The controlled hyperthermia technique has been postulated to provide greater heat adaptation than the constant work-rate or self-regulated work-rate methodologies. The results of this study support the idea that core temperature elevation is a key consideration for successful heat acclimation associated with high skin temperature and sweating response.

In our previous work using a male cohort (Garrett et al., 2014), the use of a permissive dehydration stimulus, that is restricting fluid intake during acclimation has received recent attention (Hessmer et al., 1986). In contrast, there has been evidence to demonstrate no thermoregulatory effect during exercise (Taylor and Cotter, 2006). The controlled hyperthermia technique has been postulated to provide greater heat adaptation than the constant work-rate or self-regulated work-rate methodologies. The results of this study support the idea that core temperature elevation is a key consideration for successful heat acclimation associated with high skin temperature and sweating response.

In our previous work using a male cohort (Garrett et al., 2014), the use of a permissive dehydration stimulus, that is restricting fluid intake during acclimation has received recent attention (Hessmer et al., 1986). In contrast, there has been evidence to demonstrate no thermoregulatory effect during exercise (Taylor and Cotter, 2006). The controlled hyperthermia technique has been postulated to provide greater heat adaptation than the constant work-rate or self-regulated work-rate methodologies. The results of this study support the idea that core temperature elevation is a key consideration for successful heat acclimation associated with high skin temperature and sweating response.

In our previous work using a male cohort (Garrett et al., 2014), the use of a permissive dehydration stimulus, that is restricting fluid intake during acclimation has received recent attention (Hessmer et al., 1986). In contrast, there has been evidence to demonstrate no thermoregulatory effect during exercise (Taylor and Cotter, 2006). The controlled hyperthermia technique has been postulated to provide greater heat adaptation than the constant work-rate or self-regulated work-rate methodologies. The results of this study support the idea that core temperature elevation is a key consideration for successful heat acclimation associated with high skin temperature and sweating response. 
MATERIALS AND METHODS

Experimental Design and Overview

Ten moderately trained female participants undertook a 5-day STHA regime with no fluid replenishment during each daily acclimation session. Participants were thermoregulatory cardiovascular and fluid-regulatory status at measured at rest and in response to an intermittent, exercising HST, administered the week before and after the 2nd day of the STHA regime to ensure 1 day of rest. Participants were asked to refrain from strenuous exercise for 24 h prior to HSTs and using a food diary to follow a consistent food intake. They were asked to refrain from caffeine and alcohol consumption 24 h before all testing procedures.

A general overview of the STHA protocol for the moderately trained females is shown in Figure 1.

Participants

Ten, moderately trained females (Mean [SD]; age 22.6 [2.7] years; stature 165.3 [6.2] cm; body mass 61.5 [8.7] kg; cardiac output 5.5 [1.3] L·min⁻¹; and VO₂peak 43.9 [8.6] mL·kg⁻¹·min⁻¹) participated. They were gamers and oral contraceptive pill users (combined). Participants completed pre-exercise medical questionnaires and informed consent to participate in the study. All participants were in good health. The study had ethical approval (No. 1516177) from the University of Hull’s ethics committee following the World Health Organization declaration of Helsinki guidelines.

Protocol

Experimental Standardization

All participants were fully informed of all experimental procedures (orally and written). Prior to experimental testing, participants completed pre-exercise medical questionnaires and informed consents. Each female participant was on a monophasic oral contraceptive pill (OCP) and had a hormone concentration differing between individuals depending on their specific medication. All participants were previously unacclimated to the heat and this study was completed outside the British summertime to minimize seasonal acclimatization effects. To minimize circadian rhythm effects, HSTs and acclimations occurred on the same time of day. Participants were asked to refrain from strenuous exercise for 24 h prior to HSTs and using a food diary to follow a consistent food intake. They were asked to refrain from caffeine and alcohol consumption 24 h before all testing procedures.

A methodological control of participants completed HST2 and HST3 in the same phase of their menstrual cycle (luteal phase), in the active pill portion of the OCP. This was reported by menstrual cycle questionnaire. This was detailed in the start of the menstrual cycle, premenstrual symptoms and contraceptive medication. This was confirmed by baseline measurement of plasma 17β-estradiol (Table 1).

