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

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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; \( \bar{\text{VO}_2} \text{ peak} \) 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 (\(-8; -16 to -1 b\cdot min^{-1}; d = 1.11\)) 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 in whole or substantial environmental conditions sometimedifficult to simulate. To this end, it has been suggested that a controlled hyperthermia technique (Taylor and Cotter, 2006) has been postulated to provide a greater heat adaptation than the constant work-rate or self-regulated work-rate methodology. In this context, it has been suggested that a controlled hyperthermia technique that is isothermic and fixed intensity heat acclimation methods and produces similar heat adaptation than a short and long-term. However, it is suggested controlled-hyperthermia is more efficient and practical than the controlled adaptation, especially for athletes tapering before competition (Gibson et al., 2015). The addition of a permissive dehydration stimulus, that is restricting fluid intake during heat acclimation, has recently been of interest (Hessmer et al., 1986). In contrast, there has been evidence that demonstrates the minimal changes in endurance performance across acclimation methods and similar heat adaptation than a short and long-term. However, it is suggested controlled-hyperthermia is more efficient and practical than the controlled adaptation, especially for athletes tapering before competition (Gibson et al., 2015). In our previous work using a male cohort (Garrett et al., 2014), a permissive dehydration stimulus during heat acclimation has been shown to improve the fluid regulatory mechanisms by improving their reabsorption (Avellini et al., 1979; Tenaglia et al., 1999) resulting in PV expansion. This study indicated that the female’s response was enhanced rather than impaired by dehydration acclimation and this work had an eutonic dehydration control. Furthermore, we have demonstrated that the disruption of eutonic dehydration with STHA (5-days) has been shown to provide heat adaptation for moderately (Garrett et al., 2009) and highly trained (Garrett et al., 2012) male athletes. From a practical perspective, it provides a lightweight exercise model that minimizes additional exercise strain and the disruption of quality training during the tapering period before competition (Garrett et al., 2011).

Most heat acclimation protocols have been conducted using male cohorts. Furthermore, the limited research that has been conducted using female participants has shown that the controlled hyperthermia technique (Taylor and Cotter, 2006) has been postulated to provide a greater heat adaptation than the constant work-rate or self-regulated work-rate methodology. In this context, it has been suggested that a controlled-hyperthermia technique that is isothermic and fixed intensity heat acclimation methods and produces similar heat adaptation than a short and long-term. However, it is suggested controlled-hyperthermia is more efficient and practical than the controlled adaptation, especially for athletes tapering before competition (Gibson et al., 2015). In our previous work using a male cohort (Garrett et al., 2014), a permissive dehydration stimulus during heat acclimation has been shown to improve the fluid regulatory mechanisms by improving their reabsorption (Avellini et al., 1979; Tenaglia et al., 1999) resulting in PV expansion. This study indicated that the female’s response was enhanced rather than impaired by dehydration acclimation and this work had an eutonic dehydration control. Furthermore, we have demonstrated that the disruption of eutonic dehydration with STHA (5-days) has been shown to provide heat adaptation for moderately (Garrett et al., 2009) and highly trained (Garrett et al., 2012) male athletes. From a practical perspective, it provides a lightweight exercise model that minimizes additional exercise strain and the disruption of quality training during the tapering period before competition (Garrett et al., 2011).

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MATERIALS AND METHODS

Experimental Design and Overview

Ten moderately trained female participants undertook a 5-day STHA regime within normal fluid replenishment during each daily acclimation session. Participants' thermoregulatory, cardiovascular, and fluid-regulatory status were 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 recovery. 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. All participants 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 questionnaires. This detailed the start of the menstrual cycle, premenstrual symptoms, and contraceptive medication. Plasma 17β-estradiol 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 (Monark 824E, Monark Exercise AB, Varberg, Sweden) against a self-selected resistance at 60 rpm attaining a \( T_{re} \) of 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, followed by an increase in workload progressively during the week. The fluid-regulatory hormone aldosterone and electrolytes (\( Na^+ \), \( K^+ \), and \( Cl^- \)), proteins (total protein [TP], Albumin [alb]), and cortisol were measured.

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; \( VO_2 \) peak 43.9 [8.6] mL·kg⁻¹·min⁻¹) participated. They were games players and oral contraceptive pill users (combined). 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 used a monophasic oral contraceptive pill (OCP) and dose of hormone concentration differed 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 at 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. All participants were asked to refrain from caffeine and alcohol consumption 24 h before all testing procedures.

As a methodological control, 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 questionnaires. This detailed the start of the menstrual cycle, premenstrual symptoms, and contraceptive medication. Plasma 17β-estradiol was confirmed by baseline measurement of plasma 17β-estradiol (Table 1).

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

<table>
<thead>
<tr>
<th>Menstrual cycle week (( n = 8 ))</th>
<th>HST2</th>
<th>HST3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat stress test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma 17β-estradiol (pg·mL⁻¹)</td>
<td>29.7 ± 16.4</td>
<td>28.7 ± 8.0</td>
</tr>
</tbody>
</table>

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 after each day of acclimation

**Urinary measures**

Urine samples were obtained before and after each day of acclimation. Urine samples were centrifuged at 3,000 rpm for 10 minutes and then stored at −20°C. The percentage change in plasma volume (%PV) was determined using a Hawksley Microhematocrit centrifuge (Sussex, United Kingdom) and a Micro-capillary reader (Damon/IEC, Tokyo, Japan). The percentage change in plasma volume (%PV) was calculated as follows:

\[ \%PV = \frac{V1 - V2}{V1} \times 100 \]

where \( V1 \) is the initial plasma volume and \( V2 \) is the plasma volume after acclimation.

**Blood measures**

Plasma aldosterone, aldosterone, and cortisol were measured by ELISA (R&D Systems, Minneapolis, USA) and stored at −20°C. Measurement of aldosterone and cortisol was performed using a Coat-ACount aldosterone procedure. The intra-assay coefficient of variation for aldosterone was 8.8% and 12.1%, respectively, for duplicate measures. The percentage change in plasma aldosterone and cortisol was calculated using the following equation:

\[ \%C = \frac{C1 - C2}{C1} \times 100 \]

where \( C1 \) is the initial concentration and \( C2 \) is the concentration after acclimation.

**Aerobic Fitness Testing and Cardiac Output**

Participants performed an incremental ramp exercise test on a cycle ergometer (Wattbike Ltd., Nottingham, United Kingdom). Each 5-min block consisted of intermittent treadmill running and treadmill jogging. A nose clip (Innovision, Odense, Denmark) was used to prevent any expired air escaping. Participants were instructed to breathe in synchronization with the on-screen demonstration until measurement was complete.

**Heat Stress Test (HST)**

The HST took place in an environmental chamber (Type SSR 60-20H, Design Environment, Gwent, United Kingdom). The experiment was conducted at a temperature of 38.5°C and 50% relative humidity. Participants were instructed to breathe in synchronization with the on-screen demonstration until measurement was complete.

**Body temperature**

Core body temperature was measured using an oral rectal thermometer (Suitex, United Kingdom). Each 5-min block consisted of intermittent treadmill running and treadmill jogging. A nose clip (Innovision, Odense, Denmark) was used to prevent any expired air escaping. Participants were instructed to breathe in synchronization with the on-screen demonstration until measurement was complete.

**Data analysis**

Sample size was based on power results from a previous limited research on females (Mee et al., 2015; Kirby et al., 2019) using our permisive dehydration protocol during STSA (Garrett et al., 2014). Where no statistical differences were observed in primary outcomes or stress responses, dependent measures were analyzed for normal distribution by

\[ F = \frac{\text{SS between groups}}{\text{SS within groups}} \]

where SS between groups is the sum of squares between groups and SS within groups is the sum of squares within groups.

**References**

- Willoughby, Dill, and Costill (1974)
- Dill and Costill (1974)
- Venin and Dill (1974)
- Willoughby et al., 2002
- Metalyzer 3B, Cortex (2020)
- Sawka et al., 1996
- Ramanathan, 1964
- Rhee, 2002
- Sawa et al., 1996
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 3 weeks at the menstrual cycle (luteal phase) of all ten participants. Due to issues with venepuncture measures blood parameters were analyzed for eight participants only. Similarly, eight participants had baseline plasma 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 output in Figure 2.