Short-Term Heat Acclimation (STHA)

The STHA protocol consisted of five consecutive days of heat exposure (39.5°C, 60% RH) for 90 min a day, using the controlled hyperthermia technique (Garrett et al., 2009) with permissive dehydration (Garrett et al., 2014). Participants cycled on Monark 824E (Monark Exercise AB, Varberg, Sweden) against a self-selected resistance at 60 rpm attaining a Tre 38.5°C as quickly as possible and maintained for the 90 min. Exposure by regular adjustment of workload. However, an initial workload of 60 watts for the first 5 min duration was the same for all participants at the start of each day. Elevation of Tre to the same point during heat exposure was used to increase workload progressively during the week. Fluid-regulatory hormones (aldosterone), electrolytes (Na⁺, K⁺, and Cl⁻), proteins (total protein, TP), Albumin (Alb), cortisol (Cortisol), and aldosterone were measured in plasma at baseline (pre) and after HST (post).

Table 1: Mean ± SD plasma 17β-estradiol in HST2 (pre-) and HST3 (post-) STHA in the luteal phases of the menstrual cycle.

| Menstrual cycle week (n = 8) | HST2 | HST3 |
|-----------------------------|------|------|
| Heat stress test            |      |      |
| Plasma 17β-estradiol (pg·mL⁻¹) | 29.7 ± 16.4 | 28.7 ± 8.0 |

Figure 1: Schematic model of the short-term heat acclimation (STHA) protocol for moderately trained females.
and percentage change in plasma volume (%PV) were measured at the end of acclimation on day 1 and day 5. Time (minutes) and mean temperature, of 28.5°C and work (J) were recorded each day of acclimation.

**Urineary measures**

Urine samples were obtained before and after acclimation on day 5. Sexplicate volumes were analyzed – in sexplicate – for [Hb] (Willoughby Dickinson Vacutainer Systems) by phlebotomy without stasis and vein (Vacutainer Precision Glide 21-gauge needle, Becton Dickinson) from an antecubital vein. Blood samples (5 mL) were taken from an antecubital vein (Dill and Costill, 1974). During HSTs and calculated using a mathematical equation of the OCP for all participants. Baseline measures of plasma volume were analyzed from day 1 to 5 of the acclimation regime, respectively, for duplicate measures. All samples for a given individual were analyzed within the same assay. Plasma aldosterone (200 µg, New Jersey, United States) was stored using chilled K-EDTA tubes (1.6 mg ml⁻¹). Measurement of aldosterone and cortisol used the Coat-A-Countaldosterone procedure. The intra-assay coefficient of variation for aldosterone and cortisol was 9.8% and 2.1%, respectively, for duplicate measures. Plasma aldosterone concentration and cortisol were measured using radioimmunoassay kits (Dammann Cardiovascular Research, Helsinki, Finland) and [Hct] (using fresh urine samples, Urine specific gravity was measured using a calibrated refractometer (Uricon-N, Urine Specific Gravity refractometer, Atago Co., Tokyo, Japan) and Urine color chart (Armstrong et al., 1998) respectively. Urine volume was recorded and urine osmolality was analyzed after the experiment.

**Blood measures**

Plasma aldosterone measurement is a clue to regulatory hormone aldosterone (200 µg, New Jersey, United States) was stored using chilled K-EDTA tubes (1.6 mg ml⁻¹). Measurement of aldosterone and cortisol used the Coat-A-Countaldosterone procedure. The intra-assay coefficient of variation for aldosterone and cortisol was 9.8% and 2.1%, respectively, for duplicate measures. Plasma aldosterone concentration and cortisol were measured using radioimmunoassay kits (Dammann Cardiovascular Research, Helsinki, Finland) and [Hct] (using fresh urine samples, Urine specific gravity was measured using a calibrated refractometer (Uricon-N, Urine Specific Gravity refractometer, Atago Co., Tokyo, Japan) and Urine color chart (Armstrong et al., 1998) respectively. Urine volume was recorded and urine osmolality was analyzed after the experiment.

**Aerobic Fitness Testing and Cardiac Output**

Participants performed a 4-min incremental ramp exercise test on a treadmill (h/p/Cosmos, Model Pulsar 3p, h/p/Cosmos, Traunstein, Germany). Participants were seated on a cycle ergometer (Wattbike Ltd., Nottingham, United Kingdom) and each minute was measured using a metabolic equation of the OCP for all participants. Baseline measures of plasma aldosterone (200 µg, New Jersey, United States) was stored using chilled K-EDTA tubes (1.6 mg ml⁻¹). Measurement of aldosterone and cortisol used the Coat-A-Countaldosterone procedure. The intra-assay coefficient of variation for aldosterone and cortisol was 9.8% and 2.1%, respectively, for duplicate measures. Plasma aldosterone concentration and cortisol were measured using radioimmunoassay kits (Dammann Cardiovascular Research, Helsinki, Finland) and [Hct] (using fresh urine samples, Urine specific gravity was measured using a calibrated refractometer (Uricon-N, Urine Specific Gravity refractometer, Atago Co., Tokyo, Japan) and Urine color chart (Armstrong et al., 1998) respectively. Urine volume was recorded and urine osmolality was analyzed after the experiment.