Measurements of ambient temperature ($T_a$) and RH indicated that the thermal stress was similar on days 1 and 5 of acclimation. Similarly, the thermal strain was consistent between days 1 and 5 as illustrated by mean cardiac frequency ($f_c$) and rectal temperature ($T_r$) responses. Time to reach 38.5°C was longer on Day 1 than Day 5 (Table 2; $P = 0.04$). Therefore, less work was performed on day 1 compared to day 5 (Table 2 and Figure 2; $P = 0.02$).

Urinary Measures
To determine hydration status, urine color (color$_u$), urine osmolality (osm$_u$), and protein and gravity (SGu) 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$_u$, osm$_u$, 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.

Heat Stress Test
Measurements were taken at rest and across the 45-min period. There was no main effect ($P > 0.05$) or interaction across time ($P > 0.05$) for color$_u$, osm$_u$, SGu, and body mass on day 1 and 5 of STHA.

Control Study
The HST2 trial took place 1 week before the STHA protocol with no intervention. There was no significant change in $T_a$, $T_r$, $f_c$, and %PV ($P > 0.05$). Similarly, in the prim temperature test, the PPO and MPO demonstrated limited change ($P > 0.05$).

Intervention Study
The HST2 trial took place 1 week before the STHA protocol 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 ($T_r$), mean skin temperature ($T_{sk}$) and mean body temperature ($T_{b}$) pre- and post-acclimation in hot conditions ($31^\circ{}C; 50\%$ RH). There was no significant effect on $T_r$ ($F = 0.711; P = 0.75$) after STHA; but there was a significant interaction across time ($F = 2.835; P = 0.04$). Bonferroni-corrected post-hoc comparisons showed a significant main difference at 40 min ($P < 0.01$) and 45 min ($P = 0.007$) after $T_{sk}$ reduced by $0.2^\circ{}C$ (95% CI = 0.03 to $0.10^\circ{}C$; effect size = 0.77; Moderate). The $T_b$ was a significant main effect on $T_{sk}$ ($F = 3.25; P = 0.05$) 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 (colori, osmi, SGi) and nude body mass, at rest and end-exercise, on day 1 and 5 of short-term heat acclimation.

<table>
<thead>
<tr>
<th>Day 1:rest</th>
<th>Day 1:end</th>
<th>Day 5:rest</th>
<th>Day 5:end</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colori (units)</td>
<td>2 ± 1</td>
<td>4 ± 2</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>osmi (mOsm/kg)</td>
<td>379 ± 292</td>
<td>447 ± 181</td>
<td>379 ± 267</td>
</tr>
<tr>
<td>SGi (units)</td>
<td>1.008 ± 0.007</td>
<td>1.012 ± 0.007</td>
<td>1.008 ± 0.006</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>62.3 ± 9.9</td>
<td>61.2 ± 9.8</td>
<td>62.5 ± 9.8</td>
</tr>
</tbody>
</table>

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.

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

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.
no main effect for

\( P \)

\( \text{mean difference at } 15 \) (Bonferroni-corrected post-hoc comparisons showed a significant mean difference from 10 to 20 and 25 min (\( P = 0.001 \)).

Cardiac frequency and percentage change in plasma volume

\( %\text{PV} \)

There was a significant main effect for cardiac frequency (\( F = 0.702 \text{SP} = 0.02 \)) after STHA and interaction across time (\( F = 0.485 \text{SP} = 0.02 \)). Bonferroni-corrected post-hoc comparisons showed a significant mean difference from 20 to 45 min (\( P < 0.05 \)). Cardiac frequency reduced at rest (\( -13 \text{b} \text{min}^{-1} \); \( d = 0.14 \text{Moderate} \)) and 35 min (\( -8 \text{b} \text{min}^{-1} \); \( d = 0.11 \text{Moderate} \)). There was an increase in %PV from baseline post STHA by 0.0% (\( -0.4 \text{d} \text{in} 14.5\%; \text{eff} = 0.27; \text{Large} \)).