**Heat Stress Test (HST)**

The HST took place in an environmental chamber (Type SSR 60-20H, Design & Environment, Gwent, United Kingdom) set to ambient temperature of 31°C, 40% RH. Pre-exercise urine and blood measures (%PV) were taken prior to entering the chamber. The HST consisted of 5 min blocks of intermittent exercise on a treadmill (Pulsar 3p, h/p/Cosmos, Traunstein, Germany) and cycle ergometer (Wattbike Ltd., Nottingham, United Kingdom) at each minute lock consisted of intermittent treadmill running (standing recovery), walking (50% HRmax), jogging (60% HRmax), slow (70% HRmax), moderate (85% HRmax) and high intensity (95% HRmax) ending with treadmill running and treadmill recovery. The HST consisted of 90 breaths, 15 s each, in synchronization (WinBlast, Germany) and cycle ergometry (SprintTreadmill velocity was changed every 15 s. Percentage change in plasma aldosterone concentration and cortisol was measured using a calibrated refractometer (Uricon-N, Urine Specific Gravity refractometer, Atago Co., Tokyo, Japan) and Urine color chart (Armstrong et al., 1998) respectively. Urine volume was recorded and urine osmolality was analyzed after the experiment.

**Body temperature**

Core body temperature was measured using a rectal thermistor (Bioheterm, Bioengineering Instruments Ltd., Cambridge, United Kingdom) and placed on the forehead (Forehead, N---K---, United Kingdom). Each 5-min block consisted of intermittent exercise on a treadmill (Pulsar 3p, h/p/Cosmos, Traunstein, Germany) and cycle ergometer (Wattbike Ltd., Nottingham, United Kingdom) at each minute lock consisted of intermittent treadmill running (standing recovery), walking (50% HRmax), jogging (60% HRmax), slow (70% HRmax), moderate (85% HRmax) and high intensity (95% HRmax) ending with treadmill running and treadmill recovery. The HST consisted of 90 breaths, 15 s each, in synchronization (WinBlast, Germany) and cycle ergometry (SprintTreadmill velocity was changed every 15 s. Percentage change in plasma aldosterone concentration and cortisol was measured using a calibrated refractometer (Uricon-N, Urine Specific Gravity refractometer, Atago Co., Tokyo, Japan) and Urine color chart (Armstrong et al., 1998) respectively. Urine volume was recorded and urine osmolality was analyzed after the experiment.

**Data analysis**

Sample size was based on previous results from our previous confined research on females (Mee et al., 2015; Kirby et al., 2019) using an unpaired t-test on saliva samples (Garrett et al., 2009) and we acknowledge that our average somatic variability in the magnitude of response (Waldron et al., 2019). To control for menstrual cycle phase in HST2, post STHA trials were performed in week 1 of the menstrual cycle (Post-), whereas pre STHA trials were performed in week 3 of the menstrual cycle (Pre-). The semen was collected via a venous metabolic cardia system (Cortex Metalyzer 3B, Cortex Biophysics, Leipzig, Germany). Participants performed 17 breaths with a mouthpiece connected to a bacterial filter (Innovoxygen, Odense, Denmark) and were placed over the participant’s nose to prevent any expired air escaping. Participants were instructed to breathe in synchronization (∼15 breaths/min), with the on-screen demonstration of the test. The measure was complete.
RESULTS

All ten participants completed the 5-day STHA protocol and three HSTs (HST1; HST2; HST3). The HST1 versus HST2 was a control trial taken 1 week apart with no intervention. The HST2 versus HST3 with the STHA intervention took place over 2 weeks of the menstrual cycle (luteal phase) for all ten participants. Due to issues with venepuncture measures blood parameters were analyzed for eight participants only. Similarly, eight participants had baseline plasma 77 estradiol measured before HST2 and HST3 trials in the luteal phase.

Acclimation

Thermal Stress and Strain

Thermal stress and strain from days 1 to 5 of heat acclimation are presented in Table 2 and work undertaken is illustrated by mean cardiac frequency ($f_c$) and rectal temperature ($T_{re}$) responses. Time to 38.5°C was longer on Day 1 than Day 5 (Table 2; $P = 0.04$). Therefore, less work was performed on day 10 cf. day 5 (Table 2 and Figure 2; $P = 0.02$).

Urinary Measures

To determine hydration status, urine color (color_u), urine osmolality ($\text{osmu}$), urine specific gravity ($\text{SGu}$) and body mass were measured at rest and 90 min after day 1 and 5 of acclimation (Table 3). There was no main effect ($P > 0.05$) and interaction across time ($P > 0.05$) for color_u, osmu, $\text{SGu}$, and body mass on day 1 and 5 of STHA.

Blood Measures

Blood measures and percentage change on the first day (Day 1) to the last day (Day 5) of acclimation after 90-min heat exposure are presented in Table 4. There was no main effect for [aldol]_p between days (Day 1; $F = 0.583; P = 0.25$) nor interaction across time ($F = 0.755; P = 0.41$). Similarly, there was no main effect between day 1 and 5 for [Na+]_p ($F = 0.106; P = 0.32$) nor interaction across time ($F = 0.200; P = 0.64$). The measure [TP]_p demonstrated no main effect between day 1 and 5 ($F = 0.083; P = 0.77$) nor interaction across time ($F = 0.036; P = 0.84$). There was no main effect between day 1 and 5 for cortisol ($F = 0.530; P = 0.01$) and interaction across time ($F = 0.775; P = 0.02$). Bonferroni-corrected post-hoc comparisons showed no significant difference between pre- and post-measures within days (1 and 5) ($P = 0.02$) but between day 5 ($P = 0.57$).

Heat Stress Test

Measurements were taken at rest and across the 90-minutes of HSTs. Data is presented for ten female participants unless otherwise stated.

Control Study

The HST2 was a control trial taken 1 week apart with no intervention. There was a limited change in $T_{sk}$, $T_{re}$, $f_c$, and %PV ($P > 0.05$). Similarly, in the primiparous test, the PPO and MPO demonstrated limited change ($P > 0.05$).

Intervention Study

The HST2 trial took place 1 week before the STHA (5 days), with no fluid intake intervention. The post-HST3 occurred within 7 days of the last acclimation.

Body temperatures

Figure 3 presents the mean ± SD rectal temperature ($T_{re}$), mean skin temperature ($T_{sk}$) and mean body temperature ($T_{b}$) before ($T_{b}$) and post-acclimation in the hot conditions (31°C, 50% RH; $P = 0.00$). There was no main effect for $T_{re}$ ($F = 0.141; P = 0.27$) after STHA, but there was a significant interaction across time ($F = 0.299; P = 0.004$). Bonferroni-corrected post-hoc comparisons showed a significant main difference at 40 ($P = 0.01$) and 45 min ($P = 0.007$), but not 30 min ($P = 0.77$). Additionally, $T_{sk}$ reduced by 0.2°C (95% CI: −0.30 to 0.09; $P = 0.10$; Moderate). There was no significant main effect for $T_{sk}$ ($F = 0.252; P = 0.05$) after STHA.
**FIGURE 2** | Work output on the first day (Day 1) to the last day (Day 5) of acclimation after 90-min heat exposure. Data are mean ±SD are for ten moderately trained females. Significant difference *p < 0.05; Day 1 to the last day of acclimation analyzed using one-way analysis of variance (ANOVA) with repeated measures and Bonferroni correction t-tests to isolate differences between days.

**TABLE 3** | Urinary measures of hydration (coloru, osmu, SGu) and nude body mass, at rest and end-exercise, on day 1 and 5 of short-term heat acclimation.

|            | Day 1:rest | Day 1:end | Day 5:rest | Day 5:end |
|------------|------------|-----------|------------|-----------|
| Coloru (units) | 2 ± 1      | 4 ± 2     | 3 ± 1      | 4 ± 2     |
| osmu (mOsm/kg) | 379 ± 292  | 447 ± 181 | 379 ± 267  | 396 ± 271 |
| SGu (units)   | 1.008 ± 0.007 | 1.012 ± 0.007 | 1.008 ± 0.006 | 1.010 ± 0.008 |
| Body mass (kg) | 62.3 ± 9.9  | 61.2 ± 9.8 | 62.5 ± 9.8 | 61.4 ± 9.7 |

Data presented as mean ±SD for ten female participants. A two-way repeated measures ANOVA and post-hoc Bonferroni correction t-tests when appropriate was used to determine the differences from rest to end-heat exposure, on day 1 and 5 of short-term heat acclimation.