Psychophysiological

There was a significant main effect for thermal comfort (\( F = 0.716 \text{SP} = 0.001 \)) after STHA and interaction across time (\( F = 0.378 \text{SP} = 0.001 \)). Bonferroni-corrected post-hoc comparisons showed a significant mean difference from 20 to 45 min (\( P < 0.05 \)). Thermal comfort reduced at 45 min by 1 bmin (\( -1.5 \text{b} \text{min}^{-1} \text{units} \text{d} = 0.89 \text{Moderate} \)). There was a significant main effect for thermal sensation (\( F = 0.462 \text{SP} = 0.002 \)) after STHA and interaction across time (\( F = 0.433 \text{SP} = 0.006 \)). Bonferroni-corrected post-hoc comparisons showed a significant main effect for thermal sensation from 0 to 10 and 20 to 45 min (\( P < 0.05 \)). There was a significant main effect for RPE (\( F = 0.831 \text{SP} = 0.04 \)) after STHA and interaction effect across time (\( F = 0.853 \text{SP} = 0.006 \)). Bonferroni-corrected post-hoc comparisons showed a significant mean difference from 20 to 45 min (\( P = 0.04 \)); \( b \text{min}^{-1} \text{d} = 1.11 \text{Moderate} \)) and at 45 min (\( P = 0.01 \)) RPE increased at 45 min by 2 (\( -4 \text{to } 0 \text{units} \); \( d = 0.70 \text{Moderate} \)).

Repeated sprint performance

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

Discussion

Effectiveness of Short-Term Heat Acclimation

The adaptations from short-term (5-d) heat acclimation with fluid intake during acclimation, using the controlled hyperthermia technique, reduced exercising cardiovascular strain in females controlling for menstrual cycle phase. The cardiovascular stability was enhanced rather than...
than lower heat content (~resting core temperature). At the time of daily hyperthermia, HSTs (this study), with our previous work that has used no fluid intake during acclimation but with moderately trained males (Garrett et al., 2012). However, in contrast with the limited work on STHA with females, using controlled-hyperthermia with permissive dehydration (Mee et al., 2015; Kirby et al., 2019).

Adaptation to Exercise in the Heat and Menstrual Cycle Phase

The post acclimation HST3 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; Janso de Jonge et al., 2012). Therefore, to control for menstrual cycle phase the HST3 (Post-) STHA trials were performed in week 3 of menstrual cycle (luteal phase). All participants were using oral contraceptive pills (combined). This was determined by menstrual cycle questionnaire and baseline measures of plasma 17β-estradiol. This was measured prior to HST3 and HST3 intervention trials and there was no statistical difference observed (Table 1). It has previously been reported that heat adaptation in females is affected by menstrual cycle phase (Leicht et al., 2017; Leicht and Mundel, 2018). For the use of 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 setpoint range (Charkoudian and Stachenfeld, 2016) but a 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 $T_r$ by $-0.2^\circ C$ (Figure 3 top panel), $T_{sk}$ by $-0.5^\circ C$ (Figure 3 mid panel) and $T_b$ by $-0.2^\circ C$ (Figure 3 lower panel). This was observed in similar body temperature measures that have previously been reported by the authors using the hyperthermia-control technique 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 $T_r$ during a 30-min run in the heat after 5 days in males ($-0.39^{\pm0.36}^\circ C$) but not in females ($-0.07^{\pm0.18}^\circ C$). Yet after a further 5 days of acclimation the females’ $T_r$ response was similar to males ($0.48^{\pm0.27}^\circ C$) (Mee et al., 2015). This indicates that the female population requires a longer-term intervention than the 5-days STHA we employed (Mee et al., 2015). Similarly, Kirby et al. (2019) determined nine-, but not four-day STHA improved self-paced endurance performance in females using hyperthermia-controlled with permissive dehydration (Kirby et al., 2019). However, this was over a shorter 4-day STHA. Furthermore, in these two studies there were methodological differences with the present work. The exercise protocols were shorter in duration ($\sim30$ min) and self-paced/fixed speed trials and importantly the menstrual cycle phase was not controlled.

End-exercise $f_e$ decreased by $6.2^{\pm3.6}$ 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, $\sim65\%$ of intravascular fluid volume expansion from baseline was observed in this study. Previous research suggests that intravascular
Repetitive Sprint Performance

This study demonstrated that an increase in MPO was associated with significant changes across the entire maximal sprint test after STHA for five days (Figure 4). This improvement in intermittent performance is supported by Sunderland et al. (2008) who describe elevated heat-acclimation protocol (4 days) for female teams. These results indicate that heat acclimation modifies the hormonal and metabolic responses to heat stress (Sunderland et al., 2008). They reported a reduced rate of rise in rectal temperature and a 3.5% improvement in distance run during a repeated, Shuttle run performance test after STHA for a female cohort. From a practical perspective, this improvement in performance is valuable in team sports situations. Work rate during team sports matches are largely determined by the opposition's playing style, and the ability to maintain repeated sprint performance can determine when the games player gets too close to their opponents.