**TABLE 4** | Blood measures and percentage change from rest to end-exposure on the first day (Day 1) versus the last day (Day 5) of acclimation after 90-min heat exposure.

|          | [aldol]p (pg·mL⁻¹) | [Na⁺]p (mmol·L⁻¹) | [TP]p (mg·mL⁻¹) | [albp] (mg·mL⁻¹) | [cortisol]p (ug·dl⁻¹) |
|----------|---------------------|-------------------|-----------------|------------------|------------------------|
| **Day 1** |                     |                   |                 |                  |                        |
| Acclimation |                   |                   |                 |                  |                        |
| Rest      | 216 ± 131           | 140 ± 2           | 72.8 ± 3.2      | 670 ± 36         | 172 ± 63               |
| End       | 417 ± 99            | 141 ± 1           | 78.3 ± 3.0      | 716 ± 33         | 307 ± 47*              |
| %Change   | 48%                 | 1%                | 7%              | 6%               | 44%                    |
| **Day 5** |                     |                   |                 |                  |                        |
| Acclimation |                   |                   |                 |                  |                        |
| Rest      | 187 ± 64            | 139 ± 1           | 71.6 ± 4.8      | 666 ± 41         | 190 ± 47               |
| End       | 332 ± 143           | 142 ± 2           | 77.6 ± 5.7      | 717 ± 52         | 200 ± 67               |
| %Change   | 44%                 | 2%                | 8%              | 7%               | 5%                     |

Data are mean ±SD for eight moderately trained females. A two-way repeated measures ANOVA and post-hoc Bonferroni correction t-tests when appropriate was used to determine the differences from rest to end-heat exposure, on day 1 and 5 of short-term heat acclimation. *P < 0.05 at rest versus end-heat exposure (90 min) on day 1 and 5.
was a significant interaction across time (F = 32.942; P = 0.001). Bonferroni-corrected post-hoc comparisons showed a significant mean difference at 5 (P = 0.01), 30 (P = 0.002) and 45 min (P = 0.001), with a mean reduction by 0.25 (−0.35 to −0.15°C; d = 0.92; Moderate).

Cardiac frequency and percentage change in plasma volume (%PV)

There was a significant main effect for cardiac frequency (F = 47.702; P = 0.001) after STHA and interaction across time (F = 2.485; P = 0.02). Bonferroni-corrected post-hoc comparisons showed a significant mean difference at 5 min (P = 0.001), 30 (P = 0.001) and 45 min (P = 0.003). Cardiac frequency reduced at rest (−13 ± 18 to −7 b·min⁻¹; d = 0.04; Moderate) and 45 min (−8 ± 16 to −1 b·min⁻¹; d = 0.11; Moderate). There was an increase in %PV from baseline post STHA by 0% (−0.40 to 14.5%; d = 0.27; Large).

Psychophysiological

There was a significant main effect for thermal comfort (F = 27.156; P = 0.001) after STHA and interaction across time (F = 3.378; P = 0.001). Bonferroni-corrected post-hoc comparisons showed a significant mean difference at 0 min (P < 0.05). Thermal comfort reduced at 45 min by −10 (−1.5; −0.5 units; d = 0.89; Moderate). There was a significant main effect for thermal sensation (F = 19.462; P = 0.002) after STHA and interaction across time (F = 4.533; P = 0.001). Bonferroni-corrected post-hoc comparisons showed a significant mean difference at 0 to 10 and 20 to 45 min (P < 0.05). Thermal sensation reduced at 45 min by 1 (−1.5; −0.5 units; d = 0.67; Moderate). There was a significant main effect for RPE (F = 8.831; P = 0.04) after STHA and interaction effect across time (F = 2.835; P = 0.06). Bonferroni-corrected post-hoc comparisons showed a significant mean difference at 20 (P = 0.04), 45 min (P = 0.004) and 45 min (P = 0.02) with STHA and interaction across time (F = 5.332; P = 0.001). Bonferroni-corrected post-hoc comparisons showed a significant mean difference at 0 to 10 and 20 to 45 min (P < 0.05). There was a significant main effect for RPE and an increase in %PV from baseline post STHA by 0% (−0.40 to 14.5%; d = 0.27; Large).

Repeated sprint performance

The PPO and MPO were measured across all nine, 6-s maximal sprints in the 45-min protocol (Figure 4).

There was no significant main effect for PPO (F = 1.458; P = 0.26) after STHA or significant interaction effect across all nine sprints (F = 0.397; P = 0.91). There was no significant main effect for MPO (F = 0.643; P = 0.12) after STHA or interaction across all nine sprints. MPO increased across all nine sprints by 6 W (−26 ± 139 W; d = 0.69; Moderate; d = 0.69).

DISCUSSION

Effectiveness of Short-Term Heat Acclimation

The adaptations from short-term 5-d heat acclimation with no fluid intake during acclimation using the controlled hyperthermal technique reduced exercising cardiovascular strain in females controlling for menstrual cycle phase. The cardiovascular stability was a reduced increase in heat loss rather than...
FIGURE 4 | Mean ± SD of mean power output (MPO) in maximal sprint performance, pre- to post acclimation in hot conditions (31°C; 50% RH; n = 9).

Adaptation to Exercise in the Heat and Menstrual Cycle Phase

The post acclimation HST was performed within a week of the final acclimation day to prevent the decay of acclimation (Garrett et al., 2009). It has long been recognized that the menstrual cycle plays a significant role in athletic performance (Avellini et al., 1979; Tenaglia et al., 1999; Janeiro et al., 2012). Therefore, to control for menstrual cycle phase the HST (Pre) and HST (Post) STHA trials were performed within a week of the menstrual cycle (luteal phase), with all participants using oral contraceptive pills (combined). This was determined by menstrual cycle routine and baseline measures of plasma 17β-estradiol. This was measured prior to HST 1 and HST 3 as per the intervention trials and there was no statistical difference observed (Table 1). It has previously been reported that heat adaptation in females is not affected by menstrual cycle phase (Lei et al., 2017; Lei and Mundel, 2018). For this study, we used oral contraceptive pills (Armstrong et al., 2005). However, menstrual cycle phase and the associated changes in female sex hormones can influence core temperature (Inoue et al., 2005), the overall thermoregulatory set point range (Charkoudian and Stachenfeld, 2016) but the limited effect on whole body heat loss has been reported (Notley et al., 2018).

The present results using a female cohort and controlling for menstrual cycle phase undergoing STHA of daily controlled hyperthermia with no fluid intake demonstrated that the participants experienced adaptation to the heat. This was indicated by the characteristic features of acclimation. A decrease in $T_{re}$ by $-0.2 \pm 0.35°C$ (Figure 3, top panel), $T_{sk}$ by $-0.5 \pm 0.25°C$ (Figure 3, mid panel) and $T_b$ by $-0.25°C$ (Figure 3, lower panel) was observed. Similar body temperature measures have previously been reported by the author, using the hyperthermia-controlled techniques but with male participants (Garrett et al., 2009, 2012, 2014). In contrast, the research group of Mee et al. (2015) reported that the employment of the controlled hyperthermia model with permissive dehydration successfully attenuated $T_{re}$ during a 30-min run in the heat after five days in males ($-0.39 \pm 0.36°C$) but not in females ($-0.07 \pm 0.18°C$). Yet, after a further five days of acclimation, the females’ $T_{re}$ response was similar by $0.48 \pm 0.27°C$ (Mee et al., 2015). This indicates that a female population requires a longer-term intervention than the five-day STHA we employed (Mee et al., 2015). Similarly, Kirby et al. (2019) determined that five- but not four-day STHA improves self-paced endurance performance in females using hyperthermia control with permissive dehydration (Kirby et al., 2019). However, this was over a shorter four-day STHA. Furthermore, in these two studies, there were methodological differences with the present work. Both exercise protocols were short in duration (30 min) self-paced/fixated trials and importantly the menstrual cycle phase was not controlled.

End-exercise $f_c$ decreased by $5 \pm 2$ b·min$^{-1}$. Increased cardiovascular stability is recognized as one of the most rapidly occurring adaptations to the heat (Garrett et al., 2009, 2011). Furthermore, $7.0\%$ PV expansion from baseline was observed in this study. Previous research suggests intravascular
Repeated Sprint Performance

This study demonstrated that an increase in MPO was close to significance across all maximal sprints after STHA of 5 days (Figure 4). This improvement in intermittent performance is supported by Sunderland et al. (2008) who developed a protocol (4-days) for female team sport athletes (Sunderland et al., 2008). They reported a reduced rate of rise in rectal temperature and a 33% improvement in distance run during a repeated shuttle run performance test after STHA in female athletes. From a practical perspective, an improvement in sprint performance can be valuable and can result from team sport situations. Work rate during team sport matches are largely determined by the opposition’s playing style of the opposing team and individual (Ozgunen et al., 2010). The ability to maintain repeated sprint performance can determine when a game’s player gets to the ball first and can outrun the opposition.