Fluid Regulation Response to Repeated Heat Stress

In this present study, participants experienced the same thermal load and this is similar to the basis of using the controlled-hyperthermia technique for heat acclimation. Individual experience of mild hypohydration of 2% body mass (Table 2). This is similar to the imposed hypohydration administered by Judelson et al. (2008). The results of this study are consistent with the findings of Franchi et al. (1993), Allsup et al. (1998), and who reported a strong relationship between increased Na+ with [ald] in response. In the present study, using the AP method (Dill & Costill, 1974), the technique of the study was acclimation induced increased in resting %PV across HSTs by 7.0 ± 6% in the present study. Franchi et al. (2008) who successfully induced plasma volume expansion in a female cohort following 5 days of high-intensity heat acclimation (Pethick et al., 2018).

Fluid Regulatory Hormones, Electrolytes and Plasma Volume Expansion

In the present study, after 90-min exercise [ald] did not change significantly across the acclimation bouts (Table 4). Only this study is increased contrast to what has previously been reported by Judelson et al. (2008). The principal effect of aldosterone are the retention of Na+ and therefore water from the urine output to maintain extracellular fluid volume and thus blood volume. However, in this present study exercise-induced response was increased [Na+] was clearly evident after the no fluid intake acclimation regime (Table 4). Therefore, this study is in contrast with previous findings (Brandenberger et al., 1989) and Francesconi et al. (1993), Allsup et al. (1998) who reported a strong relationship between increased Na+ with [ald] in response. In the present study, using the AP method (Dill & Costill, 1974), the technique of the study was acclimation induced increase in resting %PV across HSTs by 7.0 ± 6% in the present study. This is similar to the findings of Pethick et al. (2018) who successfully induced plasma volume expansion in a female cohort following 5 days of high-intensity heat acclimation (Pethick et al., 2018).

Stress Hormone Response

In the present study, the time to reach 38.5°C significantly increased to 16.6% from day 1 to 5. The cortisol level was associated with increased in work (21.3%) (Table 2). This study demonstrated a reduced rate of rise in rectal temperature and a 3.5% improvement in distance run during a repeated, Shuttle run performance test after STHA for a female cohort. From a practical perspective, this improvement in performance is valuable in team sports situations. Work rate during team sports matches are largely determined by the opposition's playing style, and the ability to maintain repeated sprint performance can determine when the games player gets too close to their opponents.

LIMITATIONS AND FUTURE DIRECTIONS

In order to standardize menstrual cycle phase each female participant used a nonhormonal oral contraceptive pill (OCP), but a potential limitation was that the dose of hormone concentration and differences between individual's depending on their specific medication.

For future directions, information is limited on the physiological mechanisms of fluid regulation in females following STHA. Therefore, a comparison of fluid regulation in females following STHA may provide a greater understanding of this area. To the authors' knowledge, our earlier work (Garrett et al., 2009, 2012, 2014; Neale et al., 2015) on the stress hormone cortisol significantly increased during acclimation on day 1 but this response was not observed on 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 a male cohort suggesting heat acclimation reduces cortisol levels during exercise in the heat (Francisconi et al., 1983; Armstrong et al., 1989) but such findings are not universal (Finberg and Berlyne, 1977; Sunderland et al., 2008).

CONCLUSION

In summary, this work has established 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 increasing fluid volume expansion in females for high-intensity heat acclimation in a female cohort enhances thermoregulation and cardiovascular stability.
during intermittent exercise and heat. These improvements may provide protection against exercise-related illness associated with exercise performance. This work had some limitations. A large body of literature is available and this is particularly important given the 2020 Olympics will be held in humid and hot conditions of Tokyo in Japan.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Hull Ethics Committee. All patients provided their written informed consent to participate in this study.

REFERENCES


AUTHOR CONTRIBUTIONS

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

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