Fluid Regulation Response to Repeated Heat Stress

In the present study, participants experienced the same thermal load and this was the basis for using the controlled hyperthermia technique for heat acclimation. Individual’s experience of mild hypohydration (1-2% body mass) (Table 2). This is similar to the imposed hypohydration administered by Judelson and colleagues who reported a modification in their hormonal and metabolic responses to resistance exercise influencing their post-exercise circulatory milieu (Judelson et al., 2008). The research design of this study is supported by recommendations from earlier work with females on sexual hormones and fluid regulation by the Stachenfeld research group (Stachenfeld et al., 1999; Stachenfeld, 2008).

Fluid Regulatory Hormones, Electrolytes and Plasma Volume Expansion

In the present study after 90-min exercise, aldosterone increased significantly across all acclimation bouts (Table 4). This is in contrast to what has previously been reported (Judelson et al., 2008). The principal effect of aldosterone is the retention of sodium and therefore the retention of extracellular fluid volume and plasma volume. However, in the present study, exercise-induced response of increased Na⁺ was not clearly evident after the no fluid intake acclimation regime (Table 4). Therefore, this is in contrast with previous findings (Brandenberger et al., 1989).

Stress Hormone Response

In the present study, the time to reach 38.5°C significantly increased (21.6%) from day 1 to 5 resulting in associated increase in work (21.3%) (Table 2). Mean time to reach 38.5°C has been shown to be longer during STHA using the controlled hyperthermia technique for females (51.9 ± 7.9 min) (Mee et al., 2015). Similarly, in the present study, cortisol was significantly increased (23.5%) from day 1 to day 5. Despite the greater time to 38.5°C and more work being completed, hence indicating a heat-adaptive response (Table 4). This agrees with previous observations in male cohorts suggesting heat acclimation decreases cortisol levels during exercise in heat (Francesconi et al., 1993; Armstrong et al., 1989). Future studies should be universal (Finberg and Berlyne, 1977; Sunderland et al., 2008).

LIMITATIONS AND FUTURE DIRECTIONS

In order to standardize menstrual cycle phase, female participants were given monophasic oral contraceptive pill (OCP) but the potential limitation was that the dose of hormone concentration and difference between individual’s depending on their specific medication.

Future directions information is limited on the physiological mechanisms of fluid regulation in females following STHA. Therefore, there is a need for studies that focus on dehydration during STHA. This may provide a greater understanding of this area. To the authors’ knowledge, our earlier work (Garrett et al., 2014) is the only study to have done this but with male participants.

CONCLUSION

In summary, the effectiveness of STHA for 5 days using the controlled hyperthermia technique with no fluid intake (Garrett et al., 2009, 2012, 2014) on intermittent activity in hot environments with a female cohort controlling for menstrual cycle phase. The current research suggests these methods of heat acclimation in a female cohort enhances thermoregulation and cardiovascular stability.
during intermittent exercise in the heat. These improvements may provide protection from exertional heat-related illnesses associated with exercise performance. This work used data obtained from a limited body of literature available and, thus, is particularly important given the 2020 Olympic Games will be held in the hot and humid conditions of Tokyo in Japan.

DATA AVAILABILITY STATEMENT

The dataset generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AG conceived and designed the research. GB, SB, and JS conducted the experiments. JB, HJ, DG-S, and RB contributed to the blood handling and analysis. GA and AG analyzed the data. AG and JB wrote the manuscript. All authors read and approved the manuscript.

ACKNOWLEDGMENTS

Special thanks to the participants in this study and the technical expertise provided.

REFERENCES

Akerman, A. P., Tipton, M. J., Johnson, J. M., and Stachenfeld, N. S. (2016). Sex hormone effects on autonomic responses to exercise in thermoneutral and intense heat acclimation program. J. Sports Med. 10(38–42):doi: 10.1055/s-2007-1024878.

Armstrong, L. E., Herrera Soto, J. A., Hacker, F. T., Casa, D. J., Kavouras, S. A., Allsopp, A. J., Sutherland, R., Wood, P., and Wootton, S. A. (1998). The physiological responses of physically fit men and women to acclimation to humid heat. J. Appl. Physiol. 85, 254–261. doi: 10.1152/jappl.1980.49.2.254

Avellini, B. A., Kamon, E., and Krajewski, J. T. (1979). Physiological Responses of physically fit men and women to acclimation to humid heat. J./DIC. Doc. 49: 254–261. doi:10.1132/jappl.1980.49.2.25.

Borg, G. A. (1982). Psychophysical Basis of Perceived Exertion. Med. Sci. Sports Exerc. 14,77–381.

Brandenberger, G., Candas, V., Reilly, M., and Kahn, J. M. (1989). The influence of initial state of hydration on autonomic responses to exercise in the heat. J. Appl. Physiol. 58,74–679. doi:10.1152/jappl.1981.55.6.838.

Brunvoll, J., Burdjen, I., Brown, N., Richards, C., and Dluznar, D. (2016). Effect of heavy Menstrual bleeding on Renin-Menincombination: Non-Elite Athletes. J. Appl. Physiol. 123(2):020117.1976–2017.

Charkoudian, N., and Johnson, J. M. (1997). Modification of active cutaneous vasodilation by oral contraceptive hormones. J. Appl. Physiol. 83,820–2017. doi:10.1152/jappl.83.6.2017.

Charkoudian, N., and Stachenfeld, N. S. (2016). Sex hormone effects on autonomic mechanisms of thermoregulation in humans. J. Auton. Neurosci. 196,75–80. doi:10.1016/j.jautneu.2015.11.009.

Constantini, N. W., Dubnov, I., and Lebrun, C. M. (2005). The menstrual cycle and sport performance. J. Sports Sci. 24,51–82.

Daen, J. H., and Jerreweijer, J. J. (2015). Effectiveness of indoor preparation program to increase thermal resilience in elderly for heat waves. J. Build. Environ. 83, 15–119. doi:10.1016/j.buildenv.2014.04.010.

Dill, D. B., and Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma renin and aldosterone concentration. J. Appl. Physiol. 37, 247–248. doi:10.1152/jappl.1974.37.2.247.

Finnerg, J. P., and Berlyne, D. E. (1977). Modification of renin and aldosterone response to heat by acclimatization in man. J. Appl. Physiol. Respir. Exerc. Physiol. 42,554–558. doi:10.1152/jappl.1977.42.4.554.

Francisconi, R. N. and Armstrong, L. E., Leva, N. M., Moore, R. J., Zalky, P. C., Matthew, W. F. (2019). Endocrinological Responses to Dietary Salt Restriction During Heat Acclimation. Nutritional Needs in Hot Environments. Washington, D.C., U.S. National Academy Press. 299–275.

Francisconi, R. N. and Pandolf, K. B. (1983). Hydypohydration and heat acclimation: plasma renin and aldosterone during exercise. J. Appl. Physiol. Respir. Exerc. Physiol. 55,790–794. doi:10.1152/jappl.1983.55.6.790.

Garrett, A. T., Goosens, N. G., Rehrer, N. J., Patterson, M. J., and Cotter, J. D. (2011). Induction and decay of heat acclimation in man. J. Thermol. Bio. 36,115–119. doi:10.1016/j.jtherbio.2015.02.005.

Garrett, A. T., Rehrer, N. J., Patterson, M. J., and Cotter, J. D. (2012). Effectiveness of short-term heat acclimation in highly trained athletes. Eur. J. Appl. Physiol. 112,282–287. doi:10.1007/s00421-011-2153-6.

Garrett, A. T., Goosens, N. G., Rehrer, N. J., Patterson, M. J., and Cotter, J. D. (2009). Induction and decay of short-term heat acclimation. Eur. J. Appl. Physiol. 107,595–609. doi:10.1007/s00421-009-1182-7.

Garrett, A. T., Goosens, N. G., Rehrer, N. J., Patterson, M. J., Harrison, J., Sammut, L., et al. (2014). Short-term heat acclimation is effective and may be enhanced rather than impaired by dehydration. Am. J. Hum. Biol. 26,311–320. doi:10.1002/ajhb.22509.

Garrett, A. T., Rehrer, N. J., and Patterson, M. J. (2011). Induction and decay of heat acclimation in moderately and highly trained athletes. Sports Med. 41,577–771. doi:10.2165/11587320-000000000-00000.

Gerrett, N., Kingma, B. R. M., Sluijter, R., and Daanen, H. A. M. (2019). Ambient temperature and exercise performance. Front. Physiol. 10,414. doi:10.3389/fphys.2019.00414.

Gibson, S. G., Mee, G. N., and Pandolf, K. B. (2015). Hemodynamic and saline extravasation during heat acclimation in exercise and non-exercise heat acclimated women. J. Appl. Physiol. 119,3389–3394. doi:10.1152/japplphysiol.00414.2014.

Goto, M., Okazaki, K., Kamijo, Y., Ikegawa, S., Masuki, S., Miyagawa, K., et al. (2010). Protein intake and thermoregulatory adaptation during heat stress in young men. J. Physiol. 588,927–944. doi:10.1113/jphysiol.2009.181746.
null